LPN1 Genetic Test Result Interpretation
(October 2020)

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 three possible genotypes are listed below.

LPN1-N/N: A **clear** dog has no copies of the LPN1 gene mutation (this is also referred to as being homozygous normal). LPN1-N/N dogs do not have LPN1. 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 and LPPN3) that this test cannot detect. An LPN1-clear dog cannot produce LPN1-D/D offspring.

LPN1-D/N: An **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 and LPPN3). LPN1 at-risk dogs 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 the neurological disease at or before 3 years of age (average 2.2 years of age), and clinical signs tend to be severe, often requiring a surgical intervention of laryngeal paralysis. LPN1-affected dogs will pass one copy of this mutation on to all of their offspring.

Further Information on LPN1

The LPN1 gene test is a so-called direct genetic test. This means that the causal specific DNA segment change in the *ARHGEF10* gene is detected directly. This situation is different from other types of genetic tests that describe only the identification of a DNA marker that could be far away from the true disease gene, and not be as highly reliable as a direct genetic test.

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 or LPPN3. With either inheritance model, producing a puppy with severe, early-onset LPN caused by the mutant LPN1 gene would require that both parents are either LPN1-D/N carriers or LPN1-D/D affected. About 8% of all diagnosed cases of Leonberger polyneuropathy were LPN1-D/D.

Data from our research population indicates that the LPN1-D/D dogs have a life expectancy of at least 1.5 years less than the average Leonberger. At present, ~23% 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 ~11%. The average age that clinical signs are first noted in these LPN1-D/N dogs if they develop at all is 6 years.

LPN2 Genetic Test Result Interpretation

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We have designated the letter D to indicate the mutant form of the LPN2 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 three possible genotypes are listed below.

LPN2-N/N: A **clear** dog has no copies of the LPN2 gene mutation (this is also referred to as being homozygous normal). LPN2-N/N dogs do not have LPN2. 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 and LPPN3) that this test cannot detect. An LPN2-clear dog cannot produce LPN2-D/D offspring.

LPN2-D/N: A heterozygous **affected/susceptible** dog has one copy of LPN2 gene mutation. The heterozygous dogs begin showing polyneuropathy signs on average at the age of 6 years, if they develop at all. On average, LPN2-D/N heterozygous affected dogs will pass 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 available homozygous affected dogs, the average age of onset is 4.5 years. LPN2-D/D homozygous affected dogs will pass one copy of this mutation on to all of their offspring, and all will be LPN2-affected/susceptible.

Further Information on LPN2

The LPN2 gene test is a so-called direct genetic test. This means that the causal specific DNA segment change in the *GJA9* gene is detected directly. This situation is different from other types of genetic tests that describe only the identification of a DNA marker that could be far away from the true disease gene, and not be as highly reliable as a direct genetic test.

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. LPN2-D/N and LPN2-D/D dogs are both considered affected/susceptible to polyneuropathy 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. This mutation explains ~21% of all diagnosed cases of Leonberger polyneuropathy.

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. The severity of disease in the parent may not be indicative of the severity observed in their offspring. LPN2 is often a disease of old age, thus many of the dogs submitted for our study were of advanced age. It is likely that these dogs do not have an unusually long life expectancy, but rather that our cohort of dogs represent those that lived long enough to show signs of disease. This is particularly problematic for breeding dogs, which may be bred many times before the onset of clinical signs.

LPPN3 Genetic Test Result Interpretation

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We have designated the letter D to indicate the mutant form of the LPPN3 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 three possible genotypes are listed below.

LPPN3-N/N: A **clear** dog has no copies of the LPPN3 gene mutation (this is also referred to as being homozygous normal). LPPN3-N/N dogs do not have LPPN3. 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 and LPN2) that this test cannot detect. An LPPN3-clear dog cannot produce LPPN3-D/D offspring.

LPPN3-D/N: A **carrier** dog has one copy of the LPPN3 gene mutation (this is also referred to as being heterozygous). Having one copy of the mutated form of the LPPN3 gene does not rule out the possibility that a dog may have a polyneuropathy caused by another mutation not detected by this test (including LPN1 and LPN2). LPPN3 carriers will, on average, pass the LPPN3 gene mutation on to half of their offspring.

LPPN3-D/D: An **affected** dog has two copies of the LPPN3 gene mutation (this is also referred to as being homozygous affected). Affected dogs typically develop the neurological disease at or before 5 years of age (average 3.4 years of age), and clinical signs tend to be severe, often requiring a surgical intervention of laryngeal paralysis. LPPN3-affected dogs will pass one copy of this mutation on to all of their offspring.

Further Information on LPPN3

The LPPN3 gene test is a so-called direct genetic test. This means that the causal specific DNA segment change in the *CNTNAP1* gene is detected directly. This situation is different from other types of genetic tests that describe only the identification of a DNA marker that could be far away from the true disease gene, and not be as highly reliable as a direct genetic test.

The LPPN3 mutation is inherited in an autosomal recessive manner meaning that two copies of the mutation are required to show signs of disease. To produce a puppy with severe, young-onset polyneuropathy with laryngeal paralysis caused by the mutant LPPN3 gene, it would require that both parents are either LPPN3-D/N carriers or LPPN3-D/D affected. This mutation explains ~3% of all diagnosed cases of Leonberger polyneuropathy.

The LPPN3 type of polyneuropathy-affected dogs typically show breathing difficulties, often described as noisy or raspy breathing. Additional clinical signs, which were noted variably among the dogs, included difficulty swallowing, changes in barking frequency and quality, high-stepping and uncoordinated gait, stumbling and tripping, exercise intolerance, and muscle atrophy. Like many neurological diseases, there is no effective treatment for LPN. Since in most cases the dog is not in pain but is strongly restricted in its quality of life, especially due to the frequent loss of normal function of the larynx, owners are encouraged to ask a veterinarian for advice.

Breeding Recommendations

LPN1: Until other potential disease-causing mutations are discovered, it remains impossible to determine with certainty why some LPN1-D/N dogs develop the disease. It could be due to the single copy of the LPN1 mutation, or it could be due to another form of the 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 the neurological disease, we do not recommend an automatic exclusion of these dogs from the breeding population.** We recommend testing potential breeding puppies in litters of LPN1-D/N × LPN1-N/N matings, and if all other considerations are equal, preferentially the N/N pups should be kept for future breeding. However, if the LPN1-D/N pup is preferred it is okay to keep them for **limited use** in future breeding. In our global biobank of more than 9,000 Leonbergers, ~11% were LPN1-D/N carriers. Immediately eliminating all LPN1-D/N dogs from breeding may have negative consequences for the genetic diversity of the breed.

LPN2 is a dominantly inherited form of polyneuropathy, requiring only a single copy of the mutation to produce disease phenotype. 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.

LPPN3 is a recessively inherited form of polyneuropathy. In general, LPPN3-D/D dogs should not be used for breeding. We do recommend avoiding matings that have the potential to produce LPPN3-affected (D/D) offspring. Due to the low frequency of LPPN3-D/D dogs (<1%) in the breed at present, **we recommend immediate removal of LPPN3-D/D dogs from the breeding population and pairing the LPPN3-D/N dogs only with LPPN3-N/N dogs** to prevent the production of LPPN3-affected/susceptible offspring. **We do not recommend an automatic exclusion of LPPN3-D/N dogs from the breeding population.** Among our global biobank of Leonbergers tested to-date, ~11.5% were LPPN3-D/N carriers. Immediately eliminating all LPPN3-D/N dogs from breeding may have negative consequences for the genetic diversity of the breed.

Within the Leonberger breed LPN1, LPN2, LPPN3, and LEMP genotypes must all be considered when selecting breeding pairs. No affected dogs (LPN1-D/D, LPN2-D/N & LPN2-D/D, LPPN3-D/D, LEMP-D/D) should be bred. Within each mating pair, at least one parent should be LEMP-clear (N/N), LPN1-clear (N/N), LPPN3-clear (N/N), and both parents should be LPN2-clear (N/N).

One final word of caution

Since the introduction of the LPN genetic tests, polyneuropathy has not been completely eliminated from the Leonberger population. It is important to remember that these tests are diagnostic for only three (LPN1, LPN2, LPPN3) 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 LPN mutations. This may be a form of neuropathy with similar clinical and histopathological signs due to other as-yet-unidentified mutations. However, the three LPN gene tests can reliably detect three forms of severe LPN and significantly reduce the overall incidence of polyneuropathy in the Leonberger breed. **Finally, we strongly recommend both dogs in a breeding pair to be free of any signs of laryngeal paralysis or other neurological disease, regardless of genotype.**

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

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

*We recommend limited use of LPN1-D/N dogs.

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

LPPN3 genotypes of parents	Average probability **LPPN3-N/N** puppies	Average probability **LPPN3-D/N** puppies	Average probability **LPPN3-D/D** puppies
N/N × N/N	100%	0%	0%
N/N × D/N	50%	50%	0%
N/N × D/D	0%	100%	0%
D/N × D/N	25%	50%	25%
D/N × D/D	0%	50%	50%
D/D × D/D	0%	0%	100%