UNDERSTANDING AND APPLYING THE GENETIC TEST FOR DEGENERATIVE MYELOPATHY

At the end of the synopsis of Dr. Long's presentation on Degenerative Myelopathy (DM), the Health Committee requested questions from the Chessie community to help in the understanding and applying of the new test for DM. We received quite a number of questions and would like to thank the individuals who took the time to respond. We then presented those questions to respected canine geneticist Dr. Jerold Bell for answers. Dr. Bell is a practicing veterinarian as well as Clinical Associate Professor of Genetics at Tufts Cummings School of Veterinary Medicine in MA. Dr. Bell received his BS from Michigan State and his DMV from Cornell University, NY State College of Veterinary Medicine in 1982. His areas of interest are Genetic Disorders, Epidemiology of Defective Genes in Purebred Populations, and Breed Improvement/Breeder Education. We hope these questions and answers will assist breeders and owners in the utilization of this new test.

Are all DNA tests the same?
No. There are several different types of genetic tests. To fully understand them, you must also understand that genetic tests exist for disorders with different modes of inheritance.

Tests of the Phenotype: Tests of "what you can see." This usually only test for affected status, and only after the onset of the disease process. They can be for polygenic/complexly inherited traits and diseases (i.e. hip and elbow radiographs, CERF eye exam, thyroid profile with auto antibodies), or for simple one-gene diseases where the mutation or defective gene has not been identified (i.e. von Wilibrand's disease blood factor in Chesapeake Bay Retrievers).

Tests of the Genotype: These are tests that are based on identifying segments of DNA.

Linked-marker based test: A type of genetic test that identifies a DNA sequence that is closely linked (lies close on the chromosome) to a defective gene, but not the actual defective gene (as usually it has not been identified yet). As the paired maternal and paternal chromosomes exchange material during insemination, a cross-over between a linked-marker and a defective gene can occur with these types of tests and can result in false negative and false positive results. If this occurs, all descendants of an individual with false results that inherit this portion of the chromosome will also have false results. Examples: the original linked-marker test for prcd-PRA, cerebellar ataxia in Italian Spinoni, Fanconi syndrome in Basenji.

Direct mutation-based test: This type of test identifies a specific mutation in the DNA of the defective gene. The majority of these tests identify simple Mendelian disease-causing (versus susceptibility) genes. There are no false results with this type of test (unless the proper dog is not sampled). Direct mutation-based genetic tests for simple (one-gene) genetic disorders are the easiest to understand. For recessive diseases, the results are normal (homozygous for the normal gene), carrier (heterozygous; one copy of the normal and defective gene), and affected (homozygous for the defective gene). Examples: prcd-PRA, vWD in some breeds, Mdr-1 (Drug and ivermectin sensitivity) in many breeds.

In order to have a complete discussion of the different genetic test, there needs to be some understanding about susceptibility genes. These are usually involved in polygenic/complexly inherited disorders. Some susceptibility genes are directly necessary for a disease process to occur. This is what occurs with the current **direct mutation-based test for DM**, the cataract test in Australian Shepherds, and the cord-1 PRA test in English Springer Spaniels.

There are susceptibility genes that can be directly tested for (direct mutation-based test), but they are NOT absolutely causative for a disease state. Many genes that are involved in immunity have been associated with specific diseases, however not all affected dogs have the specific

gene. Presence of the gene is considered a risk factor for the disease. Examples would be certain immune related genes linked to diabetes mellitus in many breeds, anal fistula/furunculosis in German Shepherd dogs, and the FLASH test for DM in German Shepherds from the University of FL. There are also linked-marker based susceptibility tests such as the RAPD test for DM from the University of FL. Again, for these susceptibility tests, they are not specific for the disease state (affected dogs can be free of the gene or linked marker).

Therefore, with any genetic test you must understand what is being tested for, how it relates to the disease, and how to properly use the results of that test.

How does the DM test compare to the PRA test we have for Chessies?
Both the prcd-PRA test and the DM test are direct mutation-based tests. The difference is that the prcd-PRA test is for a simple autosomal recessive disease-causing mutation. ALL Chesapeake Bay Retrievers that have two copies of the defective gene will develop this form of progressive retinal atrophy.

The DM test is a direct mutation-based test for a susceptibility gene. A genetic mutation has been found that MUST BE PRESENT in order to have the disease. All clinically confirmed dogs with DM (from all breeds, including cross-bred dogs) have two copies of the defective gene. However, other factors must also be present to cause a dog with two copies of the defective gene to be clinically affected with degenerative myelopathy

What is a susceptibility test and what is its use in a breeding program?
See above. Based on the test results posted and periodically updated on the OFA website, 390 Chesapeake Bay Retrievers have been tested for the susceptibility gene. Of those tested, 42% test clear (homozygous normal), 43% test carrier (heterozygous), and 15% test “At-risk” (homozygous for the susceptibility gene).

With 58% of the breed testing either “At-risk” or carrier, it would be devastating for breeders to eliminate this large a portion of the population’s gene pool and expect the breed to remain genetically healthy and robust. There are <u>two different categories of dogs</u> that will have different genetic counseling recommendations:

<u>For those dogs where it is known that a closely related dog was clinically affected with DM</u>: If the dog tests as a carrier or “at-risk”, it should only be bred to a dog that tests normal. Because these dogs had close relatives with the disease, they are at high risk of carrying the other gene or genes necessary to produce the disease. Based on this research, one expects that dogs homozygous for the susceptibility gene can become affected, so dogs from this category should only be bred to dogs testing normal for the susceptibility gene. They should not produce “at-risk” (homozygous) offspring when bred.

<u>For those dogs where closely related dogs with clinical DM have not been identified</u>: The susceptibility gene should be looked at as a fault, just as other behavioral, conformational, or performance faults. When deciding on the breeding quality and prospective mate of any dog, their positive traits and their faults need to be weighed and considered. Breeding toward DM normal dogs is a long-term goal; however quality dogs should not be discarded based on a single testable gene.

In order for the breed to move away from the disease, offspring testing normal for the DM susceptibility gene should be preferentially selected between comparable quality offspring to represent the next generation of breeding dogs. In this way, you lose the single susceptibility gene, while maintaining the quality genes and diversity of the breeding line.

Have not some susceptibility tests been pulled from use?? Didn't Rhodesian Ridgebacks have one test where this occurred?

Some linked-marker based tests have been retired when the actual mutation is identified and a direct mutation-based test has been developed. This occurred with prcd-PRA. To my knowledge, there are no direct gene tests that have been "pulled from use."

Is DM known to be simple recessive or is it polygenic? And if so please explain the difference.
DM has always been known to be polygenic, also known as a complexly-inherited disease. More than one gene pair controls its inheritance. No one believes that DM is a simple recessive disease. The DM test is for a recessive mutation that controls part of the inheritance of the disease.

Do we know that the gene currently tested for in DM is the right gene?
Yes, we know that this is a definitive susceptibility gene for DM. The gene where this mutation occurs is also the causative gene for amyotrophic lateral sclerosis (Lou Gehrig's disease – the human corollary to DM) in humans.

The specific genetic mutation in this gene occurred a very long time ago in an ancestral dog that lived long before the separation of breeds. This is why this specific mutation has been identified in over 23 divergent breeds, as well as mixed-breed dogs. As dog breeds developed, some lost this mutation, and others kept it, which allowed the expression of DM.

Is there the possibility of another form of DM? What are the chances of that occurring (even ALS in humans is defined as having an inherited form and a sporadic form)?
There is always a chance that other forms of canine DM exist. However, it appears that the disease in all breeds and mixed-breed dogs is associated with this ancient mutation. At this time, no dogs clinically confirmed with DM tested other than "at-risk" (homozygous) for the susceptibility gene.

What about the DM test from FL?
I do not have the background information concerning the location of the RAPD DM linked-marker, and whether it is located close to the DM susceptibility gene. If this is the case, then the DM test should replace the RAPD linked-marker test from FL. We do know that the susceptibility gene tested for with the current DM test is specific, and absolutely necessary to produce a DM affected dog.

Was a scientific peer review done? Where can one find the paper presented on this test with the research results and conclusions?
The peer-reviewed paper was published in the Proceedings of the National Academy of Sciences. It is an open access paper, and is available on-line. A link to the paper appears on the OFA website (Under OFA DNA Tests – DM).
http://www.pnas.org/content/early/2009/02/02/0812297106.full.pdf+html

For the DM test dogs coming back "At-Risk" is the probability that the dog will later develop the disease known? Are there any statistics on frequency or number seen of affected dogs in the general Chesapeake population?
As we do not know the other factors that cause the clinical onset of DM, we cannot predict what percentage of dogs testing "At-Risk" (homozygous for the susceptibility gene) will develop the disease. Dr. Coates' research of the Veterinary Medical Database (VMDB) showed that 13 of 1,567 (0.83%) Chesapeake Bay Retrievers presenting to veterinary teaching hospitals had clinical DM. Presently, 15% of Chesapeake Bay Retrievers test "At-Risk" for DM. It is obvious that the vast majority of "At-Risk" dogs will not develop DM.

Advice is conflicting about how to use the test results. If some A/A dogs never develop the disease, how can this be a definite test for DM? Is this result a false positive?

Dogs testing A/A ("At-Risk") have definitive positive results for the susceptibility gene. As this is a direct mutation-based test, there are no false positives. It is important to remember that this is not a definitive test for dogs that will develop clinical DM.

Since this test is for one gene involved in DM, can dogs testing with the A gene lack other genes that cause the disease and yet have other genes which might be important to maintain?
Yes.

Does a normal or carrier result mean that the dog will not contract DM?
Based on this research, yes, it would be highly unlikely. In this study, dogs confirmed with clinical DM have tested "at-risk" for the susceptibility gene

Should I ever breed my dog if it tests as a carrier or At-Risk?
If you considered that your dog is of quality to breed it prior to knowing the DM test result, then you must breed it regardless of the DM test result. The only change may be in who you choose to breed to.

Is the result of this test for one gene for DM enough to advise breeders to only breed only Carrier and At-Risk dogs to only normals?
No. While this recommendation guarantees that no DM affected dogs will be produced, it also requires that all matings be conducted with at least one member of a class of 42% of your gene pool. This significantly skews the gene pool in their direction, and reduces the influence of almost 60% of the breed's gene pool. For a disease that affects less than one in one-hundred Chesapeake Bay Retrievers, this severe a restriction on breeding will significantly limit the breed's genetic diversity.

Should carrier to carrier breedings be avoided even if there are no reports of DM having occurred in the pedigree?
Breeding two carriers of the DM susceptibility gene together (where no clinically affected close relatives are known) does produce a low risk for producing clinically affected offspring. This should be weighed as a fault that must be superseded by the positive traits of the two dogs being considered for breeding.

Genetic diversity seems to be very important in maintaining overall health in a breed. In a nutshell, what is genetic diversity? How can we increase it and still breed away from DM?
Genetic diversity is the total variation in types of genes between individuals in the gene pool. Breeds have closed stud books. Because of this, genes can be lost to the breed by eliminating segments of the gene pool, and there are no "new dogs" to infuse new genes. For most breeds, this does not cause a problem if the breed is relatively healthy with a large breeding population.

The greatest risk to genetic diversity is the "popular sire" syndrome. This is where a limited number of males are used for breeding, causing an inordinate amount of their genes to be spread in the gene pool. This causes other quality males to not be used, thus losing their influence and genes from the gene pool.

The next "popular sire(s)" might well be dogs testing normal for DM. What happens to a breed where the number of breeding individuals is relatively small like the Chesapeake and then we breed to a few males?
Any "popular sire" effect where an inordinate number of matings are being done to a limited number of males will significantly impact the genetic diversity of the breed, and possibly increase the frequency of other inherited diseases.

What are the potential consequences if only the limited amount of DM normal individuals is breed to and from?

Consequences include; a significant restriction of the genetic diversity of the breed, loss of other positive genes from the gene pool, and the increase of other yet undetermined disease-causing genes.

What is needed to refine the current test if necessary?
Only knowledge of what other genes are also necessary for the development of clinical DM.

The Health Committee sincerely thanks Dr. Bell for taking time to answer our questions. These answers will hopefully aid our Chessie community when making their breeding decisions.

The following is a glossary of some terms commonly used in the above article.

Chromosome - A chromosome is an organized structure of DNA and protein that is found in cells. Chromosomes are the vectors of heredity. There are two types of chromosomes: autosomes and sex chromosomes. Human cells have 22 different types of autosomes, each present as two copies, and two sex chromosomes. This gives 46 chromosomes in total. Dogs have a total of 78 chromosomes (2 sex chromosomes and 38 pairs of autosomes = 78)

Gene - A gene is the basic unit of heredity in a living organism. All living things depend on genes. Genes hold the information in DNA to build and maintain their cells and pass genetic traits to offspring. In general terms, a gene is a segment of nucleic acid (part of a chromosome) that, taken as a whole, specifies a trait. In cells, a gene is a portion of DNA that contains both "coding" sequences that determine what the gene does (see “genotype” below), and "non-coding" sequences that determine when the gene is active (expressed).

Mutation - In biology, mutations are changes to the nucleotide sequence of the genetic material of an organism. Mutations can be caused by copying errors in the genetic material during cell division, by exposure to ultraviolet or ionizing radiation, chemical mutagens, or viruses, or can be induced by the organism, itself, by cellular processes such as hypermutation. In multicellular organisms with dedicated reproductive cells, mutations can be subdivided into germ line mutations, which can be passed on to descendants through the reproductive cells, and somatic mutations, which involve cells outside the dedicated reproductive group and which are not usually transmitted to descendants. The sequence of a gene can be altered in a number of ways. Gene mutations have varying effects on health depending on where they occur and whether they alter the function of essential proteins. Mutations create variation within the gene pool.

Phenotype - A phenotype is any **observable characteristic** or trait of an organism: such as its morphology, development, biochemical or physiological properties, or behavior. Phenotypes result from the expression of an organism's genes as well as the influence of environmental factors and possible interactions between the two.

Genotype - The genotype of an organism is the inherited instructions it carries within its genetic code. Not all organisms with the same genotype look or act the same way, because appearance and behavior are modified by environmental and developmental conditions. Similarly, not all organisms that look alike necessarily have the same genotype. This genotype-phenotype distinction was proposed by Wilhelm Johannsen in 1911 to make clear the difference between an organism's heredity and what that heredity produces.

The interaction between genotype and phenotype has often been conceptualized by the following relationship: genotype + environment → phenotype. Genotypes often have much flexibility in the modification and expression of phenotypes; in many organisms these phenotypes are very different under varying environmental conditions.

Polygenic - Polygenic inheritance refers to the inheritance of a phenotypic characteristic that varies in degree and can be attributed to the interactions between two or more genes and their environment.

Marker - A genetic marker is used in molecular biology to determine if a piece of DNA has been successfully inserted into the host organism, or simply to identify (screen for) a piece of DNA. A marker for screening will make cells containing the gene look different. There are three types of screening commonly used: Green fluorescent protein, GUS Assay, and Blue/white screening

False Positive - The error of rejecting the null hypothesis given that it is actually true; e.g., A court finding a person guilty of a crime that they *did not* actually commit. (A dog showing a result from DNA testing that it *has* the gene identified to be related to DM when it really *does not*.)

False Negative - the error of failing to reject the null hypothesis given that the alternative hypothesis is actually true; e.g., A court finding a person not guilty of a crime that they *did* actually commit. (A dog showing a result from DNA testing that it *does not* have the gene identified to be related to DM when it really *does* have the gene.)