



STANDARD ARTICLE

Juvenile-onset polyneuropathy in American Staffordshire Terriers

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Background: The only hereditary neurologic disorder described so far in American Staffordshire Terriers is adult-onset cerebellar degeneration secondary to ceroid lipofuscinosis. We have seen several dogs with a newly recognized neurological disease characterized by locomotor weakness with or without respiratory signs and juvenile onset consistent with degenerative polyneuropathy of genetic origin.

Objectives: To characterize a novel polyneuropathy in juvenile American Staffordshire Terriers.

Animals: Fourteen American Staffordshire Terriers presented with clinical signs consistent with juvenile-onset polyneuropathy at 5 veterinary hospitals between May 2005 and July 2017.

Methods: Case series. Dogs were included retrospectively after a diagnosis of degenerative polyneuropathy had been confirmed by nerve biopsy. Clinical, pathological, electrophysiological, histological data, and outcome were reviewed and a pedigree analysis performed.

Results: All dogs displayed clinical signs of neuromuscular disease with generalized motor and sensory involvement, associated with focal signs of laryngeal paralysis (10/14 dogs) and megaeosophagus (1/14 dogs). Histopathological findings were consistent with degenerative polyneuropathy. Follow-up was available for 11 dogs, and 3 dogs were euthanized shortly after diagnosis. In these 11 dogs, the disease was slowly progressive and the animals maintained good quality of life with ability to walk. Pedigree analysis was mostly consistent with an autosomal recessive mode of inheritance.

Conclusions and Clinical Importance: Juvenile polyneuropathy, associated with laryngeal paralysis, is a newly described entity in American Staffordshire Terriers, and results from degenerative neuropathy. When surgery for laryngeal paralysis is performed, lifespan may be similar to that of normal dogs even though affected dogs have locomotor disturbance.

KEYWORDS

Charcot-Marie-Tooth, dog, electrodiagnostics, laryngeal paralysis, peripheral nervous system

Catherine Escriou and Marco Rosati contributed equally to this work.

Abbreviations: AR, autosomal recessive; AST(s), American Staffordshire Terrier(s); CMAP, compound muscle action potential; CMT, Charcot-Marie-Tooth; EMG, electromyography; LP, laryngeal paralysis; MNCV, motor nerve conduction velocity; MRI, magnetic resonance imaging; PNLP, polyneuropathy and laryngeal paralysis; PNOAV, polyneuropathy with ocular abnormalities and neuronal vacuolation; SNCV, sensory nerve conduction velocity

1 | INTRODUCTION

Degenerative polyneuropathies (PNs) have been described in more than 20 breeds of dogs, most of the disorders being hereditary, with a demonstrated familial link among cases. An autosomal recessive (AR) mode of transmission often is suspected.¹ Juvenile hereditary PNs associated with laryngeal paralysis (LP) have been identified in

the Dalmatian, Leonberger, Pyrenean Mountain Dog, Greyhound, Alaskan Malamute, Boxer, Black Russian Terrier, and Rottweiler, with variable ages of onset, clinical signs, and prognosis.^{2–10} So far, such a disease has not been described in American Staffordshire Terriers (ASTs).

Inherited PNs can either be syndromic, as part of a degenerative process involving the central and peripheral nervous system, or non-syndromic, with clinical signs only related to PN.¹ In the Black Russian Terrier, Rottweiler, and Boxer, the disease is syndromic with vacuolation in the neuronal cell bodies, axons and adrenal cells, and ocular abnormalities, similar to Warburg syndrome in humans.^{8,9,11} A causative mutation in the *RAB3GAP1* gene has been identified in the Black Russian Terrier and Rottweiler.^{9,11} In the other breeds, nonsyndromic forms lead to a disease similar to Charcot-Marie-Tooth (CMT) disease in humans, with axonal degeneration being a dominant finding.^{2–7,12,13}

Mutations have been found for the Alaskan Malamute and Greyhound (*NDRG1* gene), and for the Leonberger (*ARHGEF10* and *GJA9* genes), which allows for genetic screening.^{5,14,15}

In most breeds, disease prognosis is reported to be poor, with death or euthanasia occurring shortly after diagnosis.^{1,2,4,5,7} Some Alaskan Malamutes and Leonbergers have been reported to have progressive disease with survival ranging from several months to several years.^{7,12}

To date, the only inherited degenerative disorder described in the AST is ceroid lipofuscinosis. In this breed, an arylsulfatase G (*ARSG*) mutation leads to a sulfatase deficiency and therefore to neuronal ceroid lipofuscinosis, eventually resulting in neuronal apoptosis and adult-onset cerebellar ataxia.^{16,17} We have observed a progressive neurological disorder with juvenile-onset locomotor and respiratory signs and neurological examination findings consistent with PN in the AST. The aims of our study were to establish the phenotype of this newly described disorder, to determine its prognosis and to investigate the possibility of a genetic origin.

2 | MATERIALS AND METHODS

2.1 | Case selection

Fourteen ASTs that showed clinical signs consistent with a PN with or without respiratory signs including inspiratory stridor and dyspnea, consistent with LP, of juvenile onset were presented between May 2005 and July 2017, and retrospectively included in the study. All dogs were confirmed to have degenerative PN after histological evaluation of peripheral nerve biopsy samples.

2.2 | Clinical and pathological evaluation

Clinical records were analyzed and results of clinical and neurological evaluations were reviewed. Results of additional diagnostic evaluation, such as hematology, serum biochemistry profile, assessment of thyroid function, cerebrospinal fluid analysis, infectious disease testing and results of diagnostic imaging, including thoracic radiographs, magnetic resonance imaging (MRI) and laryngoscopy were reviewed when

available, as well as information regarding outcome after several months to years.

2.3 | Electrodiagnostic testing

The results of an electrophysiological examination, when performed, were reviewed. Any abnormal spontaneous activity, such as fibrillation potentials and positive sharp waves, was reported. Compound muscle action potentials (CMAP), motor nerve conduction velocities (MNCV), late latency action potentials (F-waves), sensory nerve conduction velocities (SNCVs), and results of repetitive nerve stimulation were analyzed. Electrophysiological data were interpreted in comparison with published values.¹⁸

2.4 | Histopathology

The results of the histological examination of muscle biopsy samples were reviewed. Biopsy samples were obtained by an open procedure, then immediately frozen in isopentane, cooled in liquid nitrogen (−130°C), and stored at −80°C until processed. Transverse cryosections (10 µm thick) were stained by standard protocols, including hematoxylin-eosin (H&E), modified Gomori trichrome (TG), ATPase pH 9.4, ATPase pH 4.65, ATPase pH 4.35, and 2,4-dinitrophenylhydrazine stains. Depending on the dog, at least 1 of the following muscles: tibialis cranialis (8 dogs), biceps femoris (6 dogs), triceps brachii (6 dogs), and extensor carpi radialis (5 dogs), was sampled. For each biopsy sample, the degree of myofiber atrophy was evaluated and graded as mild (+), moderate (++), or marked (+++), defined as follows: mild (rare angular myofibers); moderate (multifocal diffuse angular myofibers); marked (myofibers reduced to pyknotic nuclear clumps or fascicular atrophy). Fiber type grouping and intramuscular nerve branches also were evaluated.

Nerve biopsy samples were taken from all dogs. Peroneal nerve biopsies were available for all dogs. Further biopsy sites were the recurrent laryngeal nerve (dog 9) and the ulnar nerve (dogs 9 and 14). All nerve biopsy samples, except those from dog 2, were routinely fixed in 2.5% glutaraldehyde and contrasted with osmium tetroxide. One sample was embedded in epoxy resin whereas another underwent nerve fiber teasing, for longitudinal analysis.¹⁹ The embedded tissues were sectioned at 0.5 µm, stained with azure II methylene blue-safranin O and examined microscopically according to standard algorithms.¹⁹ For dog 2, the nerve was routinely stained with H&E. Based on the extent of nerve fiber lesions (including fiber loss, demyelination changes, node-paranode disruption and axonal degeneration) and regenerative changes (including remyelination, axonal sprouts, and regenerative clusters), the overall severity of nerve lesions was graded semiquantitatively as mild (+), moderate (++), or marked (+++).

One dog (dog 9) was euthanized because of respiratory distress, and the entire nervous system was obtained and analyzed post mortem.

2.5 | Pedigree analysis

All available pedigrees were compiled and the familial relationships among the dogs were investigated. Information about the disease status of the parents and littermates was reviewed when available.

3 | RESULTS

A summary of the data obtained from individual dogs is available in Supporting Information Table S1.

3.1 | Signalement, history, and clinical signs

Fourteen dogs were included in the study. Nine dogs were males and 5 were females. Median age at onset of clinical signs was 5 months (range, 1-6 months). Information regarding age at onset of clinical signs was not available for 2 dogs, which both were reported to have a chronic history of juvenile-onset locomotor signs. Median age at consultation was 15 months (range, 5-73 months).

All of the dogs had histories of chronic abnormal gait with or without inspiratory dyspnea. Ten dogs had both respiratory and locomotor signs. For 5 dogs, inspiratory dyspnea was the first clinical sign to be observed. These dogs subsequently developed locomotor signs, with a delay of 1-6 months (median, 3 months) between the respiratory and locomotor signs. One dog also had regurgitation. One dog (dog 1) was presented with a chronic history of locomotor signs and acute respiratory signs with inspiratory dyspnea 4 days before presentation. In the other 4 dogs, respiratory and locomotor signs appeared at the same time. Four dogs had only locomotor signs. Clinical signs were always slowly progressive.

Physical examination disclosed inspiratory dyspnea and stridor consistent with LP in the 10 dogs with respiratory signs. All of the dogs were presented with gait abnormalities, including ambulatory flaccid tetraparesis and 4-limb ataxia (12 dogs) with the pelvic limbs often more affected than the thoracic limbs (9/12 dogs), paraparesis (2 dogs), high-stepping pelvic limb gait, compensating for cranial tibial muscle atrophy by dropping of the hock, or pseudo-hypermetria of the hock (8 dogs), ankylosis of the knee and tarsus (3 dogs), and palmar-plantigrade (4 dogs) and plantigrade (3 dogs) stance. Atrophy of the distal musculature was reported in 8 dogs. Postural reactions were delayed in all dogs, the pelvic limbs being more affected than the thoracic limbs. Spinal reflexes were decreased or absent in all dogs. Withdrawal reflex in the pelvic limbs was the most consistently affected spinal reflex (all dogs). Withdrawal reflex in the thoracic limbs was decreased in 8 dogs. Nociception was decreased in 9 dogs when pressure of a tissue forceps was applied to a digit as a noxious stimulus. One dog (dog 2) had retinal dysplasia. See Supporting Information videos S1 and S2.

3.2 | Clinicopathological findings

Results of routine biochemical analyses and CBC, available for 10 and 6 dogs, respectively, were within reference ranges, as was the albumin to globulin ratio. Creatine kinase activities were mildly but increased in 3 dogs (median, 515 UI/L; range, 457-878 UI/L; reference range, 47-370 UI/L) and within reference ranges in 4 dogs. Serum cholesterol concentration (5 dogs), triglyceride concentrations (4 dogs), and serum thyroxine and thyroid-stimulating hormone concentrations (6 dogs) were within reference ranges. Results of acetylcholine receptor antibody titer serology were within reference range in 1 dog. Cerebrospinal fluid was obtained from 2 dogs by cisternal puncture, and

analysis was within reference ranges. Serological tests were performed in 2 dogs for neosporosis and 3 dogs for toxoplasmosis, and results were negative.

3.3 | Imaging

Magnetic resonance imaging of the brain and cervical spinal cord was performed in 2 dogs and results were normal in 1 dog. In the other dog, MRI disclosed a decreased cerebellar size associated with increased size of the cerebellar sulci. Thoracic radiographs, performed in 3 dogs, were normal in 2 dogs and showed megaesophagus in 1 dog (dog 9) with regurgitation. Laryngoscopy was performed in 7 dogs with respiratory signs and was consistent with bilateral LP. Endoscopy of the esophagus was performed in dog 9 and was consistent with megaesophagus, with decreased esophageal motility reported.

3.4 | Electrodiagnostic testing

Electrodiagnostic tests were performed in 13 dogs and results were consistent with generalized, predominantly axonal and demyelinating, motor and sensory PN. Electromyography (EMG) identified spontaneous activity with fibrillation potentials and positive sharp waves in most appendicular muscles in all dogs except 1 (dog 13). The intensity of spontaneous activity was greater in the distal muscles in 4 dogs, greater in the proximal muscles in 1 dog and equal in all muscles in the other dogs. Laryngeal muscles were tested in 7 dogs, including 6 with LP and 1 without LP, all of which showed abnormal spontaneous activity.

Motor nerve conduction velocities and CMAP amplitudes were recorded in at least 2 nerves in different limbs in the 13 dogs. The results are presented in Table 1 and compared with published data.¹⁷ A summary of the data for individual dogs is available in Supporting Information Table S2.

For some dogs, MNCV could not be determined because no CMAP could be obtained despite maximal nerve stimulation (2 of 10 dogs for the peroneal nerve, 7 of 12 dogs for the tibial nerve, 2 of 7 dogs for the radial nerve, and 7 of 13 dogs for the ulnar nerve; overall, 43% of the conduction studies performed). Motor nerve conduction velocities and CMAP amplitudes were consistently decreased, the CMAP amplitudes showing a greater decrease in proportion when compared to the reference values than MNCVs. Typical features are presented in Figure 1. F-wave latencies were obtained in 5 dogs and were increased in 2 of them. Sensory nerve action potentials could only be obtained for the radial nerves of 2 dogs (dogs 1 and 13), and the SNCVs (33 m/s and 37.1 m/s) were markedly decreased in comparison with published data. Repetitive nerve stimulation was performed in 4 dogs and gave normal results.

3.5 | Histopathology

The results of histopathological analysis of the muscle and nerve biopsy samples are presented in Table 2.

Twenty-six muscle biopsies were performed in 11 dogs. Histological analysis identified abnormalities consistent with denervation, including variability in myofiber size with atrophic and angular fibers of both fiber

TABLE 1 Motor nerve conduction velocities and compound muscle action potential amplitudes for proximal and distal stimulations when recorded for radial (n = 5), ulnar (n = 6), tibial (n = 5), and peroneal (n = 8) nerves, in comparison with published values¹⁷

			Range	Mean	Reference
MNCV (m/s)	Radial		16.9-37	24	72.1 ± 1.9
	Ulnar		17-37.5	25.1	58.9 ± 1
	Tibial		20.5-54.5	38	68.2 ± 1.4
	Peroneal		14-52.3	19.5	79.8 ± 1.8
Amp (mV)	Radial	Proximal	3-11.9	4.5	23.4 ± 1.5
		Distal	1.1-13.1	6	21.6 ± 1.6
	Ulnar	Proximal	0.4-13.3	2.1	22.9 ± 1.6
		Distal	0.9-16.1	1.7	25.8 ± 1.8
	Tibial	Proximal	0.1-21.8	14.1	20.1 ± 1.6
		Distal	0.2-16.9	8.3	23.3 ± 2.3
	Peroneal	Proximal	1-26.6	7.9	19.8 ± 1.4
		Distal	2-15.4	3.35	19.5 ± 1.5

Amp, amplitude; MNCV, motor nerve conduction velocity.

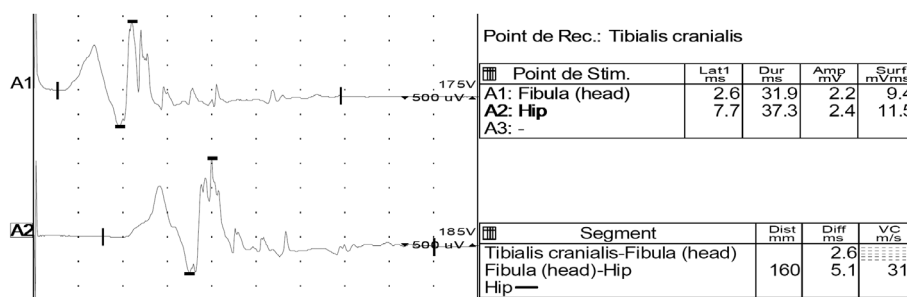


FIGURE 1 Recordings of compound muscle action potentials with proximal (A2: hip) and distal (A1: fibula) stimulations of the peroneal nerve of an affected dog (dog 7) with recording in the tibialis cranialis muscle. Note the marked decrease in compound muscle action potential (CMAP) amplitudes (reference range: proximal: 19.8 ± 1.8 mV, distal: 19.5 ± 1.5 mV), the polyphasic aspect of the CMAP and the mild decrease in motor nerve conduction velocity (reference range: 79.8 ± 1.8 m/s), demonstrating predominantly axonal but also demyelinating involvements

types, variable degrees of fibrosis, and irregular distribution of oxidative enzyme activities. Fiber type grouping occasionally was seen and type II fiber predominance was frequent. No inflammation was detected. Dorsal cricoarythenoideus muscle biopsy samples, obtained from 2 dogs with LP, displayed the same features. When several muscle biopsy samples were taken from the same limb (6 dogs), the distal muscles were more affected (3 dogs), or equally affected (2 dogs) as compared to the proximal muscles. Intramuscular nerve branches displayed decreased myelinated fiber density with features of axonal degeneration. Muscle histological features are presented in Figure 2.

Nerve histology allowed diagnosis of PN in all cases. Myelinated fiber loss was severe in 6/14, moderate in 6/14, and mild in 2/14. Residual nerve fibers showed mild (2/14), moderate (1/14), or severe changes (11/14). Thus, 2 cases were consistent with intermediate neuropathy (2/14) and 2 cases (2/14) were affected by a diffusely demyelinating nerve disease. In the other 9/14 dogs, a compound nodo-paranodal neuropathy predominated, with (4/9) or without (5/9) lymphohistiocytic infiltrates confined to the nodal-paranodal area. Other demyelinating features were identified. A mild demyelinating neuropathy was reported for dog 2. Primary axonal features resembled active Wallerian degeneration with some dystrophic axons. Rare fibers undergoing Wallerian degeneration stages III-IV as well as axonal atrophy in fibers, featuring extensive demyelinating changes, were considered to be secondary

axonal involvement. In 8/11 cases, myelin ballooning was identified and 4 of these dogs also had tomacula. A mild increase in endoneurial lymphocytes and histiocytes was seen in all dogs with nodal infiltrates and in 3 dogs without nodal infiltrates. Onion bulbs were evident in 3 dogs. Regenerative fiber clusters occurred in 1 dog only.

In 1 dog, complete neurodissection of the central nervous system was normal. Nerve histological features are presented in Figures 3 and 4.

3.6 | Follow-up

Three dogs were lost to follow-up. Follow-up (up to 6 years after diagnosis) was available for 11 dogs. Three dogs were euthanized shortly (1 day to 2 months) after diagnosis. Two of these dogs, including the 1 with megaesophagus, had locomotor signs and LP, and 1 dog (dog 1) without LP was euthanized 4 days after diagnosis. Among the remaining 8 dogs, 5 had LP. Three dogs with LP underwent surgery for arythenoid cartilage lateralization. One dog (dog 7) is still alive and ambulatory 2 years after surgery. One dog (dog 4) was euthanized 6 years after surgery for reasons unrelated to the disease. One dog (dog 14) died for an unrelated reason (rodenticide toxicosis) 5 months after diagnosis. Among the 2 remaining dogs that did not undergo surgery, 1 dog (dog 13) was reported to have mild improvement with decreased stridor after corticosteroid administration 4 months after

TABLE 2 Degree of muscle and nerve alteration on biopsies

Dog	Muscle biopsies	Nerve biopsies
1	BF: +++, ERC: +	Neuropathy type: NPN Severity: +++ Fiber loss: +++ Inflammation: –
2	TC: +	Severity: + Fiber loss: + Inflammation: –
3	TC: ++	Neuropathy type: NPN Severity: +++ Fiber loss: ++ Inflammation: +
4	BF: ++, TB: +, ERC: +	Neuropathy type: INP Severity: + Fiber loss: + Inflammation: –
5	BF: +, TC: +++, TB: ++, ERC: ++	Neuropathy type: NPN Severity: +++ Fiber loss: +++ Inflammation: +/-
6	BF: ++, TC: ++, TB: ++	Neuropathy type: NPN Severity: +++ Fiber loss: ++ Inflammation: +
7	BF: ++, TC: +++, TB: ++	Neuropathy type: INP Severity: ++ Fiber loss: ++ Inflammation: +
8	Not performed	Neuropathy type: NPN Severity: +++ Fiber loss: +++ Inflammation: -
9	Not performed	Neuropathy type: NPN Severity: Fibular: ++, recurrent laryngeal: +++, ulnar: +++ Fiber loss: ++ – +++ Inflammation: +
10	TC: +++, ERC: ++	Neuropathy type: NPN Severity: +++ Fiber loss: +++ Inflammation: ++
11	Not performed	Neuropathy type: NPN Severity: +++ Fiber loss: ++
12	TB: +++)	Neuropathy type: DNP Severity: +++ Fiber loss: ++ Inflammation: +
13	ERC: +, BF: +, TC: +	Neuropathy type: NPN Severity: +++ Fiber loss: ++ Inflammation: +
14	TB: +++, TC: +++)	Neuropathy type: NPN Severity: Fibular: +++, ulnar: +++ Fiber loss: +++ Inflammation: +

BF, biceps femoris; ERC, extensor carpi radialis; TB, triceps brachii; TC, tibialis cranialis; NPN, nodo-paranodopathy; INP, internodopathy; DNP, demyelinating neuropathy.

+, mild; ++, moderate; +++, severe.

diagnosis and the other dog (dog 11) is stable 1 year after the diagnosis, according to its owners. The 3 other dogs with only locomotor signs are still alive 6 months to 2 years after diagnosis. All of these dogs are still ambulatory. According to their owners, the disease is either stable or slowly progressive.

3.7 | Pedigree analysis

The 14 dogs came from 11 different litters and 3 dogs were siblings. Six pedigrees (for 8 dogs) were reviewed and a 5-generation pedigree analysis was performed. The family pedigree of the dogs is shown in Figure 5. Genetic analysis determined that 6 dogs had a common ancestor. The other 2 dogs were not related to this family. Many inbreeding loops were identified and 1 dog was a backcross. Both males and females were affected, and none of the parents was reported to have had clinical signs. Data concerning disease status could not be obtained for all the littermates. Nevertheless, the prevalence of the disease in 2 litters of the family pedigree with littermates was at least 25%.

4 | DISCUSSION

We describe a novel motor and sensory, distal and mainly axonal, degenerative nonsyndromic PN of juvenile onset in AST with fair prognosis in comparison with data reported for other breeds.

The disease is very likely to be nonsyndromic in AST, whereas in the Rottweiler, Black Russian Terrier, and Boxer, PN with LP is a syndromic disease. In these breeds, affected dogs also exhibit ophthalmologic abnormalities such as microphthalmia, cataracts, miotic pupils, and persistent pupillary membranes.^{8-11,20} None of the dogs included in our study exhibited microphthalmia, miotic pupils, or persistent pupillary membranes although full ophthalmological examination was available only for 3 dogs. Retinal dysplasia was identified in 1 dog. This finding has not been reported in PN with ocular abnormalities and neuronal vacuolation and is in itself thought to be an inherited ocular disorder in ASTs.²¹ Brain MRI examinations are not reported in syndromic polyneuropathy of Rottweiler, Black Russian Terrier, and Boxer, except for 1 case, a Rottweiler, in which brain MRI was normal.²² This examination, available for 3 dogs from our cohort, disclosed central nervous system involvement in only 1 case. In this dog, slight cerebellar atrophy was observed without clinical signs of cerebellar dysfunction. This anomaly is probably not associated with AST PN and could represent an incidental finding. This cerebellar atrophy remains of unknown origin because neither genetic testing for the canine arylsulfatase G mutation nor histopathology of the cerebellum was performed. The parents of this dog were not tested either. Regarding the age of the dog, it is unlikely the result of ceroid lipofuscinosis, and congenital anomaly should be considered. Another argument against syndromic PN is that complete histology of the entire nervous system in 1 affected dog failed to identify any lesions in 1 affected dog.

Nonsyndromic juvenile-onset hereditary motor and sensory polyneuropathies with LP have been reported in the Dalmatian, Leonberger, Pyrenean Mountain Dog, Alaskan Malamute, and Greyhound with various ages of onset and clinical signs (Table 3).¹⁻¹⁰ The dogs in our study shared many of the clinical features previously reported in these other breeds. Age at onset of clinical signs in our cohort varied from 1 to 6 months for 12/14 affected dogs, allowing classification of the disease as juvenile. A similar age was reported for Dalmatian, Pyrenean Mountain Dog, and Greyhound in which onset of clinical signs

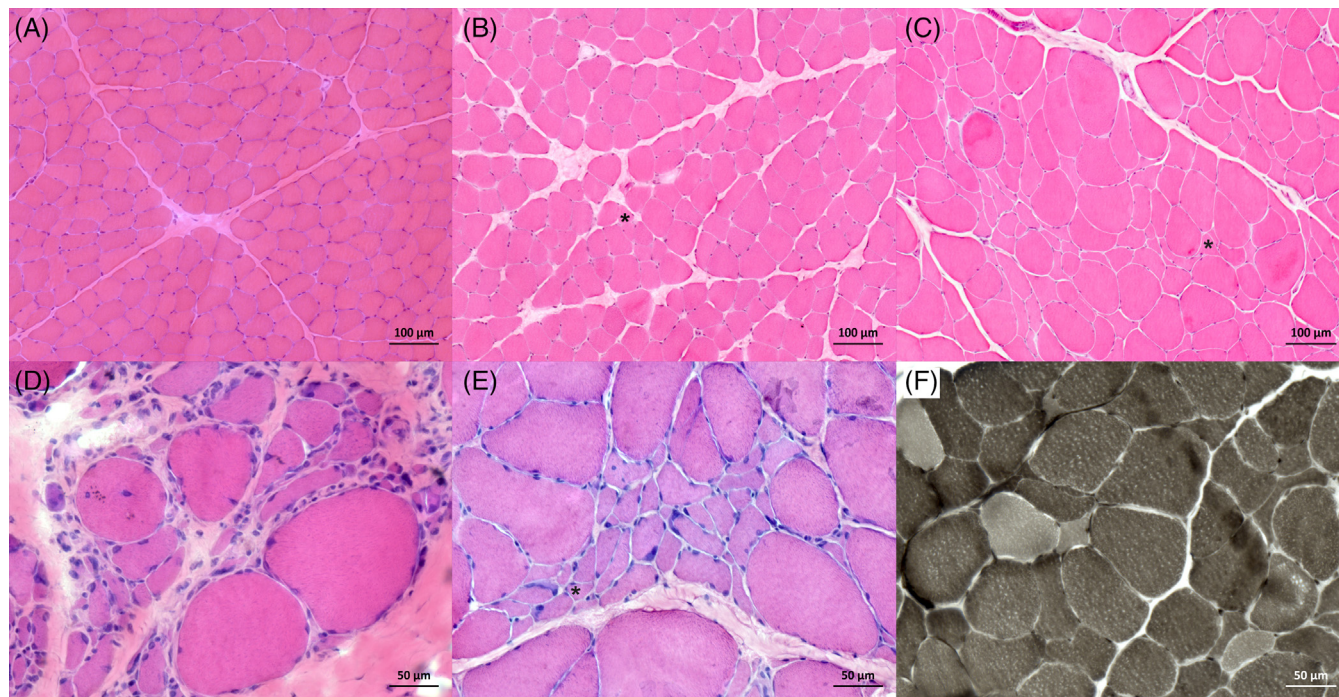


FIGURE 2 Light microscopies of sections from fresh frozen muscle biopsies. A, Normal muscle, H&E stain. B, Mild myofiber atrophy. Note the rare angular myofibers (asterisk). M. Extensor carpi radialis, dog 10, H&E stain. C, Moderate myofiber atrophy. Note the multifocal diffuse angular myofibers (asterisk). M. Biceps femoris, dog 6, H&E stain. D, Marked myofiber atrophy. Note the myofibers reduced to pyknotic nuclear clumps. M. Biceps femoris, dog 1, H&E stain. E, Marked myofiber atrophy. Note the fascicular atrophy (asterisk). M. *tibialis cranialis*, dog 7, H&E stain. F, Atrophy of type 1 (light) and type 2 (dark) myofibers. M. *tibialis cranialis*, dog 2, ATP 9.4 stain

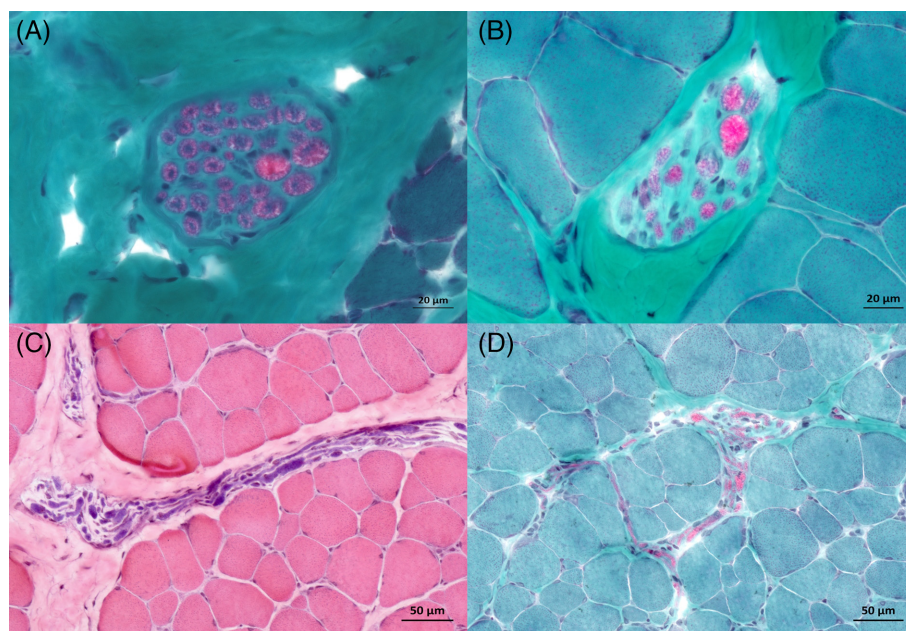


FIGURE 3 Light microscopy of a section from a fresh frozen muscle biopsy, nerve features. A, Transverse section of a normal intramuscular nerve branch. M. Biceps femoris, TG stain. B, Transverse section of an intramuscular nerve branch showing myelinated fiber loss. M. Biceps femoris, dog 6, TG stain. C, Transverse muscle section showing a longitudinal section of an abnormal intramuscular nerve branch with axonal degeneration. M. Biceps femoris, H&E stain. D, Transverse muscle section showing a longitudinal section of an intramuscular nerve branch showing axonal degeneration. M. *tibialis cranialis*, dog 10, TG stain

occurs during the first year of life.^{1,2,4,5} In the Alaskan Malamute, clinical signs occurred between 3 and 19 months of age.^{7,14} Finally, in the Leonberger, clinical signs occurred between 1 and 3 years of age in 70% of the animals examined.^{12,23} In 2 dogs (dogs 1 and 4) from our

study, presentation was at 60 and 73 months of age, respectively, and although no exact information was available about the age at onset of clinical signs, the chronicity of the signs favored classification as juvenile onset. Dogs were presented with various degrees of the classical

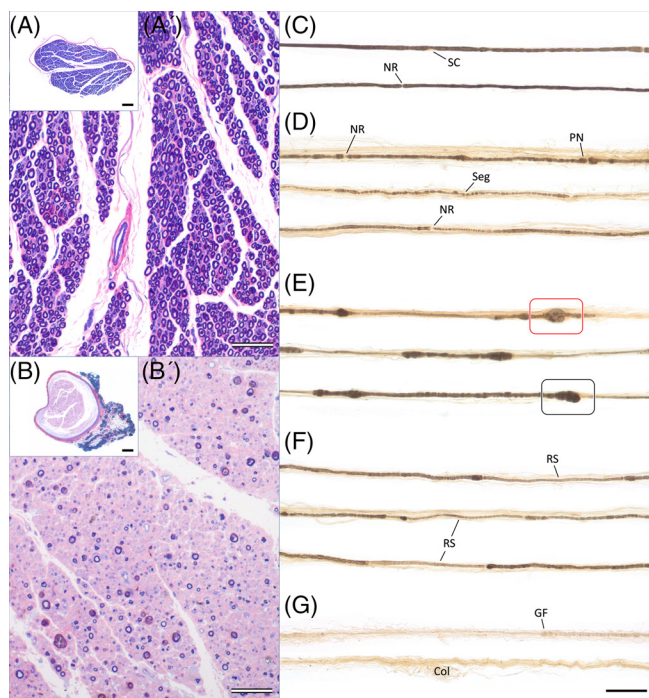


FIGURE 4 A-G, Peripheral nerve findings. Compared to advanced stages of myelinated fiber loss (B,B'), mildly affected nerves appear next to normal on histological slides (A,A') and teased fiber preparations (C), with physiological myelination and myelin compaction, conformation of the nodes of Ranvier (C: NR) and Schwann cells (C: SC). Moderately affected animals show fiber atrophy with myelin sheath segmentation (D: Seg) with ovoid formation, thickened paranodes (D: PN) and enlarged nodal gaps because of paranodal retraction and tipped paranodes. In advanced cases, myelin sheath shows ballooning (E: red frame) and tomaculous paranodal thickening (E: black frame) next to demyelinated segments. Remyelinated segments (F: RS) are occasionally seen whereas in other cases progression results in osmiophobic ghost fibers (G: GF) and fiber residues surrounded by collagen bundles (G: Col). Scale bars: A, B: 150 µm; A', B', C-G: 100 µm

motor and sensory signs of degenerative PN, reported in all affected breeds.^{1,24} Locomotor signs included flaccid paresis, which involved only the pelvic limbs or were more prominent in the pelvic limbs in most of the dogs. High stepping pelvic limb gait was reported in half of the dogs. The withdrawal reflex in the pelvic limbs always was decreased or absent at least at the level of the hock. This finding is consistent with distal PN and with an early clinical manifestation being involvement of branches of the sciatic nerve. In the case of tibialis cranialis muscle dysfunction (denervation), the hock joint cannot flex properly and a typical compensatory gait, characterized by

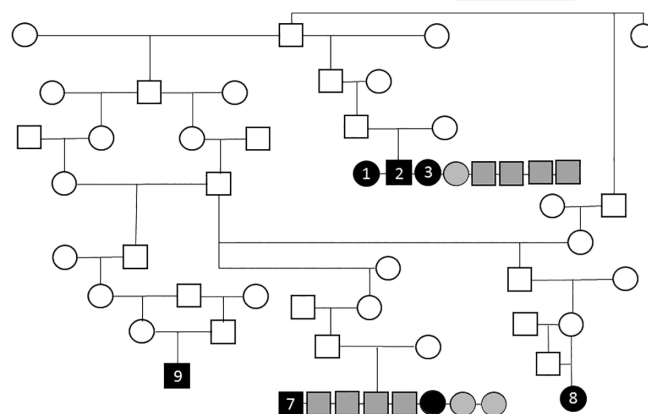


FIGURE 5 Family pedigrees of the dogs. Squares indicate males and circles indicate females. Filled symbols indicate affected dogs. Unfilled symbols indicate unaffected animals. Gray symbols indicate animals for which the clinical status is unknown

hyperflexion of the proximal part of the limb, becomes apparent.¹ Muscle atrophy mainly involved the distal appendicular muscles, as reported in all of the other breeds. Sensory signs such as ataxia, proprioceptive deficits and decreased nociception also were seen in all dogs.

Focal signs of LP with or without regurgitation have been reported in many PNs and were present in most of the dogs in our cohort. Respiratory distress also has been reported to occur before locomotor signs in the Dalmatian and Leonberger, as in numerous dogs with acquired LP.^{2,12,25–30} Laryngeal paralysis was diagnosed in most of the dogs and, in some, was the first clinical sign. Like the sciatic nerve, the recurrent laryngeal nerve contains very long axons and LP secondary to denervation can be the first clinical sign of more diffuse PN.¹ In the 5 dogs that initially were presented with locomotor signs, subsequent acute respiratory signs explained by LP were observed. Laryngoplasty led in many instances to clinical improvement. Megaesophagus was diagnosed by endoscopy in the sole dog that was presented with clinical signs of regurgitation. Two other dogs underwent thoracic radiography but no abnormality was identified. Clinical megaesophagus was a consistent finding in the Pyrenean Mountain Dog and Alaskan Malamute, and frequently has been reported in the Dalmatian. As in the Leonberger and Greyhound, clinical megaesophagus seems to be uncommon in affected ASTs. However, in a study that investigated esophageal function in dogs with idiopathic LP, the authors found that most of the dogs (25/32) had abnormal esophageal motility, but less than one-third of these dogs had clinical signs of dysphagia.²⁹ For these reasons,

TABLE 3 Characteristics of the reported juvenile-onset nonsyndromic polyneuropathy and laryngeal paralysis in dogs

Breed	Age of onset	Clinical signs	Prognosis	Gene
Dalmatian	2-12 months	L, LP, ME	Guarded to poor	Unknown
Leonberger	<1-11 years	L, LP	Guarded to fair	ARHGEF10 (20% of cases), GJA9 (adult onset)
Pyrenean Mountain	2-5 months	L, LP, ME	Guarded to poor	Unknown
Alaskan Malamute	3-19 months	L, LP	Fair	NDRG1
Greyhound	3-9 months	L, LP	Poor	NDRG1

L, locomotor; LP, laryngeal paralysis; ME, megaesophagus.

esophageal hypomotility or subclinical megaesophagus might have been underdiagnosed in our cohort of dogs. Postoperative aspiration pneumonia after surgery for LP is more likely in dogs with severe esophageal dysfunction diagnosed from an esophagogram.^{29,31} It may be advisable to obtain thoracic radiographs and, if surgery is planned and megaesophagus is not present radiographically, to obtain esophagograms in affected dogs. No complication was reported in the 3 dogs that underwent surgery, suggesting either no or mild esophageal hypomotility.

Electrophysiological examinations were consistent with motor and sensory, predominantly axonal, and demyelinating, distal PN. Abnormal spontaneous electrical activity more often was found in the distal muscles on EMG, which suggests distal PN. Similar findings have been reported in the Pyrenean Mountain Dog, Greyhound, Dalmatian, Alaskan Malamute, and Leonberger.^{2,4,5,7,12} Histological analyses of muscle biopsy samples were consistent with results obtained by EMG, neurogenic atrophy often being more pronounced in the distal muscles. Distal involvement of the nerve was confirmed by the small size of the intramuscular nerve branches. Variability of atrophy was not associated with chronicity of clinical signs. Type II fiber predominance often is associated with neuropathic disorders and could be explained by biopsies having been performed in areas of large type II fiber grouping.³² Electroneurography consistently identified a marked loss of CMAP amplitude and a mild to moderate decrease in MNCV in all dogs. In contrast to humans, no MNCV cut-off value can be used to separate demyelinating from axonal PNs in dogs, but these findings suggest a predominantly axonal PN. In many dogs, the amplitude of the motor response was too decreased to obtain latency measurements.¹ This finding also was reported in the Leonberger.¹² Mixed electrodiagnostic features with more pronounced distal findings, as observed in most of the dogs, are compatible with both intermediate neuropathy and nodal-paranodal neuropathy as observed on nerve histopathology.

This disorder differs from human axonal CMT types where degenerating axons intermingle with regenerative sprouts and clusters. Likewise, the observation of sparse onion bulbs centered on fibers lacking full myelination is distinct from congenital dysmyelinating neuropathy in people. The occurrence of tomacula in some cases raises the question of myelin sheath instability phenotype hereditary neuropathy with liability to pressure palsies. However, a minority of dogs showed a clinically relevant amount of tomacula, and hereditary neuropathy with predilection for pressure palsies typically is not accompanied by myelin sheath ballooning and features of nodal-paranodal neuropathy. On the contrary, the combination of myelin sheath changes in older dogs might be seen in metabolic disorders such as Cushing's disease and insulinoma. Moreover, an association with risk of immune-mediated neuropathy requires consideration.

In the other breeds, PN and LP have been reported to be associated with poor prognosis resulting in death or euthanasia a few months after diagnosis, often as a consequence of aspiration pneumonia, despite palliative treatment.^{2,4,5,7} Nine of the 11 Alaskan Malamutes included in a previous study were euthanized soon after diagnosis, because of their poor condition, but 2 dogs were still alive 36 and 42 months, respectively, after diagnosis, and thus the disease may be slowly progressive.⁷ In the Leonberger, prognosis is reported

to be fair, with gradual clinical worsening within months to a few years. In another study, 9 of the 21 dogs received surgical correction for LP, resulting in clinical improvement.¹² In 2 dogs, locomotor weakness progressed to quadriplegia. At the end of the study, 8 dogs were alive and still ambulatory, and the other dogs were euthanized, died of aspiration pneumonia or were lost to follow-up. Duration of follow-up was not specified. The prognosis was reported to be worse for dogs in which the onset of clinical signs occurred earlier and the course of the disease was more severe and progressed more rapidly.¹² The disease prognosis in AST seems to be fair in comparison to other breeds, and many dogs survive for a longer time that has been reported in other breeds. The severity of the clinical signs in our dogs was variable. Two dogs with both locomotor and respiratory signs were euthanized soon after diagnosis because of their poor medical condition. One of these dogs had megaesophagus and regurgitation. In the dogs with respiratory and locomotor signs, for which follow-up was available, LP was responsible for acute worsening of clinical signs, but both dogs improved after surgery. Regarding those dogs with locomotor signs only, 1 was euthanized at the owner's request but was still ambulatory before euthanasia, and 3 dogs were still alive, with a slowly progressive disorder, 6 months to 2 years after diagnosis. For dogs presented with locomotor signs only, the prognosis seems to be good and it seems to be fair for dogs presented with LP, once surgery has been performed. The rare occurrence of clinical megaesophagus, associated with a higher risk for secondary aspiration pneumonia, might explain the better prognosis in comparison to the Dalmatian and Pyrenean Mountain Dog.

To some extent, the progression of PNLP in AST seems comparable to the progression of CMT disease in humans, which typically occurs during the first or second decade of life and is slowly progressive with no alteration of life expectancy in most cases.^{33,34} The natural history of axonal degenerative PNs, or type 2 CMT, was studied prospectively over 5 years.³ Very few patients were nonambulatory at the beginning of the study. The disease typically was slowly progressive, but none of the initially ambulatory patients lost their ability to walk during the follow-up period.³⁵ Some patients with severe disease are unable to ambulate in infancy or early childhood.³⁴ Vocal cord dysfunction caused by vagus and laryngeal neuropathies has been reported in association with several CMT types, and this dysfunction is not related to the severity of neuromuscular weakness.³⁴ Both laryngeal and pharyngeal dysfunction can occur in hereditary and acquired neuropathy with axonal involvement, and also in acquired, immune-mediated nodal-paranodal neuropathy.¹⁹

The juvenile onset of clinical signs in our dogs is consistent with an inherited disorder. Both males and females were affected, and none of the parents was reported to have clinical signs. Some pedigrees are missing and data concerning disease status could not be obtained for all of the littermates. Nevertheless, the prevalence of the disease in the aforementioned litters was at least 25%. For all these reasons, an AR mode of inheritance can be suspected. Additional data are needed to confirm that the disease is inherited and to identify its mode of inheritance.

To our knowledge, ours is the first description of juvenile PNLP in ASTs. The disease results from degenerative neuropathy and very likely is inherited. When surgery for LP is performed, the majority of

dogs can have a longer lifespan than reported for dogs affected by other breed-related PNs, despite locomotor disturbance.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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