Ten Year Anniversary Centronuclear Myopathy (CNM)

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Part III of Three Parts

THIS IS THE LAST of a three part Tenth Year Anniversary series on CNM and DNA. June 2015 marks ten years since the CNM mutation identification was achieved by the research team headed by Dr. Laurent Tiret. The confirmation of the identification was presented by Dr. Tiret at the June 2005 National Amateur Retriever Championship business meeting. Part III will include topics such as cautions in defining and using "clear by parentage" (CBP) and pros and cons of breeding carriers. Part I was in the May issue and Part II in the June issue of Retriever News.

Using DNA to Identify Diseases in Canines

The mutation for CNM had been elusive and searched for by many universities. In 2003 the Alfort School of Veterinary Medicine in France published their achievement of finding it in a professional article on CNM. The identification of the mutation causing CNM was then published in April 2005 by the Human Molecular Genetics Journal hmg.oxfordjournals.org/content/14/11/1417.full - *(Note: for easy access to all links in this article, go to the Retriever News online version of this article.)* At the 2005 NARC Stake in Minnesota, Dr. Laurent Tiret took the first DNA samples for testing CNM in the USA. Since then his research team has analyzed CNM in over 9,500 Labradors in 23 countries. Eighty-two percent of CNM testing was done in the USA and Canada. All clear results are published on the CNM International Registry at www.labradorcnm.com . From February 2013 to June 2015 there have been almost 35,000 visits to the CNM web site. Carriers and affected are kept confidential to the owner of the Labrador.

Before 2002, Optigen at Cornell was the only nationally visible research team with something to offer clients to deal with health issues of their canines. Much of what they did was focused on canine eye diseases. There was little discussion of DNA among retriever breeders before Optigen's contributions. Browse through their website at www.Optigen.com for more information.

In 2008, the University of Minnesota published the availability of a test for Exercise Induced Collapse.

See page 19 of the May issue of Retriever News where their current research is described as well as on their web site at www.cvm.umn.edu/vbs/faculty/Mickelson/lab/EIC/retrievereic

The majority of the diseases that the above three research teams at three different universities focused on were simple autosomal recessives. An example of these recessives is described in Figure 1 (Page 29) where a chart describes the various possibilities of results when a normal or a carrier or affected male is bred to a normal or carrier or affected female.

CLEAR applies to dogs with two wild type normal (+) copies of the PTPLA gene, each inherited from his/her parents. The two functional copies are abbreviated +/+.

CARRIER applies to dogs carrying a wild type copy and a mutated copy of the PTPLA gene. This combination is abbreviated +/cnm. Because it is a recessive disease, no symptoms are ever observable in carriers, named healthy carriers.

AFFECTED applies to dogs carrying two mutated copies of the PTPLA gene, each inherited from a carrier parent. This combination is abbreviated cnm/cnm and predisposes to developing CNM. Affected pups will never be able to live a normal life. Carriers bred together statistically produce 25% of affected pups when a large number of litters is compiled; however, within a single litter, percent of affected pups can greatly vary around 25%. The following link shows the struggles an affected pup goes through daily https://www.youtube.com/watch?v=RY1DWQqoac0 (Go to the on-line version of Retriever News to easily see the link content)

Photos of affected pups can be seen in Figure 2 (Page 30) The photos were provided by owners of CNM affected Labradors. Their names and comments about raising an affected pup are included in Figure 2.

Prior to the discovery of the location of various disease mutations on chromosomes, DNA tests were not available to identify clear, carrier, or affected canines for diseases such as CNM. Breeders and owners found their pedigrees carried mutations when they saw litters that had affected pups. In addition to those litters with visible affected pups, there were often matings between some relatives of those parents. These breedings involuntarily contributed to the dissemination of the mutated copy of the gene. Without a DNA-based test, the disease-causing copies of genes were disseminated to the next generations. Breeders hoped that the bad disease would not be in the other pedigrees … but in many cases it eventually appears one day. This still happens unexpectedly due to either lack of information about these diseases or because breeders hope that the disease might not really be possible in their dogs because of showing no symptoms for multiple generations. Appearing could occur as soon as one or two generations or it might be as many as 15 or even more generations of the carrier status silently being passed on.

When the mutation for CNM was identified by Dr. Tiret's team, it was then possible to test and confirm if a Labrador was affected; and more importantly to plan and anticipate if he/she was clear or a carrier. Affected were very visible without any testing. Determining whether a canine was clear or a carrier by testing was necessary before decisions on breeding could take place.

Breeding Carriers or Never Breeding Carriers

Determining if a canine is a carrier of a disease is necessary before getting to the question of whether to breed or not. There are only two reliable ways to determine if a Labrador is a carrier. The first is to have the CNM DNA test. The second way is that if the male or female has been the father or mother of an affected pup, there is no question that both parents are carriers.

Beginning in the 1970's, while not known to the owners, many talented Labradors were bred that were carriers of CNM. It was when the carriers started to cross with each other in breedings to produce affected pups that the visibility of the problem became apparent. By the 1990's many nationally recognized Labradors were starting to be identified as carriers due to having affected litters. There was no CNM test yet in the 90's but it was clear that something undesirable was in the gene pool. However, if those carriers, many that were famous and people wanted their names in their pedigrees, had never been bred; today's competitive field trial and hunting test Labradors would look much different.

We now have the ability to remove all of the carriers if we wished to do so. However, if that was done; then the characteristics of the competitive field Labradors would soon look considerably different.

While some people might think that is a good idea to remove all carriers; many others would object. There are several examples of various breeds in which abruptly removing carriers resulted in more genetic diseases by reducing the gene pool and hence allowing other recessive mutations; creating new and perhaps very scary characteristics, to start to meet each other.

The other problem that could occur would be that in removing all carriers from the gene pool, we might easily also mistakenly remove characteristics we highly value in field Labradors along with what we don't want to keep. Marking ability, the desire to retrieve, trainability, and many other natural characteristics could be diminished or even lost.

Another view in this breeding dilemma is the view of some that it is acceptable to breed carriers as convenient as long as the breeder makes sure only one of the two dogs mated is tested as clear. This can introduce other problems.

Consider that in each breeding of carrier to clear, statistically about 50% of the pups will be carriers and 50% clear. This means that one carrier bred to one clear will produce 4 carriers and 4 clear in a litter of 8, as an example. As you can see, if then each of the 4 carriers was bred to a clear in the next generation and there were litters of 8; then the next generation of those breedings would create 32 pups and statistically 16 carriers. As you can see, if carriers are bred too casually, the numbers of carriers could rapidly increase. Over a period of time, this could perhaps over-saturate the gene pool with carriers and start to limit breeding sometime in the future. If carriers were always bred, there is the possibility that in a decade or two, breeding options would be greatly reduced.

Therefore the challenge to breeders is to only breed carriers when the combination of the two Labradors bred will significantly contribute positively to the gene pool. Perhaps we have to get past the practices where carriers are bred just because it is possible to do so. Instead there needs to be concentration on the how the qualities of the two parents will combine to result in positive changes to the gene pool without losing the desired characteristics. Right now there are no specific criteria or rules on exactly how those decisions should be made. All we can suggest is that all breedings should be considered from multiple points of view before they happen. This not only applies to DNA diseases but to all the possible problems a canine can carry.

FIGURE 1 Breeding results of simple autosomal recessives in Labradors. The percent's are statistical averages that are true when results of multiple breedings are combined.

Expected results of breeding for CNM depending upon the genetic inferred or tested status of parents

SIRE GENOTYPE	DAM GENOTYPE		
	Clear (+/+)	Carrier (+/cnm)	Affected (cnm/cnm)
Clear (+/+)	All = Clear	1/2 = Clear 1/2 = Carriers	All Carriers
Carrier (+/cnm)	1/2 = Clear 1/2 = Carriers	1/4 = Clear 1/2 = Carriers 1/4 = Affected	1/2 = Carriers 1/2 = Affected
Affected (cnm/cnm)	All = Carriers	1/2 = Carriers 1/2 = Affected	All = Affected

Legend:

1 – Green Background = recommended mating
2 – Yellow Background = accepted mating if the carrier parent has an exceptional genetic value
3 – Red Background = mating to be avoided
4 – Grey Background = unprobable mating due to disabilities of affected dogs

NOTE: Litters from Yellow, Red and Grey matings should be systematically tested.

FIGURE 2

Lori Sprague, owner, said this about Bob Marley:

He's 10 yrs old now. We adopted him from the OC shelter at 10 mo old. He couldn't walk, too weak and was carried out of the shelter. He started out being just a foster dog but with all his health issues and the unknown, the rescue was going to put him down. We are so thankful for his diagnosis a year later. (thanks to Marilyn and Dr Laurent.) Bob Marley has been the sunshine in our lives. Big foster brother to hundreds of dogs, not always teaching them the best behavior.

Bobby has aged a lot this past year and unfortunately we take his health one day at a time now. His fragile digestive system due to mega esophagus has put a damper on things but thankfully to a good regime of meds and specialized diet, he bounces back with a fight every time.

Bob Marley has touched everyone he meets. His presence is infectious and will always make even the worst day a little better. I count my blessings every day we share with this amazing boy!

Anita and Fred Paoli, owners, said this about Della:

Della is now 7.5 yrs old. She is the best lab I have ever owned. She taught young Dakota, age 6, how to behave, retrieve and play gentle. She is now working on rudy who is 6 mo. We are upland bird hunters. Della comes on every hunt. I usually give her a short hunt at the end of the day. She is good for about 50 yards, more if its colder. She flushed a rooster this year. I shot it and she ran over to where it had fallen, but she didn't have the energy to bring it back, so she laid on it, turned to me and started barking for me to come get the prize. She usually waits in the truck, and when we return she makes us empty our vests for her. She gathers the birds and then they are her birds. She will not let the healthy dogs near them. She has helped me train 2 pups now. If a pup won't go in cover, she takes him there. If a pup won't return a bumper, she shows him how. She is a good friend.

Centronuclear Myopathy

Using Clear by Parentage Rather than Testing

The official term "clear by parentage" means that the breeder assumes that both parents are clear of the disease of concern by the following standards:

- **Both parents are tested and given a DNA Profile and a CNM Clearance.**
- **Each individual pup in the litter would be sampled and given a DNA Profile that is identified as compatible with that of the parents.**

These three documents from the parent and the pup(s) are necessary to provide the suffix of CBP to the pups' clearance. And, even if the above criteria are used, it is only considered useable for one generation.

As a general rule it is always best to re-test any dog that is being bred rather than to depend on CBP. The same would be true for any disease in any breed. While chances of errors are low in CBP, they are found sufficiently often that it makes it worth-while just test and not to take a gamble. Costs for CNM DNA tests are relatively low at $55 plus a few dollars of postage for the analysis. The test only has to be done one time in the life of each dog mated.

It should be noted that in the ten years that CNM at Alfort School of Veterinary Medicine has been doing canine DNA analysis, there have been zero Labradors submitted to the Alfort School of Veterinary Medicine for the purpose of having CBP initials listed officially as part of their clearance number.

As another example of CBP standards, OFA has a page in their website (offa.org) that has the statement that is found in Figure 3 (Page 31) as their criteria for Clear by Parentage. While OFA's definition is organized slightly differently than the one used by the CNM Project, the content is very similar in criteria in both the OFA criteria for CBP and the CNM Project CBP definition.

While it remains relatively rare that there is an error when both parents are thought to be clear, we are finding situations where there has been an error. The list of situations to consider can be found in the **Genetics** link in the left menu of www.labradorcnm.com . Go to the first page of the website and then to the tab on the top titled "Breeding Your Labrador." ■

Conclusion Ten Year Anniversary Three-Part Article

It has been an exciting and productive ten years of involvement with the CNM Project directed by Professor Laurent Tiret.

The research continues every week. Very recently, the research team headed by Prof. Tiret was granted by the French Association against Myopathies to continue their work. Indeed, thanks to their long-term work on CNM dogs and other animal models that they developed, including genetically-modified mice; a novel pathological mechanism involved in muscle hypotrophy and weakness has been identified. This important result will be published and available online later this summer. A direct consequence of this characterization is the opportunity to prevent this mechanism from happening by using candidate therapeutical drugs in humans and dogs. This hypothesis will be tested in the next years and if proved efficient, will represent innovative therapies to treat neuromuscular disorders, including CNM.

FIGURE 3 An example of responsible clear by parentage standards found on the OFA site June 2015 at http://www.offa.org/cbp.html

OFA Clear By Parentage Policy

As a greater number of DNA based disease tests become available, a policy regarding the clearing of offspring out of DNA tested parents has become necessary.

For direct mutant gene tests only, the OFA will issue clearances to untested offspring:

- **If the sire and dam have both been DNA tested "Clear,"**
- **If the sire and dam's DNA disease test results have been OFA registered, and**
- **If all three (sire/dam/offspring) have been DNA identity profiled and parentage verified.**

The DNA profile paperwork must be submitted along with a completed OFA DNA-based disease test application. The resulting OFA certification will have a suffix of "CBP" (clear by parentage), indicating that the dog itself was not tested and that the clearance was based on the sire and dam's test results, and known science at the time. Because of the possibility of new mutations or as of yet undiscovered gene mutations, only first generation offspring will be cleared.

For linkage or marker based tests where a margin of error including both false positives and negatives exists, the OFA will not issue any clearances to untested dogs.

DNA based disease screening is an evolving area. This policy is subject to change by action of the OFA Board of Directors as technology and science advance.

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

Retriever News magazine: The links in this article can be looked at by going to the online version of Retriever News. www.retrievere-news.com

Professor Laurent Tiret PhD DVM HDR, CNM Project Director and Team Leader in Comparative Medical Genetics; Alfort School of Veterinary Medicine has reviewed and approved of all written in Part III. Dr. Tiret graduated in Molecular and Cellular Developmental Biology from the University of Paris 6 and completed his PhD at the Institute Pasteur, Paris, France, working on segmental identity in mouse embryo. He was then employed in physiology and pharmacology at the Alfort School of Veterinary Medicine, Paris, France; where he developed genetic tools, pedigrees and collaborations for mapping projects in canine genetics. He has co-authored 30 publications in peer-reviewed scientific journals and became a full Professor in 2014.

Marilyn Fender PhD, Professor Emeritus University of Wisconsin at Oshkosh:

I have worked with the CNM Project researchers at Alfort since the mutation was identified ten years ago. My commitment was initiated when I found that my 1996 NFC FC AFC was a carrier of CNM after having numerous litters without any sign of a genetic problem. When an affected litter was born, I soon devoted extensive time to finding somewhere that was researching the problem. The only researchers found with any success were Prof. Laurent Tiret's team.