# Identification of a Muscular Myopathy Mutation in French Labradors

By Laurent Tiret[1] and Stéphane Blot[2]

[1]UMR 955 ENVA-INRA of Molecular and Cellular Genetics

[2]Laboratory of Neurology

Alfort School of Veterinary Medicine, France

**Introduction**

For about two centuries, the dog has been subjected to a sustained selection which resulted in a phenotypic variation unequalled in mammals. In non-transgenic animals, selection is possible when a phenotypic trait like a smaller size, blue eyes or retrieving ability is spontaneously observed. At the molecular level, variation in shape, size or behaviour results from a plasticity in the expression of selecting genes. A recent hypothesis emphasizes that certain small DNA sequences, called SINEs1, could play a key role in modulation of gene expression. The mechanism would be based upon random integration of these mobile sequences in regions that govern gene expression. SINEs are largely represented in the canine genome and there are evidences that their expansion is still an active process (Kirkness et al., 2003). Sometimes, unfortunately, these sequences have been inserted in important controlling regions and this integration results in gene's loss of function. They act as small grains of sand that prevent a clock to operate properly. This mechanism, associated with many other mutational events, help us to understand why so many disorders segregate in canine breeds. Emergence of the approximately 300 described hereditary diseases seems correlated with the tremendous phenotypic variation observed in this species. Most of the time, breeders successfully eradicate dominant disorders by excluding affected animals from reproduction. This is why, to date, 70% of disorders are transmitted as recessive traits2. This means that dogs carrying a disease allele are phenotypically considered as healthy dogs. In the laboratory mouse, the way geneticists confirm that an animal contains a morbid recessive allele is to use a progeny test which consists in breeding two carriers. Parents are carriers if affected neonates are observed in the progeny. In the dog, progeny tests are not decided but results of unintentional matings. With this exception, there is no possibility but an analysis of DNA to ascertain that a dog is a carrier.

**Myopathies in Labrador Retrievers**

At present, two inherited myopathies have been described in the Labrador Retriever breed. One is called "Dystrophinopathy" and the other "Muscular Myopathy". Both are congenital 3 but many aspects make these diseases distinct myopathies. First, dystrophinopathy affects only males as it is transmitted by the X chromosome (recessive X-linked disorder). Second, the lack of dystrophin, a protein involved in cellular membrane integrity, in males affected by dystrophinopathy induces necrosis of muscular cells and results in an abnormally elevated level in blood of an intracellular protein called creatine kinase (CK). Third, muscles of males suffering from dystrophinopathy are subjected to an important process of necrosis4. Hopefully, dystrophinopathy in Labrador is a very rare myopathy restricted to limited pedigrees. We won't go into more details with this disease and will therefore focus on muscular myopathy in the following sections.

---

1 SINE: Short INterspersed Element. These sequences derive from cellular RNA molecules that are re-integrated in the genome of cells following reverse transcription (retroposition).

2 Strictly speaking, the expression "recessive" is property of the phenotype (what animals look like). Modern technology has allowed to find that alternative versions (alleles) of a unique gene differ only at one or few nucleotides and that different actions of various alleles can explain the phenotypes. Therefore, "recessive trait" or "recessive allele" are legitimately used.

3 Congenital disorder: formely, pups born with symptoms. By extension, a disease with symptoms appearing in the very first months of postnatal life may be considered as congenital.

*4 Necrosis : pathological and definitive death of cells or tissues.*

Following clinical, histological and genetic criterion, the myopathy we identified in French Labradors closely resembles the "Muscular Myopathy" described in the US, Canada, Switzerland and UK also called in the scientific literature " Hereditary Myopathy of the Labrador Retriever, HMLR". Clinical signs are briefly reviewed here. At birth, affected puppies are indistinguishable from their control littermates but as from two weeks of age, a progressive significant weight loss is observed. At one month of age, the absence of tendon reflexes is noticed and used as an early and reliable diagnosis. The age of onset of the disabling phenotype varies between 2 to 5 months with an awkward gait and a decreased exercise tolerance, associated with a generalized muscle weakness worsened with cold. Clinical signs are progressively accentuated and generally stabilized at one year of age. In adults, the most striking macroscopic feature of the disease progression is the atrophy of temporal, cervical and leg muscles, leading to a ventroflexion of the neck, abnormal postures and movements. As of today, the oldest affected dog is 8.5 years old and no significant premature death in our colony could be observed. Nevertheless, dogs require medical care, essentially because they suffer from respiratory complications due to megaœsophagus. A hallmark of muscles from Labradors affected by muscular myopathy is a progressive centralization of nuclei in muscular cells. This can be observed on an histological section of a biopsied muscle. In a normal muscle, some rare nuclei can be observed at the center of cells (<1%). But most of nuclei are difficult to see because they are pushed away (under the membrane) by intracellular contractile proteins. In affected muscles, 10 to 70% of fibers have centralized nuclei. We thus called the muscular myopathy "centronuclear myopathy", abbreviated CNM (Blot et al., unpublished results and Tiret et al., 2003). The main reason for deciding to change the name is that this Labrador myopathy is the only known mammalian model for a similar human myopathy, called centronuclear myopathy of man. It is also much more precise than "muscular myopathy" that, strictly speaking, should involve several distinct myopathies.

**A genetic test identifies French Labradors affected by CNM**

On two independent occasions, 5-month-old yellow hair Labrador Retrievers, from distinct litters, were referred to the Alfort Veterinary School as they were weak, hypotonic and amyotrophic. A diagnosis of centronuclear myopathy was confirmed. The onset of clinical signs in these dogs was evaluated at 3 and 6 months of age respectively. To follow the progress of this muscular disorder from birth to adulthood, an experimental mating was programmed. We have now more than 50 dogs. Thanks to this pedigree, we could initiate a genetic analysis to search for a region of the genome associated with the disease (linkage analysis). From these studies, we concluded that the disease allele is located on the second pair of canine chromosomes *5*. We then jumped to genetic information available in man and found in the corresponding homologous region a gene called a "good candidate gene". That means this gene is expressed at the right place (muscles) and at the right time (embryonic and postnatal period). We finally detected a mutation in that gene, namely a SINE insertion (see Introduction and Footnote 1), which induces many defects in the expression of the gene identified. This mutation is strongly associated with the disease. Affected Labradors have two copies of the mutation and healthy carriers of the pedigree have one copy of the mutation (Pelé et al., 2005). These results are compatible with the recessive mode of inheritance of the disease. In addition, we could never detect the mutation in healthy unrelated Labradors, nor healthy dogs from other breeds. This is a strong argument in favor of this mutation being responsible for the disease. Finally, each time we detected the mutation in a health newborn Labrador Retriever, the dog developed a myopathy in the following weeks, emphasizing the predictive value of the test in our dogs.

---

*5 Dogs have 38 pairs of "autosomes" plus X and Y.*

**Can US and Canadian Labradors be rapidly tested?**

As previously mentioned, all clinical data support a close relationship between centronuclear myopathy described in France and muscular myopathy described in North American countries. We have therefore investigated whether the same gene, and hopefully the same mutation, is involved in the appearance of myopathy in your field Labrador Retrievers. The answer is yes. As a consequence, transposition of our test to your dogs is therefore feasible. The accompanying article by Marilyn Fender describes planned milestones for a Pilot CNM diagnosis of healthy carriers.

**Conclusion**

For the last 10 years, modern molecular tools applied to canine genetics has allowed to obtain unvaluable genetic resources (Breen et al., 2004; Kirkness et al., 2003). Thanks to this international effort, more and more disease traits are characterized at the molecular level, increasing the number of available genetic tests Sutter et al., 2004). We successfully identified a mutation in a muscular gene which certainly defines a recessive allele responsible for centronuclear myopathy in French, US and Canadian Labradors. A test is available to breeders and owners and results are being integrated to become a clearance database.

**Acknowledgments**

We thank Marilyn Fender for kind and helpful discussions and also for reading of the manuscript. We thank Mark Neff for having introduced us to Marilyn Fender and for having provided buccal swabs. We also want to thank Manuel Pelé, Jean-Louis Kessler and Jean-Jacques Panthier, respectively student, technician and head of the UMR 955, for their determining experimental and intellectual contribution to this work. We thank Drs Jean-Laurent Thibaud, Nicolas Granger and the staff from the UETM for taking care of animals. We thank Matthew Breen from NCSU who helped in chromosomal assignment. This study is supported by the"Association Française contre les Myopathies" [French Association against Myopathies]

## Bibliography

- Breen, M., Hitte, C., Lorentzen, T.D., Thomas, R., Cadieu, E., Sabacan, L., Scott, A., Evanno, G., Parker, H.G., Kirkness, E.F. *et al.* (2004) An integrated 4249 marker FISH/RH map of the canine genome. *BMC Genomics*,**5,** 65. Kirkness, E.F., Bafna, V., Halpern, A.L., Levy, S., Remington, K., Rusch, D.B., Delcher, A.L., Pop, M., Wang, W., Fraser, C.M. et al. (2003) The dog genome: survey sequencing and comparative analysis. Science, **301**, 1898-903.
- Pelé, M. Tiret, L. Kessler, J. L. Blot, S. Panthier, J. J. (2005) SINE exonic insertion in the PTPLA gene leads to multiple splicing defects and segregates with the autosomal recessive centronuclear myopathy in dogs. Hum Mol Genet, 14, 1417-27.
- Sutter, N.B. et Ostrander, E.A. (2004) Dog star rising: the canine genetic system. Nat Rev Genet, 5, 900-10.
- Tiret, L., Blot, S., Kessler, J.L., Gaillot, H., Breen, M. et Panthier, J.J. (2003) The cnm locus, a canine homologue of human autosomal forms of centronuclear myopathy, maps to chromosome 2. *Hum Genet,***113,** 297-306.