Genetic Testing for Inherited Polyneuropathies in Leonbergers 2.0

Many Leonbergers suffer from forms of neurological disease collectively termed polyneuropathies. Affected dogs typically develop slowly worsening exercise intolerance, gait abnormalities such as an exaggerated hitched step, especially in the hind limbs, and often, wasting of the hind limb muscles. Additionally, these dogs often have noisy breathing, a change in their bark, or even difficulty breathing due to involvement of the larynx and laryngeal folds in the throat. Eventually the disease may progress to the point where the dog can no longer support its own weight.

A hereditary form of polyneuropathy, termed Leonberger polyneuropathy type 1 (LPN1), was genetically characterized in 2010 by the Universities of Minnesota, Bern, and California-San Diego, and a genetic test developed. The LPN1 test identifies dogs that have a severe disease with an age of onset less than 4 yr of age (mean = 2 yr). The LPN1 mutation is most likely inherited in an autosomal recessive manner. In other words, a dog must have two copies of the LPN1 mutation (i.e., a LPN1-DD genotype) to develop this form of disease, which explains approximately 20% of all diagnosed cases of polyneuropathy in Leonbergers.

We are excited to announce that a new genetic analysis of Leonbergers with polyneuropathy not attributable to the LPN1 mutation has identified a new gene mutation, termed LPN2, that is also highly associated with, and likely causative of polyneuropathy. LPN2 appears to explain approximately 25% of all diagnosed polyneuropathy cases; so, along with LPN1 we now have genetic tests that account for almost 50% of all diagnosed cases of polyneuropathy in this breed.

Unlike LPN1, LPN2 is a dominant condition, so a dog need have only a single copy of the LPN2 mutation (i.e., a LPN2-DN or a LPN2-DD genotype) to develop polyneuropathy. This has serious implications for breeders, as half of all offspring from a LPN2-DN dog will inherit a mutant D allele, and thus be genetically-susceptible to LPN2.

Another complicating fact is that the age of onset of clinical signs in LPN2 affected dogs ranges from 1 - >10 years (mean = 6 yr), with ~ 80% of dogs with a LPN2 affected genotype showing clinical signs of polyneuropathy by 8 yrs of age. This also has serious implications for breeders, as genetically-susceptible dogs may not develop clinical disease until late in life, if at all.

The Universities of Minnesota and Bern will start offering genetic testing for the newly-identified LPN2 mutation, to accompany our LPN1 testing, starting July 15th, 2014 (for more details see below). At this time we recommend that all breeding dogs should be tested for both LPN1 and LPN2.

For LPN1 we continue to recommend avoiding matings that could produce LPN1-DD dogs, which at this time will most likely come by mating two LPN1-DN parents. Having only one LPN1-DN parent, with a LPN1-NN parent, will ensure that no LPN1-DD offspring will be born. In a global group of more than 4,000 Leonbergers which have been submitted to our laboratories since the discovery of the LPN1 mutation only about 1% were DD and 15% were DN, so breeding only LPN1-NN dogs should soon be possible without adversely affecting many desirable lines or the breed as a whole.

The situation with LPN2 is quite different, since on average 50% of the puppies from even a single LPN2-DN parent will be LPN2-DN, and 75% of the puppies from two LPN2-DN parents will be LPN2-DN or even LPN2-DD. Here we recommend a more aggressive approach by not selecting LPN2-DN dogs for breeding.

We can also state that relatively few dogs carry D alleles for both LPN1 and LPN2, as these two mutations are located on different chromosomes and are therefore transmitted independently, and that
neither mutation is sex-linked. Furthermore, only ~ 4 - 6% of all Leonbergers carry the LPN2 mutation, so the carrier frequency is about 3 to 4 times lower than that of the LPN1 mutation.

Lastly, please remember that these tests diagnose only two of the possible genetic risk factors for polyneuropathy, and that affected offspring with a different genetic form of polyneuropathy can result even from a mating of dogs that both have been tested free for these mutations.

<table>
<thead>
<tr>
<th>Mode of Inheritance</th>
<th>Susceptible Genotypes</th>
<th>Age of onset</th>
<th>Heritability Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPN1</td>
<td>Autosomal recessive</td>
<td>LPN1-DD</td>
<td>&lt; 4 yr</td>
</tr>
<tr>
<td>LPN2</td>
<td>Autosomal dominant</td>
<td>LPN2-DN; and LPN2-DD</td>
<td>1 – &gt;10 yr</td>
</tr>
</tbody>
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Instructions for ordering the LPN1 and LPN2 tests

North America. Genetic testing will be performed at the University of Minnesota Veterinary Diagnostic Laboratory. The preferred sample is 2 – 3 ml of fresh blood collected in EDTA tubes. Further information on sample submission, as well as the required submission forms is available at: http://www.cvm.umn.edu/vdl/ourservices/canineneuromuscular/home.html

The samples, packaged in a padded, leak-proof container, accompanied by a submission form for each dog, should be sent by regular mail, without cooling, to the Veterinary Diagnostic Laboratory:

Veterinary Diagnostic Laboratory
College of Veterinary Medicine
University of Minnesota
Gortner Avenue
St Paul, MN 55108-1098

Phone: (612) 625-8787 or (800) 605-8787
Website: www.vdl.umn.edu
LPN-specific questions email: lpninfo@umn.edu

The price per single test (either LPN1 or LPN2) on a single sample is $65; however, the price for both tests on a single sample requested at the same time is $100. The expected turnaround time is 3 – 4 weeks. If a sample has already been submitted for the LPN1 test (August 2014 and earlier), and the result has been reported, the LPN2 test can be performed on this same sample for $40 USD provided that an updated health profile is provided. For instructions on submitting a request for this reduced fee testing option, please contact lpninfo@umn.edu. Requests sent to the VDL will be charged at $65.

Europe. For genetic testing a 2-5 ml EDTA blood sample of the animal in question is required. The use of plastic tubes is recommended. Blood samples should be sent in a padded envelope by regular mail without cooling to our laboratory. They have to arrive within 3-4 days of sample taking. Order forms can be downloaded from: http://www.vetsuisse.unibe.ch/genetic/content/service/dog/index_eng.html

Please send the samples together with a signed order form to:

Institut für Genetik
Stichwort „Leonberger“
Bremgartenstrasse 109A
CH-3001 Bern
The price per single test (either LPN1 or LPN2) on a single sample is 110 CHF or 85 EUR; however, the price for both tests on a single sample is 150 CHF or 125 EUR. The expected turnaround time is 2 – 8 weeks.

If a sample has already been submitted for the LPN1 test, and the result has been reported, the LPN2 test can be performed on this same sample for 50 CHF or 40 EUR after pre-payment onto the account of the Institute of Genetics (University of Bern): IBAN: CH5309000000603151885, BIC: POFICHBE (Postfinance, CH-3030 Bern). Please mention the lab ID (LB no.), after the receipt of your payment we will submit the LPN2 results by E-Mail or regular mail.

**Instructions for submitting Medical Questionnaires and Blood Samples for other Leonberger Research**

We are continuing to search for other genetic risk factors for heritable disease in Leonbergers, and blood samples and medical questionnaires from dogs affected by apparent disease, as well as healthy controls, will greatly enhance this research. In North America please email us at lpninfo@umn.edu for more information on how you can participate.

Dog owners who submit a blood sample from a dog affected with neurological disease, together with neurological exam or biopsy results, may receive the LPN1 & LPN2 tests for free.

**Instructions for submitting Nerve Biopsies**

In North America, muscle and peripheral nerve biopsy specimens should be submitted to-

Comparative Neuromuscular Laboratory  Phone- (858) 534-1537
University of California, San Diego  Website http://vetneuromuscular.ucsd.edu
Basic Science Building Room 2095  Questions email musclelab@ucsd.edu
9500 Gilman Drive
La Jolla, CA 92037-0705

Phone- (858) 534-1537
Website http://vetneuromuscular.ucsd.edu
Questions email musclelab@ucsd.edu