LPN1 Genetic Test Result Interpretation
(Version June, 2017)

UNIVERSITÄT BERN

LPN1-N/N: A **clear** dog has no copies of the LPN1 gene mutation (this is also referred to as being homozygous normal). However, this result does not rule out the possibility that a dog could have, or be a carrier for, a different polyneuropathy mutation (including LPN2) that this test cannot detect. A LPN1 clear dog cannot produce a LPN1-D/D dog.

LPN1-D/N: A **carrier/at risk** dog has one copy of the LPN1 gene mutation (this is also referred to as being heterozygous). Having one copy of the mutated form of the LPN1 gene does not rule out the possibility that a dog may have a polyneuropathy caused by the LPN1 mutation or another mutation not detected by this test (including LPN2). LPN1 carriers will, on average, pass the LPN1 gene mutation on to half of their offspring.

LPN1-D/D: An **affected** dog has two copies of the LPN1 gene mutation (this is also referred to as being homozygous affected). Affected dogs typically develop neurological disease at or before 3 years of age (average 1.9 years of age), and clinical signs tend to be severe, often requiring surgical intervention of laryngeal paralysis. Affected dogs will pass one copy of this mutation on to all of their offspring.

Further Information

We are testing for a specific DNA segment deletion in the gene *ARHGEF10*; therefore this can be referred to as a gene mutation test. This situation is different from other types of genetic tests that describe only the identification of a DNA marker that could be very far away from the true disease gene, and not be as highly predictive as desired. **About 11% of all diagnosed cases of Leonberger polyneuropathy were LPN1-D/D.**

We have designated the letter D to indicate the mutant form of the LPN1 gene and N to indicate the normal form of the gene. A dog's particular combination of N or D forms of the gene is known as its genotype. The genotype of a clear dog is designated as N/N, they have no copies of the LPN1 gene mutation. N/N dogs do not have LPN1; however, some dogs may develop neuropathy with similar clinical and histopathological signs due to LPN2 or other as-yet-unidentified mutations. Most dogs with the D/D genotype identified to date are affected with LPN1 and have developed clinical signs of neurologic disease, typically by 3 years of age or younger. **At present ~24% of dogs in our research population with a phenotype consistent with or diagnosis of unexplained polyneuropathy have the LPN1-D/N genotype**. The frequency of the LPN1-D/N genotype in healthy control dogs is 12.5%. The average age that clinical signs are first noted in these LPN1-D/N dogs, if they develop at all, is 6 years.

Due to other causes of neuropathy in Leonbergers, **the exact mode of inheritance of the LPN1 form of neuropathy cannot yet be stated for certain**. While it is possible that LPN1 is dominantly inherited, with a dose dependent nature to the disease (more copies = worse disease), it could also be recessively inherited, meaning that LPN1-D/N and LPN1-N/N dogs with clinical signs have another form of neuropathy, such as LPN2. With either inheritance model, producing a puppy with severe, early-onset LPN caused by the mutant LPN1 gene would require that both parents be either carriers (D/N) or affected (D/D).

Below are the chances any given puppy in a litter from the indicated mating will have the genotype of clear, carrier or affected. **Matings that produce, or are comprised of an affected dog are not recommended and are shown in red.**

LPN1 genotypes of parents	Average probability LPN1-N/N puppies	Average probability LPN1-D/N puppies	Average probability LPN1-D/D puppies
N/N x N/N	100%	0%	0%
N/N x D/N	50%	50%	0%
N/N x D/D	0%	100%	0%
D/N x D/N	25%	50%	25%
D/N x D/D	0%	50%	50%
D/D x D/D	0%	0%	100%

Breeding Recommendations

Until other potential disease-causing mutations are discovered, it remains impossible for us to determine with certainty why some LPN1-D/N dogs develop disease. It could be due to the single copy of the LPN1 deletion, or it could be due to another form of disease (yet to be elucidated), or even a combination of the two.

As long as it is not absolutely clear that LPN1-D/N dogs will develop neurological disease, we do not recommend automatic exclusion of these dogs from the breeding population. We recommend testing breeding potential puppies in litters of LPN1-D/N x LPN1-N/N matings, and if all other considerations are equal, preferentially the N/N pups (50%) should be kept for future breeding. However, if the LPN1-D/N pup is preferred it is okay to keep them for future breeding. In a global group of more than 7,000 Leonbergers which have been submitted to our laboratories, ~ 12% were LPN1-D/N carrier dogs. Immediately eliminating all D/N dogs from breeding may have negative consequences for the genetic diversity of the breed. Important lines within the breed should be maintained; for example, if an important line is about to vanish, limited use of LPN1-D/N animals may be used to preserve them.

Within the Leonberger breed LPN1, LPN2, and LEMP genotypes must all be considered when selecting breeding pairs. LPN1 affected dogs (D/D) and LPN2 affected dogs (both D/N & D/D) are not recommended to be bred. Within each mating pair, at least one parent should be LPN1 and LEMP clear (N/N).

One final word of caution

It is important to remember that this LPN1 test is diagnostic for only one of possibly several genetic risk factors for polyneuropathy. Thus, it is still possible that affected offspring with a different genetic form of polyneuropathy could occur, even from a mating of two dogs that both have been tested N/N for the LPN1 mutation. To that end, we also recommend that both dogs in a breeding pair be LPN2 clear, and free of any signs of neurological disease, regardless of genotype, because this test can only detect one polyneuropathy mutation.

LPN2 Genetic Test Result Interpretation

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LPN2-N/N: A **clear** dog has no copies of the LPN2 gene mutation (this is also referred to as being homozygous normal). However, this result does not rule out the possibility that a dog could have, or be a carrier for, a different polyneuropathy mutation (including LPN1) that this test cannot detect.

LPN2-D/N: A heterozygous **affected/susceptible** dog has one copy of LPN2 gene mutation. The average age that owners first notice clinical signs of PN in heterozygous affected dogs, if they develop at all, is 6 years. On average, heterozygous affected dogs will pass along the LPN2 mutation to half of their offspring, this half will be LPN2 affected/susceptible.

LPN2-D/D: A homozygous **affected/susceptible** dog has two copies of the LPN2 gene mutation. In a limited number of homozygous affected dogs, the average age of onset is 4.5 years. Affected dogs will pass one copy of this mutation on to all of their offspring, and all will be LPN2 affected/susceptible.

Further Information

We are testing for a specific DNA segment deletion in the *GJA9* gene; therefore this can be referred to as a gene mutation test. This situation is different from other types of genetic tests that describe only the identification of a DNA marker that could be very far away from the true disease gene, and not be as highly predictive as desired. The LPN2 mutation is inherited in a **partially penetrant autosomal dominant** manner. Autosomal dominant means that only one copy of the mutation is required to show signs of disease; partially penetrant means that among genetically affected dogs (LPN2-D/N & LPN2-D/D) not all will show obvious clinical signs in their lifetime. This mutation explains ~20% of all diagnosed cases of Leonberger polyneuropathy.

We have designated the letter D to indicate the mutant (LPN2) form of the gene and N to indicate the normal form of the gene. A dog's particular combination of N or D forms of the gene is known as its genotype. The genotype of a clear dog is designated as N/N , they have no copies of the LPN2 mutation. LPN2-N/N dogs do not have LPN2. However, some dogs may develop neuropathy with similar clinical and histopathological signs due to LPN1 or other as-yet-unidentified mutations. **LPN2-D/N and LPN2-D/D dogs are both considered to be affected/susceptible to PN caused by the LPN2 mutation.** The age of onset in LPN2 affected dogs is quite broad, with dogs beginning to show signs from 1 year of age all the way through 10 years of age, or not at all in their lifetime.

In our research population of LPN2 affected/susceptible Leonbergers, ~60% of dogs showed signs of disease by 8 years of age. Within their lifetime, four out of five LPN2 affected/susceptible dogs showed signs of disease or biopsied as affected. Severity of disease in the parent may not be indicative of the severity observed in their offspring. This is particularly problematic for breeding dogs, which may have been bred many times before the onset of clinical disease.

Below are the chances any given puppy in a litter from the indicated mating will have the genotype of clear or affected (D/N or D/D). **Matings that produce, or are comprised of an affected dog are not recommended and are shown in red.**

LPN2 genotypes of parents	Average probability LPN2-N/N puppies	Average probability LPN2-D/N puppies	Average probability LPN2-D/D puppies
N/N x N/N	100%	0%	0%
N/N x D/N	50%	50%	0%
N/N x D/D	0%	100%	0%
D/N x D/N	25%	50%	25%
D/N x D/D	0%	50%	50%
D/D x D/D	0%	0%	100%

Breeding Recommendations

LPN2 is a dominantly inherited polyneuropathy, requiring only a single copy of the mutation to produce disease. Due to the dominant nature of the mutation, and its relatively low frequency (~6%) in the breed at present, we recommend **immediate removal of LPN2-D/N and LPN2-D/D dogs** from the breeding population to prevent the production of LPN2 affected/susceptible offspring.

Within the Leonberger breed LPN1, LPN2, and LEMP genotypes must all be considered when selecting breeding pairs. LPN1 affected dogs (D/D) and LPN2 affected dogs (both D/N & D/D) are not recommended to be bred. Within each mating pair, at least one parent should be LPN1 and LEMP clear (N/N).

One final word of caution

It is important to remember that this LPN2 test is diagnostic for only one of possibly several genetic risk factors for polyneuropathy. Thus, it is still possible that affected offspring with a different genetic form of polyneuropathy could occur, even from a mating of two dogs that both have been tested N/N for the LPN2 mutation. To that end, we also recommend that at least one dog in a breeding pair be LPN1 clear, and that both dogs in a breeding pair be free of any signs of neurological disease, regardless of genotype, because this test can only detect one polyneuropathy mutation.