Library Article

Identifying and Controlling Defective Genes: Knowing How is the First Step in Tackling the Problem

(This article first appeared in The AKC Gazette, July, 1993 and was reprinted in the Beardie Bulletin, February 1996 with permission. Jerold S. Bell, DVM)

Part of every breeder's commitment to purebred dogs is to improve the health and diminish the incidence of genetic disease. In order to do this, breeders must know the tools available to them to identify, manipulate (through breeding) and, therefore, control the genes that cause genetic disorders. The inbreeding that created distinct breeds with particular characteristics also acts to isolate detrimental genes that control genetic disorders. The point from establishment of a breed to its proliferation is termed a genetic bottleneck and is the point where the least amount of genetic variation occurs. A genetic bottleneck can also occur when a few related dogs are imported to a new country. At such a time, the genes of frequently used individual dogs can have a major impact on the future of the breed. Rare recessive genes (desirable or undesirable) can increase tremendously in frequency and become fixed in the population.

The establishment of a genetic disorder in a breed due to inheritance from a common ancestor is referred to as the founder's effect. We know of such widespread examples as copper toxicosis in Bedlington Terriers, pyruvate kinase deficiency anemia in Basenjis, and abnormal purine metabolism and urate bladder stones in Dalmatians, among others. No breed is free of breed-related genetic disorders.

Determining a Genetic Basis.
When a disorder appears in a breeding program, the first question breeders must ask is, "What caused this disorder?" Is the cause genetic, environmental, a combination of both or spontaneous? Examples of purely hereditary diseases, whose occurrence are not influenced by any other factors, include progressive retinal atrophy (PRA), hemophilia A and B and muscle fiber deficiency in Labrador Retrievers. Some purely environmentally caused disorders result from toxins, injuries or infection.

Many polygenic disorders (those caused by more than one gene) will also be greatly influenced by environmental factors. Examples include hip dysplasia,

intervertebral disk disease and other musculoskeletal abnormalities. Finally, there are spontaneously occurring disorders that turn up at random without a recognized genetic or environmental cause. These include many cancers and immune-mediated disorders.

In disorders controlled by a simple autosomal dominant gene (A), all affected individuals will have at least one affected parent. The heterozygous affected (Aa) individual will pass the defective gene to half its offspring, making them affected. Males and females will be affected in equal numbers. In many disorders caused by a simple autosomal dominant gene, the homozygous state (AA) is lethal pre- or postnatally. As most affected dogs are heterozygous, affected-to-affected matings should produce some unaffected offspring. Examples include cutaneous asthenia (connective tissue disease) of English Springer Spaniels and hairlessness in many breeds.

In disorders caused by a simple autosomal recessive gene (a), both parents of affected dogs (aa) will be either heterozygous carriers (Aa) or affected (aa). This type of transmission often skips generations because only the affected dogs are readily identified, while the carriers are not. Males and females are affected in equal numbers. If the frequency of the gene in the breeding population is low, affected dogs will have a higher inbreeding coefficient than the average of the breed. When combining the results of numerous matings of similar types of parents, the expected percentages of genetically normal, carrier and affected offspring should be evident (see Figure 1). Affected -to-affected matings should produce all affected offspring. Examples include dwarfism in Alaskan Malamutes and progressive neuronal abiotrophy in Kerry Blue Terriers.

Sex-Linked Inheritance
In X-linked inheritance, females have two X chromosomes and, therefore, a pair of X-linked genes. Males have only one X chromosome and, therefore, only one X-linked gene. With disorders caused by a simple X-linked dominant gene (X'), all individuals with a single defective gene will be affected. Heterozygous affected mothers (XXa) pass on the defect to half of their sons and daughters with a particular disorder with greater frequency than other breeds. When researching possible genetic disorders, a veterinarian or specialist must first confirm the "diagnosis." For example, a growing, large-breed dog with a forelimb lameness that responds to crating does not constitute a diagnosis of osteochondrosis dessicans (OCD). "Fading puppy syndrome" and seizures" are not diagnoses, but symptoms that can be caused by many genetic or

environmental factors. In depth diagnostic studies must be performed to establish a precise diagnosis.

Table 1. Expected percentages of genetically normal, carrier or affected offspring from matings involving a simple autosomal recessive gene

Type of Mating	Expected Normals	Expected Carriers	Expected Affecteds
Normal to normal	100%	-	-
Normal to Carrier	50%	50%	-
Carrier to Carrier	25%	50%	25%
Carrier to Affected	-	50%	50%
Affected to Normal	-	100%	-
Affected to Affected	-	-	100%

Mode of Inheritance.

Once a genetic basis is established, the mode of transmission should be investigated. In determining the mode of inheritance of a genetic disorder, breeders must have information on the genders and the affected versus nonaffected status of littermates, parents and offspring of affected individuals. This information must include veterinarian-confirmed evaluations of these individuals beyond the age of onset of clinical signs of the disorder.

Many genetic disorders are caused by the effects of only one gene pair (simple Mendelian inheritance); others are caused by many gene pairs acting together. As the list of disorders increases, some may be recategorized.

A congenital disorder is one that is present at birth. Breeders see many congenital disorders in their breeding stock; not all are hereditary. There are many infectious diseases or toxins to which pregnant dams can be exposed that will cause congenital disorders. For example, toxoplasma infection in the pregnant dam can cause retinal damage in the fetus. Fetal exposure to corticosteroids can cause a cleft palate.

Not all genetic disorders are congenital. There are many late-onset hereditary disorders that will not be expressed for months or years. Some do not occur until well after breeding age, which further complicates genetic control.

Library Article

To determine if a disorder has a genetic component, first research its frequency in the breed. Has the disorder been reported previously in your breed? To find out, research veterinary Journals, breed-related publications and breed surveys. Has the disorder been reported in any other breeds, or even other species (cats, mice, humans)? Has a genetic basis or mode of inheritance been confirmed in these cases?

Some genetic disorders do not have an established mode of inheritance but do have a known breed prevalence, i.e., individuals of a breed are diagnosed. Homozygous affected mothers (X'X') have two affected parents and all affected offspring. Affected fathers (x'y) pass on the defect to all their daughters, but none of their sons. Because of this, there tend to be more affected females than males. In disorders caused by a simple X-linked recessive gene (X'), affected males need only one defective gene (X'Y) while affected females need two defective genes (XaX,). Carrier males do not occur, as males can only be normal (X'Y) or affected (X'Y). An affected male must have a carrier (X'XA) or affected (X'X') mother. An affected female must have an affected father and a carrier or affected mother.

When compiling the results of numerous matings to carrier mothers, 50 percent of all male offspring will be affected, and 50 percent of the female offspring will be carrier. Because of this, there tend to be more affected males than females. Examples include muscular dystrophy in Golden Retrievers and hemophilia A and B.

Problems in fitting data from a disorder into a simple Mendelian pattern can arise if the defect shows variable expressivity or incomplete penetrance. Variable expressivity means all individuals with the affected genotype will have the disorder but may manifest it differently. Variations can include differences in severity, age of onset or type of symptoms that may obscure the diagnosis. Incomplete penetrance means some individuals with the affected genotype will not have an affected phenotype and will appear normal. These individuals can only be recognized by the frequency with which they pass on the defective gene to their offspring or, possibly, by sophisticated genetic testing.

Modifications of Simple Inheritance
Variable expressivity and incomplete penetrance can occur because of environmental factors or by other genes altering the action of a primary gene pair. The latter would be considered a form of polygenic inheritance. Many disorders are labeled as polygenic when no clear-cut mode of inheritance is evident. In some cases, a disorder tends to follow a dominant or recessive

pattern but does not produce the expected Mendelian ratios of affected to non-affected offspring. Examples include cryptorchidism, elbow dysplasia and deafness. Such disorders are often cited as having incomplete penetrance, for lack of other identifying characteristics.

Many breed-related genetic disorders such as juvenile kidney dysplasia in Miniature Schnauzers and pancreatic insufficiency in German Shepherds have been studied and found to not follow simple Mendelian patterns. They are assumed to be controlled by polygenic inheritance.

Genetic Epidemiology
Determining the extent of the problem in a breed includes knowing the prevalence of the defect in that breed. For certain types of disorders, control agencies exist to monitor frequencies in diagnosis. The Canine Eye Registry Foundation (CERF) keeps track of hereditary eye diseases diagnosed by board-certified veterinary ophthalmologists. The Orthopedic Foundation for Animals (OFA) maintains records on hip and elbow dysplasia. The AKC lists OFA and/or CERF certification on a dog's pedigree. Some national breed clubs compile periodic breed surveys on hereditary conditions or establish registries for a specific genetic condition when that condition is recognized to be a problem in the breed.

Another way of determining the extent of a proven genetic disorder in a breed is through analyzing the dogs' pedigrees. By determining the relatedness of all confirmed affected individuals, breeders can estimate the relative age of the defective gene(s) in the breeding population. An ancestor that traces to all affected individuals is termed the closest common ancestor. The process of identifying a closest common ancestor is often mislabeled by breeders as a "witch-hunt" because, usually, the individual identified as a closest common ancestor is a popular male used extensively for breeding at one time. However, the closest common ancestor does not identify the ancestor as a carrier of the defective gene; rather, it measures the minimum age of a defective gene in the population (how far back a mutation must have happened to explain the occurrence of all affected individuals). For autosomal recessive disorders, the common ancestor must trace to all parents of affected individuals; for autosomal dominant disorders, the common ancestor must trace to only one parent of the affected individuals.

One of the fallacies breeders seem to find most difficult to let go of is that carriers of recessive genes can be identified based on their appearance multiple generations behind affected individuals. Carriers of recessive genes

can only be confirmed if they are parents or offspring of affected dogs (NOTE: you will need to obtain a copy of the original article in the AKC Gazette of the Beardie Bulletin for Figure 1 and explanatory notes). If the closest common ancestor for a genetic disorder in a breed occurs within a few generations, then the gene(s) controlling the disorder may not be widespread in the population. The problem may only concern a few kindreds, and strict breeder control should be exercised to keep it from spreading.

If the closest common ancestor for a genetic disorder is far back in the pedigree (possibly from another continent), then the gene(s) must be considered widespread the breed. In that case, control is every breeder's responsibility, and breed-wide control programs are indicated.

Identification of Carriers
For control of defective recessive genes, breeders must be able to identify unapparent carrier individuals. For some genetic disorders, biochemical tests can be done to identify carriers of recessive genes. If the gene "codes" for the production of an enzyme or biochemical substance necessary for normal metabolism, carriers (those with only one normal gene) will have approximately half the level of the substance as homozygous normal dogs (those with two normal genes). This level of gene product is enough for normal functioning, but can often be measured as a screening test for carriers. Many neurological storage diseases, bleeding disorders and hereditary anemias can be screened in this way.

Biochemical tests used to screen for recessive genes measure a gene product and not the gene itself. Therefore, these types of tests are not absolute assessments of the genotype of the dog. Some dogs will test in an indeterminate area - somewhere between the range for homozygous normal and heterozygous carriers. A small percentage of individuals will be mislabeled and receive a false test result that indicates the opposite of their true genotype. Affected dogs have no functional genes producing the biochemical substrate, and therefore are readily identified with these tests-with few false results.

Examples of genetic disorders with established screening programs include neurological storage diseases such as GM-gangliosidosis in Portuguese Water Dogs and fucosidosis in English Springer Spaniels. Hematological disorders with screening programs include pyruvate kinase deficiency anemia in Basenjis, Beagles, West Highland White and Cairn Terriers; the recessive form of von Willebrand's disease in Scottish Terriers, Shetland Sheepdogs and

Chesapeake Bay Retrievers; and the dominant form of von Willebrand's disease in Doberman Pinschers.

Recent developments in molecular genetics have led to identification of the actual physical genes causing certain defects. A direct test of a dog's genotype can be determined with gene probes and polymerase chain reaction (PCR), a laboratory technique that can be performed on a small amount of blood. Such tests significantly eliminate the problems of indeterminate or mislabeled results inherent with biochemical assays. A PCR has been developed for phosphofructokinase (PFK) deficiency anemia in English Springer and Cocker Spaniels. A research project cosponsored by the AKC, OFA and Morris Animal Foundation is underway for the development of additional canine gene probes.

Test Matings
Test matings can be performed for individuals with high carrier risk of simple recessive disorders for which no testing system exists. If a dog with an unknown genotype is bred to an affected dog and produces a litter that includes five unaffected offspring and no other affected pups, then the probability the test animal is normal is greater than 95 percent. If the litter includes seven unaffected pups and no other affected pups, then the chance of being genetically normal is greater than 99 percent. If affected dogs cannot reproduce, then test matings to confirmed carriers must be performed. Eleven unaffected offspring are required for 95 percent confidence, and sixteen unaffected offspring are required for 99 percent confidence of genetic normalcy.

Test matings can be difficult on breeders both technically and emotionally. (See I Raised a Test Litter" in the August 1991 GAZETTE.) If the disorder has a late age of onset, the offspring have to be properly identified and tracked beyond that age. Knowingly producing possibly affected dogs is burdensome. Also, all test matings will produce carrier dogs; therefore, it is the breeder's responsibility to issue these dogs limited registrations and to be sure the offspring are eventually neutered. While test matings are difficult, organized test mating programs have been used successfully by many breeds. Examples include progressive retinal atrophy (PRA) in Irish Setters and juvenile cataracts in Miniature Schnauzers.

When attempting to control genetic disorders, breeders must utilize all available information concerning confirmed affected dogs. When selecting breeding stock and screening for genetic disease, breeders must ensure the dogs are beyond the age of onset of the disorder.

Disorders that have a late onset can often leave breeders one generation behind in control: Affected dogs have already reproduced, and affected or carrier offspring have already been placed in breeding homes. If the disorder demonstrates variable expressivity, breeders must screen for all known variations. If the disorder shows incomplete penetrance, breeders may need screening tests (such as those used for carriers of recessives) or test matings to identify affected dogs without the clinical phenotype.

Dominant Disorders
Control of genetic disorders caused by a defective dominant gene is usually easy, because all individuals carrying the defective gene are affected. By removing the affected individuals from the breeding pool, all the defective genes are removed.

Dominant disorders showing incomplete penetrance, such as the dominant form of von Willebrand's disease, are more difficult to control. A blood-screening assay for plasma von Willebrand's factor antigen (vWF:Ag) can indicate the genetic potential for carrying the defective gene, but will not show which dogs are clinically affected. Many dogs with low vWF:Ag never have bleeding episodes, but pass on the liability for bleeding to their offspring. Although the vWF:Ag is reported as a quantitative number, it is a screening test for a qualitative gene product that is influenced by many genetic and environmental factors. Concurrent thyroid abnormalities, infectious disease, estrus and other environmental stresses can alter the measurable vWF:Ag. As all affected dogs with clinical bleeding episodes have low vWF:Ag, breeders should select breeding stock with high vWF:Ag and normal thyroid function.

Genetic disorders controlled by recessive genes are much more difficult to control, because there are far more normal-appearing carrier individuals than affected. The removal of all affected individuals-or even all affected and their parents-will still leave the majority of carriers in the breeding pool. These will include half the full-sibs to the parents, two-thirds of the normal appearing full-sibs to the affected dogs, half of any additional offspring of either parent and all the offspring of affected dogs. Individuals that appear in generations behind the parents may be unapparent carriers, but cannot be identified as such unless they produce affected individuals.

Assessing Matings
Most dogs affected with a simple autosomal recessive genetic disorder are produced by carrier- to-carrier matings. Sometimes a breeder will take the dam from that mating, breed to other males and produce no further affected

offspring. The common feeling is that the first stud dog had "some bad genes," but by staying away from him and using other stud dogs, healthy pups were produced. In reality, the later matings were probably carrier (the dam)-to-normal matings that multiplied the defective gene into half of all the offspring. Occasionally a breeder has a dog with a non-lethal defect that does not seem to pass it on to its offspring. If that defect is controlled by an autosomal recessive gene, those matings may all be affected to-normal matings, producing all carrier offspring. The majority of simple recessive genetic disorders have no available tests for carriers. This leaves breeders with only the knowledge of the veterinarian confirmed affected dogs and their close relatives. In such situations, the recognition, cooperation and dissemination of information about confirmed affected and carrier dogs within the breed organization is of the utmost importance. Without this information, concerned breeders have no way of knowing the carrier risk in their own dogs.

Calculating Risk Factors
With a simple autosomal recessive genetic disorder, pedigree analysis can be used to compute relative risk factors for carrier and affected status. These risk factors are based on the following relationships: Parent of affected = 100 percent chance of being carrier; offspring of affected = 100 percent chance of being carrier; full-sib to carrier = 50 percent chance of being carrier; non-affected full-sib to affected = 67 percent chance of being carrier. Similar risk factors can be calculated for X-linked recessive inheritance.

Calculating relative risk assessments involves identifying every individual in the pedigree from the above categories and calculating the risk down the pedigree to the individual whose risk you wish to identify. Although there are complicated formulas available, a good estimation of risk is found as follows: Offspring receive half the carrier risk of their parents (half the carrier risk of the sire, plus half the carrier risk of the dam). The risk of being affected is the product of the carrier risk from the parents (half the carrier risk of the sire times half the carrier risk of the dam).

Relative risk assessments can only be viewed as the minimal risk of the individual based on the known affected relatives from the pedigree. If additional affected relatives are diagnosed, the known risk can rise. Many people want to add risk factors for parents of known carriers. As only one of the parents of known carriers needs to pass on the defective gene, assigning risk to the opposite parent will falsely identify individuals and lines of descent that may not have such risk. Therefore, risk can only be assigned when the

parents of confirmed carriers are mated together or if additional affected dogs place one of the parents in a known risk category.

Breeders can use relative risk assessments in their breeding programs by trying to decrease the risk of carrier and affected status with each generation. If the average carrier risk for the breed can be determined, breeders should attempt to breed to those dogs whose carrier risk falls below this average, thus lowering the relative risk of their own dogs with each generation.

Carriers can multiply defective genes into their offspring: Numerous carrier offspring can replace the one carrier parent. Therefore, limiting the number of breeding offspring from carriers or those with high-carrier risk is equally important. If an obligate carrier is replaced in the gene pool by two offspring (each having a 50 percent chance of being a carrier), then the carrier risk in the breeding program remains the same. The use of limited AKC registration is appropriate here.

Disorders with polygenic or unknown modes of inheritance can still be controlled through selective breeding. These disorders must be viewed as having multiple genes and possibly environmental factors. A critical threshold of these factors can add up to cause the disease. As the genetic factors are the sum of,",hat is passed on from the parents, both parents of affected dogs must be viewed as carrying a "dose" of defective genes.

Selection against polygenically controlled disorders involves: (1) identifying traits that more closely represent genes being selected against; (2) the elimination of nuisance factors that can limit your selective pressure against the genes; and (3) selecting for breadth of pedigree as well as depth of pedigree.

For Example, Hip Dysplasia
Canine hip dysplasia is a polygenically controlled disorder of the hip joints that affects many breeds of all sizes. Breeders have attempted to control hip dysplasia by only breeding dogs, whose hips have been OFA certified. The lack of a rapid response to such selection indicates there is no gene for OFA-certifiable hips, and all dogs do not have hip dysplasia due to the same genetic factors. When evaluating dogs for hip dysplasia, breeders must gather all clinical data that might possibly relate to the genetic factors involved. This data includes palpable joint laxity under anesthesia, clinical signs of hip lameness and radiographic signs of hip subluxation and/or bone malformation. A dog with laxity and subluxation but normal anatomy probably has different genetic

causes for hip dysplasia than does a dog with no subluxation but malformed sockets.

Genetically controlled rapid growth in a young dog can cause an incongruity between the bone and soft tissue components of the hip joint. By selecting against such traits (as opposed to hip joint status as a whole), breeders may find a better genetic response in the offspring. It is best to perform hip evaluations after the recognized age of onset of hip dysplasia (95 percent by two years) but before the genetic potential can be altered (due to osteoarthritis, remodeling and long-term environmental influence). It is known that "genetically predysplastic hips" can be protected by restricting environmental stress, and this is wise for pet dogs. Breeding dogs, on the other hand, should be allowed to develop naturally, so as not to mask the genetic potential for dysplastic development.

Probably the most important lesson we learn from studying hip dysplasia is that the breadth of pedigree is as important as the depth of pedigree. This means that in addition to determining the hip status of the breeding dog and its parents, breeders must determine the hip status of the full-sibs of the dog and also the full-sibs of the parents. Mating the only two dogs to receive OFA certification from their respective litters doesn't necessarily reduce the risk of producing offspring with dysplastic hips. I often ask breeders which dog they would rather use: one that passed OFA certification from a litter where the rest of the litter failed, or a mildly dysplastic dog from a litter where the rest of the litter received OFA certification. I would pick the latter (although the best choice would be one of the OFA certified dogs from the second litter).

The point is, with polygenic disorders, the littermates mirror the genetic potential of the individual breeding dog. in addition, the offspring of breeding dogs should be monitored to see which are passing polygenically controlled disorders with lower frequency.

For Example, PDA
Patent ductus arteriosus (PDA) is a congenital heart defect found sporadically in many breeds. It is caused by a fetal blood vessel connecting the left and right sides of the heart. If the vessel remains open (patent) after birth, heart failure ensues.

For every pup in heart failure due to PDA, there are additional relatives with a ductus diverticulum (DD), a dead-end vessel that does not cause heart failure. Breeding studies have shown that normal-appearing individuals with DD

(identified by ultrasound or angiography) pass on as much liability for abnormal ductal closure as those with PDA.

Matings involving first-degree relatives of dogs with PDA or DD produce offspring with abnormal ductal closure similar to that of matings to affected dogs. This shows that breeders must screen for (1) subclinically affected dogs whose genetic and environmental threshold has not produced clinical disease, and (2) close relatives of affected dogs must be considered to have a higher genetic load of genes contributing to polygenically controlled disorders.

Control Programs
Once a mode of inheritance and the prevalence of a genetically controlled disorder are known, members of a breed organization can institute a control program tailored to the particular disorder. If the disorder is widespread, control may have to progress slowly so good genetic qualities are not lost along with defective gene(s). If no tests are available for carriers of a simple recessive gene, obligate carriers should be removed from breeding programs; they are the only known dogs definitely transmitting the defective gene.

Once a genetic basis for a disorder is established, breeders need to put emotions and accusations behind and deal with the problem of control. No one who loves a breed wishes to produce defective dogs or propagate deleterious genes. No one can create a defective gene through breeding practices. No matter how long or through how many dogs defective genes have been spread, concerned breeders must start from the point of recognition of the defect and work together toward the improvement of the genetic health of the breed.

(Jerold Bell is director of the Clinical Veterinary Genetics Center for the Tufts University School of Veterinary Medicine and national project administrator for numerous genetic disease control programs of Purebred dogs. He performs genetic counseling through Veterinary Genetic Consulting and practices small animal medicine in Connecticut. He and his wife own Gordon Setters.)

References
Bell, J. Clinical Veterinary Genetics Syllabus. Tufts University School of Veterinary Medicine. 1993.

Brewer, G.J. and Yuzbasiyan Gurkan, V. "Fighting Disease With Molecular Genetics," Pure-Bred Dogs American Kennel GAZETTE, January 1989.

Library Article

Brooks, M., Dodds, W.J. and Raymond, S.L. "Epidemiologic Features of von Willebrand's Disease in Doberman Pinschers, Scottish Terriers and Shetland Sheepdogs: 260 cases (1984-'88), " Journal of the AVMA, April 15, 1992.

Foley, C.W., Lasley, J.F. and Osweiler, G.D. Abnormalities of Companion Animals: Analysis of Hereditability. Iowa State University Press. 1979.

Giger, U., Smith, B.F., Woods, C.B., Patterson, D.F. and Stedman, H. "Inherited Phosphofructokinase Deficiency in an American Cocker Spaniel, " Journal of the AVMA, November 15, 1992.

Keller, G., Vogt, D., Corley, E.A., Padgett, G. and Ellersieck, M. "Progress in the Control of Hip Dysplasia, "Pure-Bred Dogs American Kennel GAZETTE, November 1991.

Patterson, D.F., Pyle, R.L., Buchanan, J.W., Trautvetter, R. and Abt, D.A. "Hereditary Patent Ductus Arteriousus and its Sequelae in the Dog, Circulation Research, January 1971.

Robinson, R. "A Bibliography: Genetic Anomalies in Dogs," Canine Practice, January/February 1991.