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A Novel Movement Disorder in Related Male Labrador Retrievers Characterized by Extreme Generalized Muscular Stiffness

A.E. Vanhaesebrouck, G.D. Shelton, L. Garosi, T.R. Harcourt-Brown, J. Couturier, S. Behr, R.J. Harvey, N.D. Jeffery, K. Matiasek, W.F. Blakemore, and N. Granger

Objectives: To describe the clinical phenotype of a new motor disorder in Labrador Retrievers.

Animals and Methods: Case series study. Seven young male Labrador Retrievers presented for evaluation of stiff gait.

Results: All affected dogs had generalized muscular stiffness, persistent at rest and resulting in restricted joint movements. They showed a forward flexed posture, festinating gait, and bradykinesia. Signs developed between 2 and 16 months of age and tended to stabilize in adulthood. Needle electromyogram in the conscious state showed continuous motor unit activity in resting epaxial and proximal limb muscles. This activity was abolished by general anesthesia. Muscle and nerve histopathology was normal. In 2 dogs necropsied, astrocytosis was evident throughout the spinal cord gray matter, reticular formation and caudate nuclei. Decreased neuronal counts were selectively found in the spinal cord Rexed's lamina VII, but not in VIII and IX. Pedigree analysis showed that the affected dogs were from 5 related litters.

Conclusions and Clinical Importance: This new hypertonicity syndrome in Labrador Retrievers is unique because of the selective distribution of the histological lesions, the lack of progression in adulthood, and its exclusive occurrence in male dogs. Pedigree analysis suggests an X-linked hereditary disease, although other modes of inheritance cannot be ruled out with certainty. We hypothesize that altered output from basal nuclei and reticular formation together with motor neuron disinhibition caused by a decreased number of spinal cord interneurons leads to the muscular stiffness.

Key words: Canine; Central nervous system; Muscle stiffness; Rigidity.

In dogs, sustained involuntary skeletal muscle contraction leading to muscle stiffness can originate from the muscles (eg, myotonia[1]), the peripheral nervous system (eg, cramps[2] or neuromyotonia[3]), or the central nervous system (CNS) (eg, tetanus, myoclonus or movement disorders such as dystonia[4]). In all of these syndromes, the electromyogram (EMG) shows abnormal electrical muscle discharges. However, a combination of the clinical description of involuntary muscle contraction (including characterization of onset, duration, and persistence with rest) and EMG characteristics (pattern of spontaneous discharges, frequency of motor unit activity, and persistence under general anesthesia [GA]) often is sufficient to attribute the origin of the rigidity to the muscles, nerves, or CNS. Indeed, continuous motor unit activity originating from the CNS typically is abolished by GA, but persists if it originates from the muscles or nerves.[5]

Abbreviations:

CNS	central nervous system
CSF	cerebrospinal fluid
EM	electron microscopy
EMG	electromyographic examination
GA	general anesthesia
GAD	glutamic acid decarboxylase
GFAP	glial fibrillary acidic protein
H&E	hematoxyline and eosin
MRI	magnetic resonance imaging
MUAP	motor unit action potential

Syndromes characterized by continuous motor unit activity of CNS origin are poorly understood in veterinary medicine. In human patients, muscle stiffness of central origin, whether or not associated with abnormal posture and movements, usually is caused by diseases involving the extrapyramidal system (eg, Parkinsonism and dystonia).[6] By contrast, lesions of the pyramidal system cause spasticity and hyperreflexia.[6] Rigidity of central origin also can result from dysfunction of inhibitory glycinergic neurotransmission or GABAergic interneuron transmission in the spinal cord as well as in the brainstem. For example, strychnine poisoning and hyperekplexia (also known as startle disease)[7] arise from glycinergic dysfunction whereas "stiff-man" or "stiff-horse" syndrome[8,9] results from GABAergic dysfunction. Tetanus rigidity results from the interference of the toxin with the release of both neurotransmitters.

This study describes the clinical, electrophysiologic, and histopathologic findings, and a pedigree analysis of 7 young related male Labrador Retrievers with generalized rigidity of CNS origin.

From the Department of Veterinary Medicine, The Queen's Veterinary School Hospital, University of Cambridge, Cambridge, UK (Vanhaesebrouck, Harcourt-Brown, Jeffery, Blakemore, Granger); the Comparative Neuromuscular Laboratory, Department of Pathology, School of Medicine, University of California, San Diego, CA(Shelton); the Davies Veterinary Specialists, Higham Gobion, UK (Garosi, Couturier, Behr); the Department of Pharmacology, The School of Pharmacy, London, UK (Harvey);and the Section of Clinical & Comparative Neuropathology, Institute of Veterinary Pathology, Ludwig Maximilians University of Munich, Munich, Germany (Matiasek); Behr is presently affiliated with the Willows Referral Service, Solihull, UK; Jeffery is presently affiliated with the Lloyd Veterinary Medical Center, College of Veterinary Medicine, Iowa State University, Ames, OH. *Part of this study was presented as an oral presentation at the 21st annual European College of Veterinary Neurology symposium, Rhodes, Greece, in September 2008. The abstract has been published in Granger N, Harcourt-Brown TR, Shelton GD, et al, "Stiff-dog" syndrome in three related young male Labrador Retrievers. J Vet Intern Med 2009; 23(2):400.*

Corresponding author: An Vanhaesebrouck, Department of Veterinary Medicine, The Queen's Veterinary School Hospital, University of Cambridge, Madingley Road, Cambridge CB3 0ES, UK; e-mail: av354@cam.ac.uk

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Material and Methods

Dogs

Seven young related male Labrador Retrievers presented with abnormal gait characterized by generalized stiffness. The dogs were investigated between 2007 and 2010 at the Queen's Veterinary School Hospital, Department of Veterinary Medicine, University of Cambridge (UK) and Davies Veterinary Specialists (UK).

Electrophysiological Studies

Electrophysiological investigations were conducted with conventional equipment.[a] Needle EMG was performed without GA (ie, consciously) in 2 dogs, under GA in 2 dogs, and before and after GA in 3 dogs. Without GA, only 1 site was tested in each of the following muscles: *biceps femoris*, *triceps brachii* and lumbar epaxial muscles; EMG was performed when the dog was standing and also when the dog was relaxed in lateral recumbency. EMG recordings under GA were systematically obtained from the appendicular, epaxial, and head muscles on 1 side of the body.[10] Motor and sensory nerve conduction studies were recorded from the tibial (n = 4), peroneal (n = 2), ulnar (n = 2), radial nerves (n = 1), or some combination of these in 4 dogs.[11] Repetitive nerve stimulation from the tibial nerve, at the level of the stifle, was performed in 2 dogs. The F-waves were recorded in the *interosseous* muscles by stimulation of the tibial nerve in 3 dogs.[12] Ulnar nerve somatosensory evoked potentials were recorded dorsal to the arch of the C1 vertebra in 1 dog.[13]

Histopathologic Studies

Muscle biopsies were collected by an open biopsy procedure from 5 dogs under GA (*biceps femoris* [n = 2], *triceps brachii* [n = 2], *tibialis cranialis* [n = 3], and *gastrocnemius* [n = 1]). Unfixed biopsy specimens were snap frozen in isopentane, precooled in liquid nitrogen, and stored at 80°C until further processing. Cryosections were cut (8 μm) and a standard panel of histochemical stains and enzyme reactions performed, including hematoxylin and eosin (H&E), modified Gomori trichrome, periodic acid-Schiff, oil red O, myofibrillar adenosine triphosphatase (ATP-ase) at pH 9.8 and 4.3, acid phosphatase, alkaline phosphatase, nicotinamide-adenine dinucleotide-tetrazolium reductase, succinate dehydrogenase, and staphylococcal protein A conjugated with horseradish peroxidase.[14] A *biceps femoris* biopsy from 1 dog was fixed in 2.5% glutaraldehyde for 1 week. The specimen then was postfixed in 2% osmium tetroxide and dehydrated in a descending alcohol series and propylene oxide before embedding in epoxy resin,[b] as described previously.[15] Ultrathin (90 nm) sections were stained with uranyl acetate and lead citrate. The sections then were viewed in a Hitachi Model H600 transmission electron microscope (EM).

Fixed peroneal nerve biopsies were obtained from 3 dogs. Biopsies were fixed in 2.5% glutaraldehyde for 1 week and processed as for the fixed muscle biopsy. Sections (1 μm) were cut and stained with toluidine blue for light microscopy before further staining for electron microscopy. Ultrathin (90 nm) sections were stained with uranyl acetate and lead citrate and sections viewed in a Hitachi Model H600 transmission EM.

In 2 dogs, post-mortem examination was performed. The brain and spinal cord were fixed in 10% neutral-buffered formalin for histopathologic study. Multiple transverse and longitudinal tissue sections from the brain to the caudal part of the spinal cord were analyzed. Sections (5 μm) were stained with H&E, Cresyl violet, glial fibrillary acidic protein (GFAP) antibodies[c] (by avidin-biotin-peroxidase method), and Gallyas silver impregnation. Neuronal cell counts at the level of the C1 spinal cord segment were performed on cross sections stained with Cresyl violet of 2 affected dogs and 1 age- and size-matched control. A neuron was only counted when it contained a visible nucleus, nucleolus, and Nissle substance. Neurons were counted in the intermediate gray zone (lamina VII) and in the ventral gray horn (laminae VIII–IX). Laminae of the gray matter were identified according to the classification of Rexed.[16]

Pedigree Analysis

Pedigree information on the 7 affected dogs and an additional 460 relatives was collected. Owners, referring veterinarians, and breeders of affected dogs were asked for any information regarding related dogs showing similar clinical signs. The final pedigree was drawn with the assistance of computer software, PEDRAW.[17]

Statistical Analysis

Where appropriate, mean ± standard deviation of results is presented.

Results

Clinical and Neurologic Description

All dogs were purebred male Labrador Retrievers. The age at presentation was 22 ± 11 months (range, 15–47 months). The mean age of disease onset, reported by the owner, was 17 ± 12 months (range, 2–41 months) and the mean duration of clinical signs was 6 ± 5 months (range, 2–16 months). All dogs presented for gait abnormality and exercise intolerance. Initially, only the pelvic limbs appeared affected. As the disease progressed, the thoracic limbs became involved. All dogs were reluctant to exercise for more than 5–10 minutes of walking, after which they would rest in sternal recumbency. There was no evidence of cardiovascular dysfunction in any of the dogs.

All dogs showed a strikingly similar gait (as illustrated in the supplementary video) that was short strided, slow, and characterized by symmetrical appendicular and truncal rigidity. The dogs experienced difficulty in turning. Running induced a "bunny-hopping" gait in the pelvic limbs. Increased gait speed also resulted in subtle ataxia, marked by a crab-like gait, in which the pelvic limbs tracked off to the side of the thoracic limbs, and with an occasional tendency to fall sideways. During locomotion, there was a shift in body weight toward the thoracic limbs. All dogs had difficulty rising from recumbency and initiation of locomotion was particularly difficult. The vertebral column appeared very rigid, fixed in an almost straight line with the neck held horizontally.

During close examination, the most prominent finding was muscle stiffness. Muscle tone was symmetrically increased, especially in the proximal limb and axial (cervical and lumbar) muscles. The proximal appendicular muscles were moderately hypertrophied. Muscle stiffness was present at rest (in standing position and in lateral recumbency) and during voluntary movements. This severe generalized rigidity resulted in a marked restriction of joint movements, most notably in the hips, stifles, shoulders, and elbows, and also limited motion during postural reaction testing (ie, hopping and hemiwalking). Mild deficits in proprioceptive positioning (ie, paw replacement test) were identified as the disease

progressed. Cranial nerves and spinal reflexes remained normal. Exaggerated startle responses were not found.

Electrophysiological Investigations

Conscious needle EMG showed continuous motor unit activity in the proximal limb and epaxial muscles (Fig 1) while the dogs were standing or lying in lateral recumbency. The morphology of the motor unit action potentials (MUAPs) was considered normal, based on amplitude (reference range for human MUAPs, 100 μV–3 mV), shape (≤4 phases), and duration (reference range for human MUAPs, 5–15 ms)[18] (Fig 1). The mean maximal amplitude during each 10-second recording was 1.2 mV ± 760 μV (range, 306 μV–2.7 mV). The discharge frequency of MUAPs ranged from 25 to 91 Hz. When firing rates were high, MUAPs became superimposed and identification of individual motor units became more difficult. The firing rate of the MUAPs recorded in the *tibialis cranialis* muscle further increased when the peroneal nerve was electrically stimulated. The recording of continuous motor unit activity, together with the signs of muscle rigidity, was abolished by GA. Fibrillation potentials were sporadically recorded in 2 dogs under GA.

Results of motor and sensory nerve conduction, repetitive nerve stimulation and late potential (F-wave) studies were normal. The ulnar nerve sensory evoked potential latency (7.30 ms; normal value, 6.20 ± 0.87 ms, unpublished data) and amplitude (0.65 μV; normal value, 0.84 ± 0.40 μV, unpublished data) measured in 1 dog was considered normal.

Laboratory Investigations

CBC and serum biochemistry results (including creatine kinase, electrolytes, and thyroid and adrenal function tests) all were within normal limits. A cervical (n = 4) or lumbar (n = 1) cerebrospinal fluid (CSF) puncture was performed in 5 dogs. The CSF nucleated cell count was normal in all dogs. The total CSF protein concentration was mildly to moderately increased in 2 dogs (cervical puncture, 0.31 g/L; reference range, < 0.25 g/L; lumbar puncture, 2.33 g/L; reference range, < 0.40 g/L).

Antibody titers to *Toxoplasma gondii* and *Neospora caninum* were negative in 4 dogs. The serum acetylcholine receptor antibody titer was negative in 2 dogs. The SINE exonic insertion in the *PTPLA* gene causing centronuclear myopathy in Labrador Retrievers was not found in 1 dog tested.[19] Plasma lactate and pyruvate concentrations, conducted before and after exercise in 1 dog, were normal.[20] In 1 dog, serum titer for voltage-gated potassium channel antibodies, with a human assay,[d] was negative.

Diagnostic Imaging

Radiographs of the vertebrae were obtained in 4 dogs. One dog had an asymmetric transitional vertebra at S1. On magnetic resonance imaging (MRI) of this dog's lumbosacral area no spinal cord compression was seen. However, computed tomography showed abnormal alignment of the L6 and L7 spinous processes in the vertical plane. This was attributed to lumbar muscle hypertonicity during growth of the dog. Brain and spinal cord MRI performed in 2 other cases was normal. One other dog underwent a cisternal myelogram that was normal.

Follow-up

In 4 dogs, 3 months to 2 years after the diagnosis (between the ages of 1.5 and 4 years) euthanasia was requested by the owner because of poor quality of life. One dog died at the age of 2 caused by a gastric dilatation and volvulus and 1 dog died at the age of 3 caused by acute respiratory failure (for which the cause could not be determined). One dog was still alive at the age of 3.5 years (2 years after diagnosis). In all cases, the clinical signs progressed over several months, then stabilized.

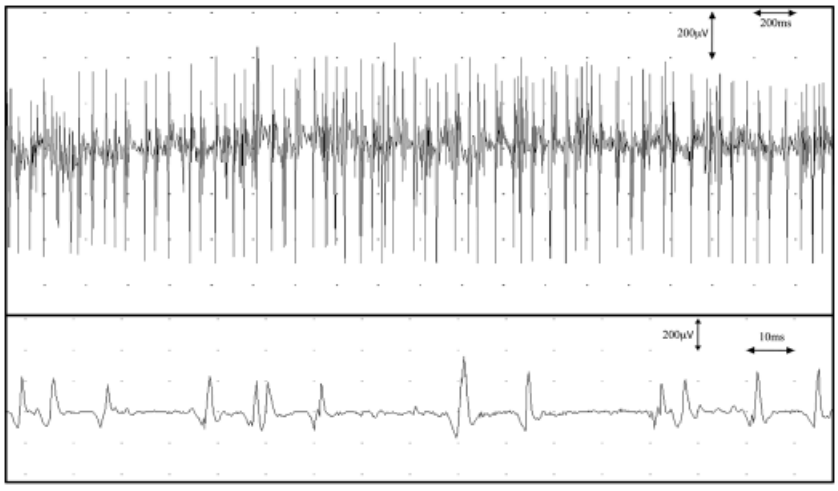

Fig 1. Conscious needle EMG of the *triceps brachii* muscle in an affected Labrador Retriever, resting in lateral recumbency. Top panel: note the continuous spontaneous motor unit activity firing approximately at 51 Hz. Bottom panel: magnified view of the top panel recording, showing motor unit action potentials of normal amplitude, shape, and duration.

Therapeutic trials with diazepam (n = 3), mexiletine (n = 1), phenobarbital (n = 1), potassium bromide (n = 2), selegiline (n = 2), dantrolene (n = 2), and corticosteroids at immunosuppressive dosages (prednisone, 2 mg/kg/d for 3 weeks, n = 2) were unsuccessful. Treatment with nonsteroidal anti-inflammatory drugs gave partial and temporary improvement (n = 3) in the dog's ability to walk for slightly longer periods.

Histopathologic Results

Histopathology, histochemistry, and EM of muscle and nerve biopsy specimens were normal. Quantitative analysis of muscle fiber size and distribution of fiber types showed a normal size and ratio of type I and type II fibers compared with historical controls for the muscle examined.[21]

Post-mortem examination and histopathologic findings were identical in the 2 dogs available for necropsy. No macroscopic lesions were evident in the brain and spinal cord. Microscopic lesions consisting of symmetrical reactive astrocytosis and astrogliosis were evident in the intermediate gray matter on H&E stained cross-sections at all levels of the spinal cord (Fig 2A), and in the brain throughout the reticular formation (Fig 2C) and caudate nuclei. Glial fibrillary acidic protein immunohistochemistry demonstrated reactive astrocytes with enlarged and densely stained cell bodies and cell processes, both in the spinal cord (Fig 2B) and the reticular formation (Fig 2D). The changes observed in the reticular formation were diffuse and not confined to a specific area. A Gallyas stain highlighted a few "empty beds" and excluded dendritic changes. No cytoplasmic inclusions were seen in the neurons or glial cells. The substantia nigra pars compacta appeared normal. Axonal degeneration, with secondary demyelination, was extensive and most pronounced in the ventro-medial area of the spinal cord white matter (Fig 3). The pyramidal tracts were clearly spared at the level of the decussation compared with the neighboring tectospinal tracts. Using Cresyl violet stain, the neuronal count on both sides of the C1 spinal cord segment in Rexed's lamina VII was 19 and 20 in the affected cases and 45 at the same level in the control case. In Rexed's laminae VIII and IX, the neuronal count was 37 and 39 in the affected cases and 40 in the control case. The white to gray matter area ratio was not different between the affected dogs (6.60 and 5.57) and the control dog (5.73) at the level of C1 spinal cord segment.

Pedigree Analysis

Pedigree analysis indicated common ancestry of all affected dogs within 14 generations (Fig 4). Three affected dogs were siblings and the other affected dogs were from 4 different but related litters. The first common ancestor for the 7 affected dogs could be identified 8 generations ago. The pedigree showed a high level of inbreeding. Because only male dogs were affected and their parents were reported as normal, an X-linked reces-

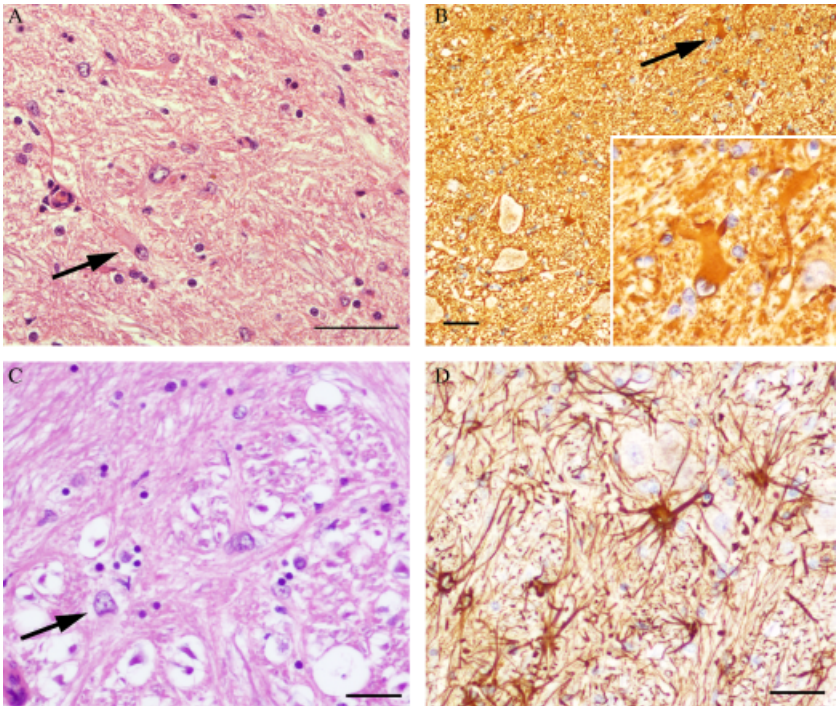

Fig 2. Photographs of the spinal cord (**A,B**) and reticular formation (**C,D**) in cross-section in one of the affected dogs submitted for autopsy. On microscopic examination of H&E stained sections (A,C), the lesions consist of reactive astrocytosis demonstrated by enlarged and visible astrocyte cell bodies (black arrow in A) and hypertrophied nuclei (black arrow in C). With immunohistochemical staining for GFAP (B,D), the reactive astrocytosis is demonstrated by the enlarged and densely stained cell bodies (black arrow in B—magnified view of the astrocyte pointed by the arrow is shown at the bottom-right corner of B) and cell processes (D). Bar = 50 μm.

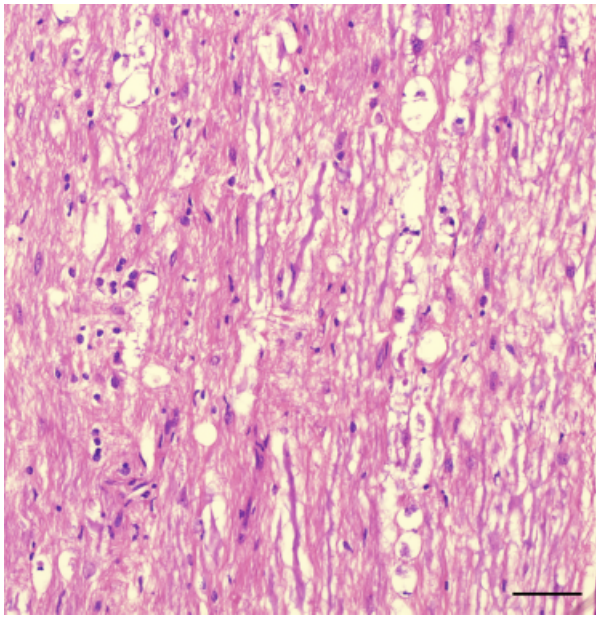

Fig 3. Photograph of the ventro-medial tracts of the spinal cord in longitudinal section, in one of the affected dogs submitted for necropsy. There is axonal degeneration with secondary demyelination demonstrated by the presence of disrupted axons and chains of digestion chambers. Bar = 50 μm.

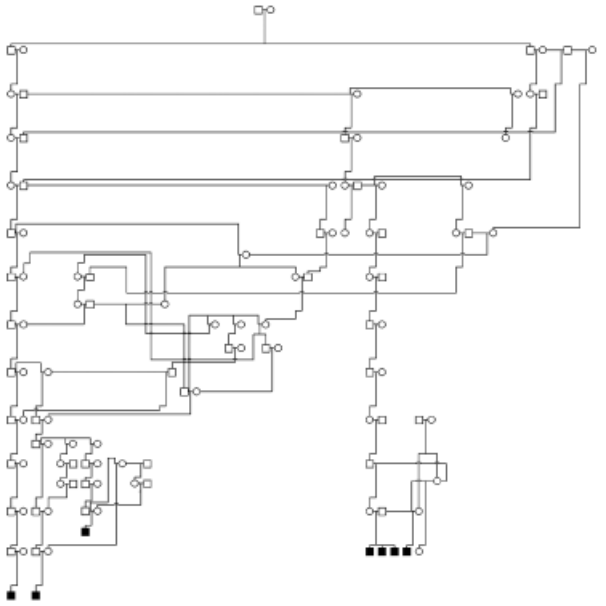

Fig 4. Family tree drawing showing the relationship between the affected dogs. The squares represent males and the circles represent females. Black-shaded squares indicate the affected dogs. Seven affected dogs belong to 5 litters, 3 dogs were from the same litter and 4 dogs shared the same father.

sive mode of inheritance is postulated. However, an autosomal recessive mode of inheritance cannot be ruled out at present.

Discussion

This study describes a new motor disorder in a family of young Labrador Retrievers. The clinical phenotype was homogeneous among the cases, affecting male Labrador Retrievers <3 years of age with a characteristic generalized rigidity and muscular stiffness that persisted at rest. This clinical phenotype is important because it aids in the differentiation of diseases characterized by paroxysmal, episodic or repetitive muscular contractions such as myotonia (congenital or acquired), myoclonus (sporadic or repetitive), or paroxysmal dystonia (ie, Scottie cramps and hypertonicity syndromes in Cavalier King Charles Spaniels and several other breeds).[2,4] An exaggerated startle reflex in response to acoustic or tactile stimuli was not noted in any of our cases. This finding helped to further clinically differentiate this disease from syndromes in animals in which an exaggerated startle reflex is expected (eg, hyperekplexia, "stiff-horse" syndrome, tetanus).[4,9,22] For example, congenital hyperekplexia has been described in Labrador Retriever puppies.[22] Although this previous description shares some clinical and EMG similarities with our cases, it differs from the syndrome described here based on the difference in age of onset, the absence of an exaggerated startle reflex in our cases, and the presence of histopathologic changes in the 2 necropsied cases.[23] Even if spinal interneuron dysfunction is presumably linked to both disorders, these differences suggest 2 different inherited diseases.

Complete electrophysiological analysis of the cases localized the origin of the muscle rigidity to the CNS. Indeed, the disease was characterized by continuous motor unit activity while the dogs were conscious, but the EMG was normal during GA. This finding is a characteristic of stiff syndromes originating from the CNS in humans.[5] Motor and sensory nerve conduction studies also were normal.

Normal histopathology of muscle and nerve biopsy specimens, and post-mortem examination in 2 dogs, confirmed disease localization to the CNS. In the 2 dogs for which a necropsy was performed, the main finding on histopathologic examination of the CNS was marked astrocytosis in the intermediate gray matter of the spinal cord, throughout the reticular formation and in the caudate nuclei. The number of neurons in the intermediate gray matter also was decreased, especially in the Rexed's lamina VII. In comparison, the relative sparing of Rexed's laminae VIII and IX was quite remarkable. These findings suggest that a category of neurons (such as interneurons) is specifically affected. Noteworthy, interneurons located at the level of the intermediate gray matter play a major role in the control of muscle tone, as demonstrated by electrophysiological studies of spinal interneurons in dogs.[24]

The clinical findings in our cases were comparable with Parkinsonism in humans. Neurodegenerative diseases causing Parkinsonism do not only include Parkinson's disease, but also other diseases of the extrapyramidal system, called "Parkinson-plus" syndromes, such as progressive supranuclear palsy, multiple system atrophy, and corticobasal ganglionic degeneration.[25] Classically,

the clinical diagnosis of Parkinsonism is based on the identification of at least 2 of the following motor signs: rigidity, bradykinesia, postural instability, and tremor.[25] All of our dogs presented with rigid hypertonia, defined as resistance to passive movement independent of speed and posture, in contrast to spasticity and dystonia, respectively.[6] As the disease progressed, difficulty rising was observed in all dogs. In human patients, this is a sign of bradykinesia or difficulty in initiating movements.[26] In our dogs, clinical signs of postural instability included a forward-flexed posture caused by a shift forward of the center of gravity and a festinating gait, defined as short steps that progressed into a gallop, presumably to prevent falling.[27] Tremor was not observed in any of our cases. Interestingly, akinesia together with a forward-flexed posture and a festinating gait has been described in Chinese Crested Dogs and Kerry Blue Terriers with canine multiple system atrophy.[28,29] In contrast to the cases presented here, cerebellar signs also were present and predominant rigidity and muscular stiffness were not reported.[28,29]

Furthermore, our cases shared some phenotypical similarities with the ''stiff-man'' syndrome, consisting of extreme rigidity, reported in human patients. Rigidity, persisting at rest but abolished with GA, as well as difficulties standing up and walking also are features of human ''stiff-man'' syndrome.[8,30] In contrast to ''stiff-man'' syndrome, an exaggerated startle response was not observed in our dogs.[8,31] In human patients, this usually is a sporadic autoimmune disease affecting the neurotransmission of GABA. In approximately 60% of those patients, antibodies to the GABA-producing enzyme glutamic acid decarboxylase (GAD) are detected.[32] A ''stiff-horse'' syndrome also has been reported in which anti-GAD antibodies were found.[9] Testing for anti-GAD antibodies was not performed in our cases. However, the occurrence of the disease in 3 young littermates exclusively in males and the familial relationship among affected dogs suggest a hereditary basis rather than an autoimmune disease. Moreover, ''stiff-man'' syndrome has not been described in members of the same family and there is no known genetic predisposition.

As in ''stiff-man'' syndrome, the clinical signs in our cases slowly progressed over months to years until the signs reached a plateau of severity.[8] This is different from the clinical course of Parkinsonism, which is progressive.[33,34]

The muscle stiffness was neither responsive to diazepam, the drug of choice for ''stiff-man'' syndrome, nor to immunosupressive dosages of corticosteroids.[8,9] A trial with selegiline also showed no therapeutic effect. Selegiline, a monoamine oxidase-β inhibitor, inhibits the breakdown of dopamine and therefore decreases rigidity and bradykinesia in the early stages of Parkinson's disease in humans, but is ineffective in ''Parkinson-plus'' disorders.[25]

Pathological changes similar to our dogs also are recognized in the brain and spinal cord of humans with Parkinsonism or ''stiff-man'' syndrome. Caudate nuclei astrocytosis, as found in our dogs, has been associated with bradykinesia in human patients with Parkinsonism[35] and in dogs with multiple system atrophy.[28,29] Bradykinesia is thought to result from failure of the basal ganglia to reinforce the motor cortex, inhibiting it from preparing and executing movements.[26] In Parkinsonism, it is further suggested that the altered basal ganglia output via the reticulospinal tract may lead to increased inhibition of the Ib spinal cord interneurons that in turn reinforce γ-motoneuron input.[36] Therefore, it is likely that the lesions found in the caudate nuclei and reticular formation in our cases contributed to the rigidity.

In ''stiff-man'' syndrome, loss of interneurons and motor neurons in the intermediate horn and ventral horn of the spinal cord respectively, together with gliosis, also are reported.[8] In those human cases, the brainstem and basal ganglia occasionally showed inflammatory infiltrates, which may be related to the immune-mediated nature of the disease. Loss of small neurons in the intermediate horn of the spinal cord also has been observed in progressive supranuclear palsy and multiple system atrophy.[37,38] However, no inclusions were observed in our cases, in contrast with the Lewy bodies in idiopathic Parkinson's disease, glial cytoplasmatic inclusions in multiple system atrophy, and neurofibrillary tangles in progressive supranuclear palsy and cortico-basal ganglionic degeneration.[35]

Although an autosomal recessive mode of inheritance cannot be ruled out, pedigree analysis suggests an X-linked mode of inheritance. However, the occurrence of the disease in only male dogs so far may only indicate that males are predisposed to the disease. For instance, a significantly higher incidence of Parkinsonism is found in men,[39] and the male-to-female ratio is 2 : 1 in ''stiff-man'' syndrome.[8]

Only a few mutations in X-linked genes have been associated with CNS rigidity in human patients. Hyperekplexia is a congenital disorder characterized by hypertonicity and an exaggerated response to sudden acoustic or tactile stimuli (startle reflex) and usually is caused by defects in autosomal genes encoding the glycine receptor α1 and β subunits (*GLRA1* and *GLRB*) or the glycine transporter GlyT2 *(SLC6A5)*.[7] Only recently, a missense mutation (p.G55A) in the collybistin gene (*ARHGEF9*) on Xq11 has been associated with hyperekplexia and epilepsy.[40] Interestingly, additional studies have indicated that rearrangements in *ARHGEF9* also can be associated with a complex range of other symptoms including anxiety, aggression and intellectual disability.[41] In animals, hyperekplexia has been described in a litter of Labrador Retrievers[22] and horses[42] and genetically confirmed in Irish Wolfhounds,[43] cattle[44,45] and in several mouse mutants.[7] However in each of these cases, an exaggerated startle response is a predominant feature and mutations are not found in genes on the X chromosome.

At the other hand, rigidity caused by X-linked Parkinsonism has always been associated with dystonia.[46] Although dystonia might be difficult to differentiate from Parkinsonian rigidity, no signs of dystonia, such as involuntary twitches, repetitive movements, or abnormal postures, were observed in our dogs.[47] X-linked dystonia-Parkinsonism is most commonly caused by

mutations in the TAF-1 gene, located on the X chromosome.[48,49] The TAF-1 protein plays an essential role in the transcription of many genes and more precisely in the dopamine receptor expression in neurons.[48]

In conclusion, the disease described here shares some clinical similarities with Parkinsonism and ''stiff-man'' syndrome in human patients. However, the suspected hereditary basis (as opposed to the autoimmune origin of ''stiff-man'' syndrome or ''stiff-horse'' syndrome), the distribution of the histopathologic lesions with the selective loss of spinal cord interneurons, and the lack of progression at the adult age (as opposed to Parkinsonism), makes this disorder unique among the group of movement disorders. Whereas mutations in the collybistin gene are unlikely to be responsible for this disorder, a lesion in the TAF-1 gene could explain the rigidity observed in this family of Labrador Retrievers. Future mapping of the causative gene with X-linked polymorphic markers will be required to confirm the genetic basis of this hypertonicity disorder.[50]

Footnotes

[a] Viking Quest and Medelec Synergy, Viasys Neurocare, Madison, WI

[b] TAAB Laboratories Equipment Ltd, Berks, UK

[c] Polyclonal rabbit anti-GFAP, 1/20 000, Ref. Z0334, Dako, Ely, UK

[d] University of Oxford, John Radcliffe Hospital, Oxford, UK

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References

1. Vite CH. Myotonia and disorders of altered muscle cell membrane excitability. Vet Clin North Am Small Anim Pract 2002;32:169–187, vii.

2. Shelton GD. Muscle pain, cramps and hypertonicity. Vet Clin North Am Small Anim Pract 2004;34:1483–1496.

3. Vanhaesebrouck AE, Van Soens I, Poncelet L, et al. Clinical and electrophysiological characterization of myokymia and neuromyotonia in Jack Russell Terriers. J Vet Intern Med 24:882–889.

4. de Lahunta A, Glass E. Uncontrolled Involuntary Skeletal Muscle Contractions, 3rd ed. Philadelphia, PA: Saunders; 2009:206–220.

5. Auger RG. AAEM minimonograph #44: Diseases associated with excess motor unit activity. Muscle Nerve 1994;17:1250–1263.

6. Sanger TD, Delgado MR, Gaebler-Spira D, et al. Classification and definition of disorders causing hypertonia in childhood. Pediatrics 2003;111:e89–e97.

7. Harvey RJ, Topf M, Harvey K, et al. The genetics of hyperekplexia: More than startle! Trends Genet 2008;24:439–447.

8. Meinck HM, Thompson PD. Stiff man syndrome and related conditions. Mov Disord 2002;17:853–866.

9. Nollet H, Vanderstraeten G, Sustronck B, et al. Suspected case of stiff-horse syndrome. Vet Rec 2000;146:282–284.

10. Chrisman CL, Burt JK, Wood PK, et al. Electromyography in small animal clinical neurology. J Am Vet Med Assoc 1972; 160:311–318.

11. Walker TL, Redding RW, Braund KG. Motor nerve conduction velocity and latency in the dog. Am J Vet Res 1979;40:1433–1439.

12. Poncelet L, Balligand M. Nature of the late potentials and F-ratio values in dogs. Res Vet Sci 1991;51:1–5.

13. Holliday TA, Weldon NE, Ealand BG. Percutaneous recording of evoked spinal cord potentials of dogs. Am J Vet Res 1979;40:326–333.

14. Dubowitz V, Sewry C. Histological and histochemical stains and reactions. In: Dubowitz V, ed. Muscle Biopsy. A Practical Approach, 3rd ed. Amsterdam: Elsevier; 2007:21–40.

15. Smith PM, Jeffery ND. Histological and ultrastructural analysis of white matter damage after naturally-occurring spinal cord injury. Brain Pathol 2006;16:99–109.

16. Fletcher F. Spinal cord and meninges. In: Evans H., ed. Miller's Anatomy of the Dog, 3rd ed. Philadelphia, PA: Saunders; 1993:806–811.

17. Curtis D. A program to draw pedigrees using LINKAGE or LINKSYS data files. Ann Hum Genet 1990;54:365–367.

18. Bowen JM. Electromyography. Section 2. Philadelphia, PA: WB Saunders; 1987:145–168.

19. Pele M, Tiret L, Kessler JL, et al. SINE exonic insertion in the PTPLA gene leads to multiple splicing defects and segregates with the autosomal recessive centronuclear myopathy in dogs. Hum Mol Genet 2005;14:1417–1427.

20. Platt SR, Garosi LS. Neuromuscular weakness and collapse. Vet Clin North Am—Small Anim Pract 2004;34:1281–1305.

21. Mehta JR, Braund KG, McKerrell RE, et al. Analysis of muscle elements, water, and total lipids from healthy dogs and Labrador Retrievers with hereditary muscular dystrophy. Am J Vet Res 1989;50:640–644.

22. Fox JG, Averill DR, Hallett M, et al. Familial reflex myoclonus in Labrador Retrievers. Am J Vet Res 1984;45:2367–2370.

23. March PA, Knowles K, Thalhammer JG. Reflex myoclonus in two Labrado Retriever littermates: A clinical, electrophysiological, and pathological study. Prog Vet Neurol 1993;4:19–24.

24. Gelfan S, Tarlov IM. Interneurones and rigidity of spinal origin. J Physiol 1959;146:594–617.

25. Ahlskog JE. Diagnosis and differential diagnosis of Parkinson's disease and Parkinsonism. Parkinson Relat Disord 2000;7: 63–70.

26. Berardelli A, Rothwell JC, Thompson PD, et al. Pathophysiology of bradykinesia in Parkinson's disease. Brain 2001; 124:2131–2146.

27. Giladi N, Shabtai H, Rozenberg E, et al. Gait festination in Parkinson's disease. Parkinson Relat Disord 2001;7:135–138.

28. deLahunta A, Averill DR Jr. Hereditary cerebellar cortical and extrapyramidal nuclear abiotrophy in Kerry Blue Terriers. J Am Vet Med Assoc 1976;168:1119–1124.

29. O'Brien DP, Johnson GS, Schnabel RD, et al. Genetic mapping of canine multiple system degeneration and ectodermal dysplasia loci. J Hered 2005;96:727–734.

30. Guardado Santervas PL, Arjona Padillo A, Serrano Castro P, et al. Stiff person syndrome (SPS), a basal ganglia disease? Striatal MRI lesions in a patient with SPS. J Neurol Neurosurg Psychiatry 2007;78:657–659.

31. Barker RA, Revesz T, Thom M, et al. Review of 23 patients affected by the stiff man syndrome: Clinical subdivision into stiff

trunk (man) syndrome, stiff limb syndrome, and progressive encephalomyelitis with rigidity. J Neurol Neurosurg Psychiatry 1998; 65:633–640.

32. Solimena M, Folli F, Aparisi R, et al. Autoantibodies to GABA-ergic neurons and pancreatic beta cells in stiff-man syndrome. N Engl J Med 1990;322:1555–1560.

33. Testa D, Monza D, Ferrarini M, et al. Comparison of natural histories of progressive supranuclear palsy and multiple system atrophy. Neurol Sci 2001;22:247–251.

34. Wenning GK, Litvan I, Jankovic J, et al. Natural history and survival of 14 patients with corticobasal degeneration confirmed at postmortem examination. J Neurol Neurosurg Psychiatry 1998;64:184–189.

35. Gibb WR. Neuropathology of Parkinson's disease and related syndromes. Neurol Clin 1992;10:361–376.

36. Delwaide PJ, Pepin JL, De Pasqua V, et al. Projections from basal ganglia to tegmentum: A subcortical route for explaining the pathophysiology of Parkinson's disease signs? J Neurol 2000; 247(Suppl 2):II75–II81.

37. Terao S, Sobue G, Hashizume Y, et al. Disease-specific patterns of neuronal loss in the spinal ventral horn in amyotrophic-lateral-sclerosis, multiple system atrophy and X-linked recessive bulbospinal neuronopathy, with special reference to the loss of small neurons in the intermediate zone. J Neurol 1994;241: 196–203.

38. Kikuchi H, Doh-ura K, Kira J, et al. Preferential neurodegeneration in the cervical spinal cord of progressive supranuclear palsy. Acta Neuropathol 1999;97:577–584.

39. Baldereschi M, Di Carlo A, Rocca WA, et al. Parkinson's disease and Parkinsonism in a longitudinal study: Two-fold higher incidence in men. ILSA Working Group. Italian Longitudinal Study on Aging. Neurology 2000;55:1358–1363.

40. Harvey K, Duguid IC, Alldred MJ, et al. The GDP-GTP exchange factor collybistin: An essential determinant of neuronal gephyrin clustering. J Neurosci 2004;24:5816–5826.

41. Kalscheuer VM, Musante L, Fang C, et al. A balanced chromosomal translocation disrupting ARHGEF9 is associated with epilepsy, anxiety, aggression, and mental retardation. Hum Mutat 2009;30:61–68.

42. Gundlach AL, Kortz G, Burazin TC, et al. Deficit of inhibitory glycine receptors in spinal cord from Peruvian Pasos: Evidence for an equine form of inherited myoclonus. Brain Res 1993;628: 263–270.

43. Gill JL, Capper D, Vanbellinghen JF, et al. Startle disease in Irish Wolfhounds associated with a microdeletion in the glycine transporter GlyT2 gene. Neurobiol Dis 2011;43:184–189.

44. Charlier C, Coppieters W, Rollin F, et al. Highly effective SNP-based association mapping and management of recessive defects in livestock. Nat Genet 2008;40:449–454.

45. Pierce KD, Handford CA, Morris R, et al. A nonsense mutation in the alpha1 subunit of the inhibitory glycine receptor associated with bovine myoclonus. Mol Cell Neurosci 2001; 17:354–363.

46. Lee LV, Munoz EL, Tan KT, et al. Sex linked recessive dystonia Parkinsonism of Panay, Philippines (XDP). Mol Pathol 2001;54:362–368.

47. Sanger TD, Chen D, Fehlings DL, et al. Definition and classification of hyperkinetic movements in childhood. Mov Disord 2010;25:1538–1549.

48. Makino S, Kaji R, Ando S, et al. Reduced neuron-specific expression of the TAF1 gene is associated with X-linked dystonia-Parkinsonism. Am J Hum Genet 2007;80:393–406.

49. Fabbrini G, Brancati F, Vacca L, et al. A novel family with an unusual early-onset generalized dystonia. Mov Disord 2005; 20:81–86.

50. Richman M, Mellersh CS, Andre C, et al. Characterization of a minimal screening set of 172 microsatellite markers for genome-wide screens of the canine genome. J Biochem Biophys Methods 2001;47:137–149.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Video Clip S1. The video (mpeg-4, H-264) consists of 3 short fragments. In the first fragment, note the short-strided gait. During locomotion, there is an abnormal forward shift of the body weight toward the thoracic limbs. In the second fragment, increased speed results in bunny hopping of the hindlegs, with the hindlegs occasionally tracking off to the side. In the last fragment, one of the affected Labradors has difficulties to rise from recumbency.