



Ten Year Anniversary Centronuclear Myopathy (CNM)

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Part II of Three Parts

PART I was in the May issue of Retriever News. It provided an overview of DNA and how DNA diseases might begin. Part II, in this issue, will cover topics such as certification levels for a DNA test, development of tests, testing laboratories and research in canine genetics and translation to human medicine. Part III in the July issue, will include topics such as issues relating to “clear by parentage” and views about breeding carriers or not breeding carriers.

The Different Certification Levels for the CNM DNA Test

Each testing laboratory develops its own website, forms, and procedure for the public to use. This contributes to some confusion since that caused each legitimate research laboratory’s forms looking slightly different from each other. Clients or the veterinarians for the clients have to carefully read directions to understand the differences in the submission processes between the major research sites. Once the directions are read, there is little problem.

The Alfort CNM Project Testing Laboratory recommends a panel of graduated authentication levels. Each sample is categorized under NOPI, PI or PIV. These identifiers are used for all CNM tests done. This level is included in the unique clearance number given to a clear dog. **Importantly, the scientific result of the CNM tests in all three categories is exactly the same; only the authentication level differs.**

Below are the three levels used to describe CNM categories:

NOPI (no permanent identification): This is the least used category. It means that there is no permanent identification such as a microchip in the dog. Lack of clear identification can cause problems both in being sure that the identity of the dog is confirmed as well returning a lost dog to their owner.

PI (Permanent Identification): This means that the owner of the male or female being sampled has confirmed there is permanent identification in the dog and it matches the number on the e-form. This level does not require being done by a veterinarian. It does provide identification of the dog if lost or if there is an argument in ownership. Many litters are done at the PI level.

PIV (Permanent Identification verified by a licensed veterinarian): This is recommended, but not required, for studs or females that are expected to be bred frequently. **The role of the veterinarian** is to confirm the micro-chip number in the dog as matching the number on the e-form as well as verifying that they have properly sealed and identified the swabs taken on each dog. This approach comes up most frequently when the mating is done at a distance by shipping semen. Distant

breeding is becoming more common as owners are becoming more cautious about flying their dogs across the USA. For males or females that are used only once in a while, PI is typically sufficient rather than PIV. It is the choice of the owners of the dogs.

Results: Clear results are published on the International Registry in www.labradorcnm.com

Because no cross-validation between distinct testing laboratories exists, only dogs tested by CNM at Alfort School of Veterinary Medicine are put on the list.

Dogs that are identified as carriers or affected are kept confidential to the owner. This confidentiality is a legal requirement for all carriers and affected canines.

Each clear canine is listed with these categories:

Registered Name	Call Name	Registration Number	Sex	Certification Level
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At the time of the publishing of this article, there have been about 35,000 hits on the International Registry. There are over 9,500 clear dogs listed that represent 23 countries. Searches are easily done to find specific clear Labradors that tested by the Alfort School of Veterinary Medicine. Inserting a call name or one word from a registered name will quickly bring up all dogs with those words in the list.

A Single Mutation for the Same Disease in a Given Breed Does Not Mean a Single Test

When medical geneticists working in the field of veterinary medicine identify a mutation that they think causes the disease they are working on, they send their results compiled in an article, submitted to an international scientific journal. The article is then peer-reviewed; very often, reviewers request additional experiments or rewording of the conclusions. The objective of this review is to make sure scientists do not over-interpret their dataset. It is very important that a scientific article provides the whole community with accurate, reliable and reproducible results. Because of this thorough examination by peers, it usually takes an average of six months to one year to have an article accepted by the Editor of the journal. In most cases, scientists who identified the mutation also write that they developed a DNA test, available for the community of breeders and owners of dogs from the breed(s) in which the mutation has been identified.

Once published, the article provides other scientists with all the information describing the mutation at the molecular level. Hence scientists from other locations have the opportunity to develop their own

test. If the mutation within the DNA molecule is the same in all dogs within a single breed (such as the mutation in the *PTPLA* gene causing CNM in Labrador retrievers), the techniques to identify this mutation and perform the DNA test may vary between testing laboratories, eventually leading to different tests with distinct sensitivity and specificity for a single mutation. The test used by the CNM staff at Alfort is in fact composed of three tests, each of them used as a control of the two others. That way, errors often due to the poor quality of some DNA molecules contaminated by food at the time of swabbing, can be more easily detected, increasing the overall reliability of the result.

Due to a USA Supreme Court decision in the last year, research units in various universities that had patents on what they had achieved found that their DNA patents no longer prevented some new groups from taking the results achieved by them, without paying royalties that prevented many of them from developing their own tests. Non-research locations quickly began to offer DNA analysis, often without knowing much about the disease or the difficulties one might encounter in maintaining reliability of results. This decision thus impacts the market of DNA testing, anyone who chooses to put up a website saying “DNA analysis” can do so more easily. In the short term, this may benefit clients as prices may fall, but behind prices, there are differences and it is the responsibility of each owner, breeder or veterinarian to make a relevant choice of where to send a sample to be tested. This is very similar to choosing the fruits you eat, or going to one choice of an MD rather than another when you face a disease.

Are All Testing Laboratories Equivalent?

For the reasons explained above, sending samples (usually cheek swabs) from a single dog to different testing laboratories may yield different results. During the last ten years, there have been several in-

stances where the CNM staff had to rescue locations where test results with errors were done by another laboratory. For example, in one of the 23 countries that CNM at Alfort serves, a popular stud was identified as clear for CNM by a unapproved “for profit” site. The stud was then bred extensively and affected CNM pups appeared in litters. Breeders contacted the laboratory at Alfort where they received help to recover from the situation. It appeared that the stud carried the mutation and had been initially mis-diagnosed as clear by the other (for profit) laboratory. Unfortunately individuals that see that CNM is offered as a test on a website often make the wrong assumption that offering being connected to our laboratory that originally identified the mutation.

Why Sending Swabs to the Research Laboratory that Initially Identified the Mutation May Yield a More Reliable Test Result

Research teams at universities and colleges are composed of tenure-track PhD permanent faculty along with graduate students working on their advanced degrees and a few assistants that have had extensive preparation. All university faculty staff have a variety of roles that contribute to follow-up of the diseased dogs. They provide in-depth information regarding every aspect of the disease in terms of epidemiology, disease history, pathological mechanisms, differential diagnosis or research through innovative treatments. Their daily job is to track every detail about the disease to try to explain every aspect of clinical signs observed by owners of dogs. **For a research laboratory, DNA samples from a dog is more than a dog: it is a patient that deserves a very specific attention.**

Because CNM is a research and diagnostic laboratory, they are more concerned about the quality of the results rather than the number of



A CNM affected dog displaying a prominent muscle hypotrophy leading to generalized weakness.
Photo by Prof. Stéphane Blot. Alfort School, EnvA, France.

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test samples done. As mentioned above, every time an individual submits a test, the identity of the dog is added to the overall data-base. Once the result is available and before it is sent back to the owner, it is triple-checked with the genetic information from the data-base.

Two more examples of responsible research centers are:

EIC (Exercise Induced Collapse) was featured on page 19 of the April issue of Retriever News. It demonstrates the continuing research expanding the knowledge of EIC by researchers at the University of Minnesota by faculty members Dr.'s Mickelson and Patterson and others. A description of EIC directly quoted from their website is: "Dogs affected with EIC develop muscle weakness, incoordination and life-threatening collapse after just five to fifteen minutes of field exercise and cannot participate in many types of strenuous activities. The condition also exists undetected in Labradors that are not routinely participating in such activity, and likely also exists to an as yet unknown extent in a number of other breeds." More on EIC can be found at <http://www.cvm.umn.edu/vbs/faculty/Mickelson/lab/EIC/retrievereic/home.html>

Optigen (www.optigen.com) at Cornell University has been well known for their excellence in researching eyes in canines. Quote directly taken from their website is: "Founding members have collaborated for decades on projects in the fields of veterinary ophthalmology, molecular diagnostics, and genetics. Veterinary ophthalmologists Gregory Acland and Gustavo Aguirre have gained international recognition for their award-winning work. They have a wide range of experience with the problems and challenges faced by dog owners and breeders."

There are many other legitimate research teams at other universities that are not included here due to space constraints. The above locations are examples of why it is always best to go with the researchers who identified the specific mutations and have the in-depth knowledge about the disease of concern. They are the ones with the strongest capabilities to expand knowledge bases due to their having extensive experience in research. They have the connections across groups of a wide cross-section of responsible researchers that will enable goals to be reached more quickly.

Besides Testing, DNA from Your Dog Sent to a Research Laboratory can have a Second Life: The Power of Participative Research!

It is a priority for research laboratories to perform as much additional research as they can. No money or personal profit is taken from testing by any of the CNM staff. One hundred percent of the benefits resulting from testing your dog goes directly into additional research. The field of DNA analysis is rapidly developing. Keeping everyone up on the best information available is critical to future research. The analysis of samples coming in from the general public not only benefits those specific canines sampled but also sometimes provides the discovery of some new disease or problem previously unknown. For example, every time you send samples to CNM at Alfort, you contribute to the future research information on canines.

Since the identification of the first mutation in the *PTPLA* gene, the laboratory has identified an additional seven new mutations in Labradors, other canine breeds or even other species while working with different teams of researchers both in their laboratory and in others. For example, thanks to their highly-informative data-base, they performed a genetic analysis allowing to track the origin of the CNM mutation (detailed information at www.ncbi.nlm.nih.gov/pmc/articles/PMC3465307); they also contributed to the identification of another mutation in the Labrador retriever breed, leading to a very similar dis-

ease named "Myotubular myopathy" (more info at www.ncbi.nlm.nih.gov/pmc/articles/PMC2930454)

For your continuing information, a web site that contains educational information as well as how to submit CNM samples for analysis is located at www.labradorcnm.com

Comparative Medicine in Animals and Humans: The One-Health Initiative

Because humans and dogs have shared a common history since the first ones domesticated the second ones nearly 30,000 years ago; we share most of our simple or complex diseases, such as myopathies or cancers (more info at www.ncbi.nlm.nih.gov/pmc/articles/PMC3508784).

Research laboratories working in the field of veterinary medical genetics are extremely important for the identification of new mutations in human diseases affecting patients worldwide. For example, thanks to the identification of the mutation in the *PTPLA* gene by the CNM team at Alfort, a group of human geneticists from Israel identified a mutation in the same gene, leading to a very similar disease in children and young adults (more info at www.ncbi.nlm.nih.gov/pmc/articles/PMC3842179). In that case, dogs clearly made a difference as they helped rush the identification of the disease-causing mutation, now used for the purpose of genetic tests in families affected by this handicapping disease.

Another example is the possibility to test new treatments in dogs affected by similar diseases. Evaluating in dogs the efficiency of new drugs, gene or cell therapies is a legal requirement before it can be safely used in patients. Thanks to the identification of the mutation in the *MTM1* gene by an international consortium including the CNM staff at Alfort, a highly-promising therapy has been tested in affected dogs which responded very positively (more info at www.ncbi.nlm.nih.gov/pmc/articles/PMC4105197). Instead of dying in the first year of their life because of a general weakness, treated dogs are living and running! Presently, human patients affected by the same disease are being recruited and a clinical trial will be set up in the very next years. **The dream of rescuing children affected by debilitating genetic disorders is not far from becoming a medical and social reality.**

More than ever before, dogs and humans are partners in life, and this now includes their close connection regarding medical issues. ■

Part III Content in the July Issue of Retriever News

In the July Retriever News topics will include: Reliability expectations for results; When can "Clear by Parentage" be believed; Planning ahead to do DNA testing; Why it is important to test before breeding; and the topic of breeding carriers of genetic diseases or never breeding carriers.

If there is a particular additional topic related to genetics and breeding you would like covered at some time; please send questions to cnminfo@centurytel.net

Footnote to article by Professor Emeritus Marilyn Fender PhD; University of Wisconsin in Oshkosh:

To my unexpected shock, in the mid-90's an affected CNM litter was born that included my 1996 National Field Champion carried CNM. Mine was one of a number of other famous Labradors assumed to be CNM carriers in the 1990's. There was no DNA test for CNM. I started searching universities for an answer. Ultimately in 2002, I found Dr. Laurent Tiret and his team who had identified the CNM mutation when no one else had been able to do so. My background skills and academic credentials as a tenured faculty Professor at University of Wisconsin at Oshkosh combined with competing in obedience, hunt tests, and field trials with Labradors allowed me to become the connection between the research done by the Alfort School of Veterinary Medicine and the needs of the Labrador gene pool.

Note: All content in this article has been reviewed and approved by Professor Laurent Tiret PhD DVM HDR, CNM Project Director and Team Leader CNM Research at the Alfort School of Veterinary Medicine.

This feature article of "Ten Year Anniversary ... Centronuclear Myopathy (CNM)" will be continued in the July issue.