

Genetic counseling in the era of molecular diagnostics

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Abstract

Veterinarians with an interest in theriogenology will often be asked by small animal clients for advice concerning hereditary diseases in their breeds. Many new DNA-based tests for analysis of genetic diseases and traits (e.g. coat color) are now available for use by both breeders and veterinarians. With appropriate interpretation, these tests can be invaluable tools in a breeding program. For example, they can be used to produce animals free of specific diseases, to quickly eliminate a disease from an entire breed, or to select for specific traits in breeding stock. Selection strategies that do not take into account maintaining genetic diversity of the breed may be detrimental and reduce the potential for future improvement.

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1. Introduction

In the new era of genomics and molecular genetics, we need to be prepared to offer the latest genetic tools, in addition to the latest techniques in reproduction and clinical medicine. In the past, when a congenital or hereditary defect occurred in a litter, veterinary professionals usually advised breeders to avoid breeding of the affected offspring, their littermates, or their parents. Alternative recommendations were to avoid a repeat breeding of the parents of the affected animal, or to “outcross” them as much as possible at the next breeding attempt. When a problem appeared multiple times within a line, the best advice that a veterinarian could give was to discontinue breeding all dogs from that line. We have come a long way since those days and

can now give much more specific advice for an ever increasing number of genetic diseases.

Disease-specific testing and advice is possible when the DNA defect (mutation) underlying a particular disease has been characterized. The first canine genetic mutation was defined in a colony of dogs with Hemophilia B [1]. Since then, mutations responsible for more than 45 traits or diseases have been identified in the dog and for at least 13 in the cat [2,3]. Over 450 inherited diseases have been recognized in dogs and over 250 in cats. Because about 70% of dogs examined at veterinary practices are purebred dogs [4], the advice given by veterinarians to breeders will have a substantial impact on the future health of these animals.

2. Recognizing genetic disease

Before counseling breeders about genetic disease, it is helpful for them to understand that the disease encountered in their puppies or kittens may, in fact, be genetic. Several different types of evidence should accumulatively lead the veterinarian to consider that a

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particular disease is genetic in origin [5] as follows: (1) The disorder has a greater frequency within a group of related individuals (e.g. within a family group, strain, or breed) than in the general population. (2) The disease is seen more often in animals with a higher degree of inbreeding (with the caveat that a pedigree may need to go back many generations to reveal inbreeding). For example, there may be no common ancestors in a five-generation pedigree, but if examined to the seventh and eighth generations, one popular sire may appear many times, so the offspring produced from the breeding may actually have a fairly high inbreeding coefficient. (3) The disease has a characteristic age of onset and clinical course, especially in young animals. (4) The same syndrome is found in another species and is known to be genetic. (5) A specific phenotypic defect or syndrome is consistently associated with a specific chromosomal anomaly. (6) The disease process can be related to a molecular defect in a single polypeptide (e.g. enzyme, structural protein, or receptor).

If the particular disease syndrome is observed in a mixed-bred animal, this does not necessarily exclude the possibility that the disease is genetic. While autosomal recessive diseases are less common in mixed-breds than in purebreds, diseases with a dominant mode of inheritance are just as common in mixed-breds as in the purebred population. This, however, may not be true for cats, due to inbreeding in roaming cat populations.

3. Inheritance patterns

The most important aspect of genetic counseling is explaining to clients how a disease is passed from generation to generation. Without this knowledge, very little progress can be made in eliminating the disease from the population. The inheritance patterns encountered in veterinary medicine include autosomal recessive, autosomal dominant, X-linked recessive, X-linked dominant, and polygenic or complex inheritance. Recessive diseases account for about 70% of diseases with a known inheritance pattern [6], although, as we learn more about heritability, diseases of more complex heritability patterns may become more easily recognized. Inheritance patterns may be difficult to determine from the limited clinical information provided by few available family members. A few salient features of the various modes of inheritance are given below.

Autosomal recessive diseases are generally characterized by affected offspring, of either gender, being born to phenotypically normal, unaffected, parents,

with the sire and dam having an ancestor in common. Tracing the pedigree back more than the usual five generations may be necessary until the common ancestor is revealed. Animals that are phenotypically normal but are heterozygous (known to carry a disease-causing allele) are often referred to as carriers. Diseases with an autosomal recessive mode of inheritance are more commonly recognized in dogs than in humans, where there exists a higher proportion of dominant diseases. This may be a result of a higher degree of relatedness in domesticated species due to popular sire effects, selective inbreeding, and bottlenecks in their populations. For example, the current pure-bred dog population in The Netherlands over the last 30 y was derived from only 3 to 5% of registered dogs [7].

When the inheritance pattern is autosomal dominant, each affected animal has at least one affected parent. The exception, which is not uncommon, is the development of a new mutation in the oldest animal in the pedigree that is recognized as affected. Most of the diseases considered to be dominant would be more correctly termed incompletely dominant because, in their homozygous state, they are often lethal or more severe in expression. However, for the purpose of this paper, the terms dominant and incompletely dominant will be used interchangeably. A common dominant trait is the short tail of the Manx cat. This trait is lethal in utero when kittens have two copies of the mutant gene.

X-linked dominant diseases are rare; the only known example in small animal veterinary medicine is X-linked Alport syndrome in an isolated family of Samoyed dogs [8]. Pedigrees of X-linked recessive diseases are usually distinguished by only males being affected, although affected females may be encountered if affected males survive to reproductive age and are bred to carrier females. Affected males are often encountered in a pedigree within a few generations of a new mutation. The first canine mutation discovered was the X-linked recessive disease, Hemophilia B [1]. Another particularly common X-linked trait is orange color in domestic cats.

Common veterinary diseases are increasingly recognized as having a genetic component. Many of these diseases are influenced by a combination of factors, including environmental conditions, and involve at least two genes (and likely many genes). Hip dysplasia and neoplasia have long been recognized as diseases that have a genetic component, but do not follow simple inheritance patterns and thus are referred to as complex. As other veterinary diseases are better understood and characterized, complex genetic disease will become an even larger portion of hereditary diseases recognized in dogs and cats.

4. Types of genetic disease testing available

Genetic testing is possible using either phenotype-based or DNA-based genetic tests. DNA-based testing is available in two forms; linked marker testing and mutation-based testing. Each method has its place in the management of genetic disease in veterinary medicine.

4.1. Phenotype-based testing

Phenotypic testing is based on the ability to detect affected or carrier animals based on an expressed characteristic, such as enzyme activity, malformation detectable by radiographs or ultrasound, unusual metabolite concentration, or other trait detectable by clinical or laboratory diagnostics. When DNA-based tests are not available, these phenotypic tests are essential for controlling both polygenic and single gene disease. Examples include hip radiographs for the Orthopedic Foundation for Animals (OFA) or PennHip, eye examinations for the Canine Eye Registration Foundation (CERF), and factor concentrations for clotting deficiencies in some breeds.

4.2. Linked-marker testing

The first type of DNA-based test is the linked-marker test. Linked-marker tests look for microsatellite markers or single nucleotide polymorphisms (SNPs) that are alleles (areas of DNA that differ between animals at the same locus or genetic address but do not cause an observable disease or phenotype), and are present on the chromosome close to the gene that has a disease-causing mutation. These markers travel with the disease from parent to offspring, and are used as surrogate indicators of the unknown disease-causing gene. Results are often provided as a pattern such as A, B, or C and must be interpreted based on information provided by the testing company. Linked-marker tests are used when the responsible gene is unknown and will subsequently be phased out when the gene and its mutation are identified.

4.3. Mutation-based testing

The second type of DNA-based genetic test is the mutation-based test. Mutation-based tests are ideal for use in the management and elimination of genetic disease. They detect the exact DNA defect (mutation) in the gene that causes the disease. These tests are breed-specific, as each breed may have a different mutation that produces the same disease. Results are generally

given as Normal or Clear (homozygous normal), Carrier (heterozygous), or Affected (homozygous mutant).

5. Recommendations for breeders

Recommendations for breeders must be based on the goals of their breeding program, the overall health of the individual litters to be produced, and the health and diversity of the breed as a whole. These recommendations will vary based on diseases and traits important to the breed, the tests available, and the inheritance pattern of each trait or disease of interest. Thinking of all breeds of dogs as endangered species may provide a useful perspective. Most breed clubs do not allow new dogs into the registry. Therefore, the alleles found among reproducing dogs of a particular breed are all of the alleles that breed will ever have, unless rare new mutations occur. Mutations are rare; although they may produce desirable traits, it is more likely that they will either produce a disease, or have no effect at all on the breed.

5.1. Goals

One of the core beliefs of every good breeder is that they want to improve their breed. Therefore, they would like each generation to be healthy and better performing or better looking than the last. These two goals are the foundation upon which we should base our recommendations.

5.2. Testing

Genetic tests are no longer just about determining if an animal is a carrier for one disease or another. We can now use this type of testing to select animals that carry desired traits. An example is coat color pattern in cats. If a breeder wants to choose a male that would give the best chance of producing patterned and unpatterned kittens, they may want to select a breeding pair where both animals are heterozygous for the agouti gene. Previously, breeders would have had to select animals that had one parent phenotypically displaying the autosomal recessive trait for solid coat to be sure their breeding stock were obligate carriers. Genetic coat pattern testing in cats is now available and several additional coat color tests are in development at the University of California-Davis (Davis, CA, USA). Canine coat color testing is also available from multiple commercial laboratories. In the future, many more of these types of tests may become available. These may include identifying genes for long or short hair, wire

hair or smooth as well as profiles for more complex polygenic traits such as temperament, hip conformation, or head shape.

Veterinarians can help breeders, not only in collecting and shipping appropriate samples for genetic testing, but also by explaining to them the correct genetic tests that are available for their breed. In the USA, there are several laboratories which currently offer genetic testing for multiple diseases. In future, there may be so many available genetic tests that breeders will need assistance in choosing which tests are most valuable for their individual animals. Veterinarians should become aware of which laboratories offer tests and reference the available options for a particular breed (Table 1). For example, Labrador retrievers, currently the most common breed in the AKC, have six mutation-based tests available, as well as several phenotype-based tests. A search of the genetic testing companies reveals the following test available from each: OptiGen *prcd*-PRA and narcolepsy tests; HealthGene Corp. – Canine Hemophilia B, narcolepsy and two tests for coat color (black/chocolate and yellow); PennGen – Cystinuria; and VetGen two

types of coat color. Phenotypic tests for the Labrador include hip evaluation, elbow evaluation, and eye certification.

Services, such as the Canine Health Information Center (CHIC), an online database maintained by the American Kennel Club and the Orthopedic Foundation for Animals, that catalog the various test results for genetic disease done on each dog, are exceptionally valuable. They allow a breeder to determine what diseases are present or absent in a particular breeding line. For example, if the breeder of Labradors knows that the parents of her bitch were both homozygous normal for the *prcd*-PRA gene, then testing her for *prcd*-PRA before breeding her is unnecessary. In this case, a paternity test might be much more economical and valuable.

5.3. Interpretation

When the results of a test are available, there is clearly a need for veterinarians to evaluate and explain the results and their meaning to the client. Explanations are most complicated for phenotypic testing, because the values of carriers may overlap with the values for normal and affected animals or the results may need to be measured against the breed mean to select for improvement in a trait.

Linked marker tests have two potentials for errors. Firstly, recombination may occur between the marker and the disease-causing allele to which it is usually linked. This can result in false positive or false negative test results. Secondly, the marker allele may also be present in the population and not linked to the mutant gene, resulting in a false positive test. Therefore, the test results must be carefully interpreted based on the frequency of the marker allele in the population that is not linked to a mutant gene, as well as the percent recombination reported for that particular test.

Mutation-based tests are rarely inaccurate. If an affected animal appears to have been produced from a mating between a carrier and a clear parent, the most likely cause (other than labeling or handling errors) is incorrectly assigned parentage. When test results are not consistent with what is known about the disease and the parents, parentage testing should be performed. In addition, there may be more than one form of the disease in the breed. For example, according to information available on the OptiGen website (http://www.optigen.com/opt9_test_prachc.html), progressive retinal atrophy in Chinese Crested dogs may have more than one cause, making a single genetic test insufficient for managing the disease in that breed.

Table 1
Useful websites relating to canine and feline genetic diseases

Genetic disease testing centers
http://www.vet.upenn.edu/pennngen (University of Pennsylvania)
http://www.optigen.com/
http://www.vetgen.com/
http://www.vgl.ucdavis.edu/ (University of California-Davis)
http://www.vetdnacenter.com/
http://www.caninegeneticdiseases.net/CGD_main.htm (University of Missouri)
http://www.healthgene.com/vdl/vets/smanimals/test-gen.asp (Canada)
http://www.aht.org.uk/sci_diag_disc_genetics_dna.html (UK)
http://www.medigenomix.com/en/genetik1E.html (Germany)
http://www.andxinc.com/productsnew.html (feline sarcoma predisposition)
Selected parentage testing centers
http://www.vgl.ucdavis.edu/
http://www.vetdnacenter.com/
http://www.vita-tech.com/dna.cfm
http://www.metamorphixinc.com/products2.html
http://www.gribbles.com.au/dna_dogTest.htm
Informational websites on genetic diseases in dogs
http://omia.angis.org.au/
http://www.vetsci.usyd.edu.au/lida/index.php
http://www.vet.cam.ac.uk/idid/
http://www.upei.ca/~cidd/intro.htm
http://www.akcchf.org/research/genetic_tests.pdf
http://home.gci.net/~divs/disease/dbpgs/a.html
http://www.petdoc.ws/BreedPre.htm
http://www.caninehealthinfo.org/

5.4. Counseling

Genetic counseling should be used to help breeders continue their valuable breeding programs with their own stock, despite recognized genetic disease. Genetic counseling should not be undertaken with the assumption that breeders are at blame for causing the mutation and, hence for causing the disease in their dogs. Recommending that clients “just start over with someone else’s dogs” is ill-advised and can be harmful; it may cause loss of that client, breed diversity, or both. That individual breeders have dissimilar opinions on how their breed should appear is good for a breed as it encourages genetic diversity [9]. Genetic diversity within a breed is important because the function of each portion of DNA in the genome is not understood. When genetic diversity is decreased, a desirable allele may be lost or the frequency of an undesirable allele may be increased.

Breeders should be aware of the importance of both making improvements in their individual dogs as well as maintaining the health of the overall population. For instance, for an individual breeder concerned about producing an affected puppy or kitten, eliminating a mutant allele entirely may be easier than testing for carriers with every new generation. However for the good of the breed, if a test to detect a carrier animal is available, keeping the mutated allele in the population might be desirable. This can be accomplished while avoiding the production of diseased animals by breeding only to known homozygous normal animals and continuing to test all the intact offspring from these carrier dogs, allowing preservation of rare genetic material that might be present in only carrier dogs. An example is the Danish Bedlington terrier registry where mandatory testing for copper toxicosis is required before breeding. A recent study comparing the population before and after dogs that carried the mutated gene were eliminated from the breeding population, showed loss of genetic diversity in the Bedlington terrier breed [10]. To avoid this, it is important to focus efforts on preventing the production of individuals with genetic disease while maintaining genetic diversity within the breed [11]. This concept closely resembles an approach for endangered species conservation and is particularly appropriate in breeds where the breeding population is small. Maintaining genetic diversity requires explaining that carriers are not inherently bad. Having one mutated copy of a recessive gene does not make a dog inferior, especially when a test to detect a carrier animal is available.

6. Management of genetic diseases

6.1. Management of autosomal recessive disorders with available genetic tests

Carrier animals should only be bred to a known homozygous normal animal; this ensures that an affected animal will never be produced. The offspring must be either tested for their status or removed from the breeding pool. When an animal is produced that has a combination of traits that is superior to those of a parent, then that animal should replace the parent, even if carrying the mutant allele. The goals of the breeding program are to never produce an affected animal and to improve the overall quality of the breed. Hopefully, this approach will eventually produce an animal that is both excellent and free of the mutant allele. Because a genetic test is available, heterozygotes can be bred to homozygous normal animals indefinitely, because we can test the offspring and ensure that another affected animal is never produced. Thus, too much emphasis should not be put on the presence of one mutated copy of a gene, permitting concentration on producing animals that are the best examples of their breed. For example, selecting for Border collies homozygous normal for neuronal ceroid lipofuscinosis only could result in animals that had suboptimal herding ability or increased incidence of hip dysplasia. The real benefit of genetic testing is the ability to know the genotype of each breeding dog, so that the best decisions on possible mates can be made.

Affected animals should be bred only if they are exceptional examples of the breed in every other way, and then they should be bred only to homozygous normal animals. Of course, all of the offspring will be carriers. This strategy should be used only sparingly to conserve desired traits. Therefore, the affected animal should be replaced as soon as possible with a homozygous normal descendant that retains the important desirable characteristics.

6.2. Management of autosomal dominant diseases with available genetic tests

An affected animal or an animal whose DNA test shows that it will become affected should not be bred. Because the genetic basis of the disease is dominant, it is likely that only one parent of the affected animal is affected with the disease. Consequently, both parents should be tested and the affected animal removed from the breeding program. Currently, there are only two diseases for which there is a commercially available

genetic test in small animals showing dominant inheritance; they are polycystic kidney disease (PKD) in Persian type cats and dominant progressive retinal atrophy in Mastiffs and Bullmastiffs.

Breeds in which a large percentage of the population is affected by an autosomal dominant disease may not be able to stop breeding all affected animals. For example, 38% of Persian cats are affected with the autosomal dominant PKD1 mutation causing feline polycystic kidney disease [12]. Because affected animals constitute a huge portion of the overall population and the effect of removing these animals on the health and diversity of the breed is unknown, immediately removing all affected animals from the gene pool may be inadvisable because inbreeding could increase the potential to unmask new diseases, a loss of desirable traits could occur, or genetic diversity could decrease. Such effects are more likely to be damaging for a breed with a small population and may have less to no impact at all on a breed with a much larger population. Therefore, especially in breeds with small numbers of breeding animals and a large percentage of affected animals, heterozygous animals may need to be bred. However, a normal replacement animal should be chosen from the next generation that has all the desirable characteristics of the parent. This is a difficult strategy because it is very likely that diseased animals will be produced in that generation. An alternative solution for the health of the breed might be to remove all affected animals from the breeding population, then selectively outcross to other breeds if the genetic diversity of the breed suffers because of this approach. Unfortunately, this is also a highly controversial approach.

6.3. Management of X-linked recessive diseases with available genetic testing

Unaffected males cannot be carriers of these diseases; therefore they only need to be tested when the disease of interest is either of late onset or difficult to diagnose. Here, any male that has produced an affected female must be affected. Similar to autosomal recessive traits, females are obligate carriers if they have produced any affected offspring. The females in a suspected line should be tested if a genetic test is available. Known carrier females should not be bred, as half of their male offspring will be affected. Unless the disease has a very late onset, new X-linked recessive diseases are often identified within a few generations of the occurrence of the original mutation and can be eliminated before the disease-causing

allele has a chance to become widespread, as was done in Corgis with severe combined immunodeficiency [13].

6.4. Management of genetic disease when no test is available

Of the top 10 diseases of concern in dogs compiled by the AKC Canine Health Foundation [14] (neoplasia, epilepsy, hip dysplasia, thyroid disease, allergies, bloat, heart disease, autoimmune disease, progressive retinal atrophy, and cataracts), only one (i.e. progressive retinal atrophy), has mutation-based genetic tests available to help in eliminating the disease. However, it is important to note that a genetic test is not essential for a breed to make progress in reducing the incidence of disease. Progress has been made in decreasing the incidence of hip dysplasia, for which the underlying genetic basis is unknown, by breeding only dogs that fall below the breed average based on distraction index and subjective hip scoring [15]. This may be possible for many diseases in which the phenotypic defect is measured by a test with numeric results.

A Utrecht study evaluated the use of a new genetic counseling service for Boxer dog breeders. This service provided breeders with a report containing the odds that pups from several sire dam combinations would develop any of four different diseases [16]. The conclusion was that breeders tended to rank exterior phenotypic characteristics and characteristics of progeny first in their decisions on what matings to perform, and the report from the counseling service second. This may have been because breeders believed the whole dog was more important than diseases when making mating decisions. Alternatively, the study noted that the counseling report was not used in select cases because a few breeders did not think that one of the diseases was genetic. This study illustrates the need for better education regarding genetic disease as a whole in the process of counseling. The type of genetic disease risk profiling used in this study may become a more accepted tool for counselling breeders in relation to the more complex diseases, especially if it proves its usefulness.

Some breeds may not be able to eliminate disease without changing the breed standard. Certain diseases are linked to traits that have been selected for in the breed or are a direct result of the breed standard. For example, it may be impossible to correct all breathing disorders in English Bulldogs without changing their facial conformation. Also, deafness in Dalmatians may be associated with the spotting pattern [17].

Some mutant alleles may be desirable in a heterozygous state, such as folded ears in cats and merle coat color in dogs, and so may never be eliminated from the population. Future testing for these traits may only be valuable for detecting animals that are heterozygous but do not show the obvious phenotype, such as “cryptic” merles.

7. Future directions

Mutations responsible for canine and feline diseases are discovered every year; with the help of advanced techniques developed from various genome-sequencing projects, the basis for more complex diseases may soon be understood. Veterinary practitioners can assist this genetic research by recognizing and accurately diagnosing genetic disease, participating in research projects, and referring breeders with genetic disease problems to researchers.

Breed societies may begin to establish rules of breeding and registration based on their genetic test results. This has been done in several countries where breeding is tightly regulated by the government. Until then, veterinarians must do their best to educate breeders about the importance of testing for genetic disease; either via tools available today, or via tools yet to be developed. Dr. George Padgett summed up this point by saying “If we want to make any impact in controlling genetic disease in dogs, we must agree that an ethical approach is based on fairness, openness, and honesty. While traditions are important to us and should remain important, they should be changed if they conflict with the exercise of our ethics as dog breeders” [18].

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