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Demyelinating polyneuropathy with focally folded myelin sheaths in a family of Miniature Schnauzer dogs ☆

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ABSTRACT

A spontaneous demyelinating polyneuropathy in two young Miniature Schnauzer dogs was characterized clinically, electrophysiologically and histopathologically. Both dogs were related and a third dog, belonging to the same family, had similar clinical signs. On presentation, clinical signs were restricted to respiratory dysfunction. Electrophysiological tests showed a dramatic decrease in both motor and sensory nerve conduction velocities. Microscopic examination of peripheral nerve biopsies (light and electron microscopy, teased nerve fibers), showed that this neuropathy was characterized by segmental demyelination and focally folded myelin sheaths. Various clinical syndromes associated with tomacula or focal thickening of the myelin sheath of the peripheral nerves have been described in humans and shown to be caused by gene mutations affecting the myelin proteins, such as the hereditary neuropathy with liability to pressure palsies or the demyelinating forms of Charcot–Marie–Tooth disease. In animals, a tomaculous neuropathy has been reported in cattle and chickens but not in carnivores. Here we report a demyelinating peripheral neuropathy with tomacula in two Miniature Schnauzer dogs.

1. Introduction

Tomacula represent a pathological focal thickening of peripheral nerve myelin resulting from excessive myelin folding [1] and abnormal compaction. Various demyelinating neuropathies in humans are associated with the formation of tomacula in peripheral nerves [2] but the term 'tomaculous neuropathy' classically refers to hereditary neuropathy with liability to pressure palsies (HNPP) caused by a mutation in the gene coding for the peripheral myelin protein 22 (PMP22) [3]. Tomacula have also been described in hereditary demyelinating forms of Charcot–Marie–Tooth disease (CMT). Demyelinating forms of CMT can result from mutations of genes coding for myelin proteins, such as PMP22 and myelin protein zero [4]. Although rare, acquired neuropathies, such as chronic inflammatory demyelinating polyneuropathy or IgM paraproteinemic neuropathy can also be associated with the presence of tomacula in humans [2]. Finally, metabolic diseases in human, such as Tangier disease, can lead to the formation of redundant myelin folding in the peripheral nerves [5]. In animals, a suspected inherited tomaculous neuropathy can occur in cattle [6] and an acquired riboflavin deficiency has also been associated with a tomaculous neuropathy in chickens [7]. Generally, canine demyelinating neuropathies have only rarely been described. A hypertrophic neuropathy with characteristic onion bulb formations, classically described as a key feature of demyelinating forms of CMT [8,9], has been documented in the Tibetan Mastiff, and a hypomyelinating neuropathy has been reported in the Golden Retriever [10].

The traditional classification of peripheral neuropathies in humans relies on electrodiagnostic tests. A significantly reduced nerve conduction velocity (NCV) is indicative of demyelination or dysmyelination as opposed to axonal degeneration and axonal loss. Further classification is then obtained by sural nerve biopsy analysis, familial history and genetic testing. In this study, both motor and sensory nerve conduction velocities were dramatically reduced. Profound segmental demyelination and focally folded myelin sheaths (tomacula) were identified on peripheral nerve biopsies at the light and ultrastructural level, as well as in teased nerve fibers. As the dogs were littermates, a hereditary basis was suspected. These observations share many similarities with demyelinating forms of CMT with tomacula in humans.

☆ [1] The clinical cases described in the following manuscript were seen at the National Veterinary School of Alfort and the Frégis Veterinary Hospital Center. Peripheral nerve and muscle analyses were conducted at the Comparative Neuromuscular Laboratory, Department of Pathology, University of California, San Diego.

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2. Materials and methods

2.1. Histopathology and histochemistry of muscle biopsies

Immediately following collection, muscle biopsy specimens (cranial tibial and gastrocnemius muscles from case 2), were flash frozen in isopentane pre-cooled in liquid nitrogen and the muscles stored at −80 °C until further processing. Frozen sections (8 μm thick) were cut and evaluated using a standard panel of histochemical stains and enzyme reactions [11], including hematoxylin and eosin (H&E), modified Gomori trichrome, periodic acid-Schiff, oil red O, myofibrillar adenosine triphosphatase (ATPase) reactions at pH 9.8 and 4.3, acid phosphatase, alkaline phosphatase, the reduced form of nicotinamide-adenine dinucleotide-tetrazolium reductase and succinic dehydrogenase.

2.2. Light microscopy, electron microscopy and teased nerve fibers of peripheral nerve biopsies

The nerve biopsy specimens (ulnar nerve from case 1 and tibial nerve from case 2) were immersion-fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer before shipment to the Comparative Neuromuscular Laboratory, University of California, San Diego. Upon receipt, nerves were rinsed, post-fixed in 1% aqueous osmium tetroxide for 3–4 h before dehydration in a graded alcohol series and propylene oxide. After infiltration with a 1:1 mixture of propylene oxide and araldite resin for 4 h, nerves were placed in 100% araldite resin overnight before embedding in fresh araldite resin. Thick sections (1 μm) were cut with glass knives and stained with toluidine blue prior to light microscopic examination. Thin sections (60–90 nm) were cut with a diamond knife and stained with uranyl acetate and lead citrate before electron microscopic examination.

Teased fibers from the ulnar nerve were processed as above except that the araldite resin used for overnight infiltration lacked hardener. After infiltration, excess araldite resin was wiped off and the nerve subsequently teased in Epon 812 resin without hardener before coverslipping and examination by light microscopy.

3. Results

3.1. Clinical examination and electrodiagnostic study

3.1.1. Case 1

A 31 month-old male intact black Miniature Schnauzer was presented to the National Veterinary School of Alfort, France, for continuous episodes of regurgitation since six months of age. Thoracic radiographs performed by the referring veterinarian showed an enlarged thoracic esophagus and aspiration pneumonia. Physical and neurological examinations were normal without clinical signs of weakness or exercise intolerance. Routine serum biochemistry evaluations, including creatine kinase (CK) activity and electrolytes, serum acetylcholine receptor antibody titer, thyroxin hormone (T4) and thyrotropin hormone (TSH) concentrations were normal.

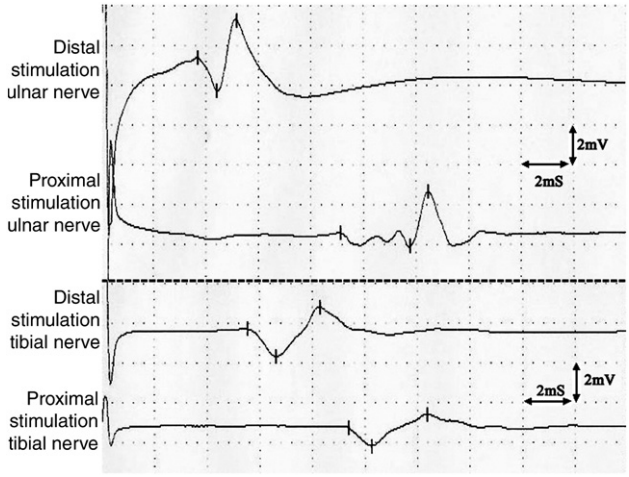

Fig. 1. Motor nerve conduction study of the right ulnar nerve and right tibial nerve of case 2. Numerical results corresponding to those traces appear in Table 1. Note the increased duration and polyphasic shape of the compound muscle action potential (CMAP) obtained after proximal stimulation of the ulnar nerve. Note also the reduction in amplitude (39%) of the CMAP obtained after proximal stimulation of the tibial nerve, compared to the distal stimulation.

Electromyography (EMG), performed under general anesthesia, showed spontaneous abnormal electrical activity consisting of fibrillation potentials (mean 2+; range: 0 to 4+) in the laryngeal, interosseous, cranial tibial, biceps femoris, and carpal flexor and extensor muscles. Electroneurography (ENG) was performed on the tibial, peroneal and ulnar nerves using previously described techniques [12]. Intramuscular monopolar needles were used as recording electrodes. The numerical results are presented in Table 1. The most prominent abnormality was a major reduction in motor and sensory nerve conduction velocities with less dramatic reductions in amplitudes of the compound muscle action potential (CMAP). The decrease in amplitude between the proximal and distal CMAP, 38% and 36% for the ulnar and tibial nerve respectively, could indicate partial conduction block or phase cancellation, although temporal dispersion was not observed. These electrophysiological findings are suggestive of a demyelinating disease affecting both the sensory and motor components of the peripheral nerves. A fascicular biopsy of the ulnar nerve was obtained.

Cefalexin, metronidazole, and metoclopramide were instituted, as well as altered feeding procedures consisting of multiple daily feedings of a dry, high-quality diet in an upright position. This therapy resolved the respiratory signs and frequency of regurgitation decreased. One year later, the dog presented with an episode of increased respiratory noise and polypnea, which, based on clinical and laryngeal examination, was due to laryngeal paresis. Neurological examination was normal at that time. One and a half years after initial presentation, regurgitation was controlled and no other neurological signs developed.

3.1.2. Case 2

A 14 month-old male intact black Miniature Schnauzer was presented to the Frégis Veterinary Hospital Center, France, for inspiratory stridor and subtle exercise intolerance beginning at four months of age. Neither regurgitation nor dysphonia was reported by

Table 1
Numerical results of the electroneurographic examination of cases 1 and 2

Tested nerve (case number)	Left tibial (case 1)	Left peroneal (case 1)	Left ulnar (case 1)	Right tibial (case 2)	Right ulnar (case 2)
MNCV	27.0	73.0	24.0	23.5	13.0
Normal MNCV (11)	64.1 +/− 2.6	82.1 +/− 2.2	61.9 +/− 3.1	64.1 +/− 2.6	61.9 +/−3.1
SNVC	NT	22	21	NT	NT
Normal SNVC (39)	/	64.4 +/− 5.2	69.4 +/− 6.9	/	/
CMAP amplitude distal stimulation	25.6	27.3	15.7	2.47	3.6
Normal CMAP amplitude distal stimulation (11)	24.5 +/− 3.5	17.1 +/− 2.0	26.5 +/− 1.8	>3	>4
CMAP amplitude proximal stimulation	16.3	27.2	9.7	1.51	2.8
Normal CMAP amplitude proximal stimulation (11)	20.2 +/− 1.6	19.2 +/− 2.2	24.0 +/− 2.6	>2	>4
Distal CMAP/proximal CMAP ratio (%)	36	<1	38	39	22
Temporal dispersion	No	No	No	No	Yes

Normal values are expressed as mean +/− standard deviation. Compound muscle action potential (CMAP) amplitudes are expressed in mV and conduction velocities in m/s. NT: not tested. MNCV: motor nerve conduction velocity. SNCV: sensory nerve conduction velocity.