

Spongy Degeneration with Cerebellar Ataxia in Malinois Puppies: A Hereditary Autosomal Recessive Disorder?

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Background: There is a high incidence of hereditary degenerative diseases of the central nervous system in purebred dogs.

Hypothesis: Cerebellar ataxia in Malinois puppies, caused by degenerative changes that predominate in cerebellar nuclei and the granular cell layer, is a hereditary disorder that is distinct from cerebellar cortical abiotrophies.

Animals: Thirteen Malinois puppies with cerebellar ataxia.

Methods: Retrospective study. Records of Malinois puppies with spongy degeneration of the cerebellar nuclei were analyzed including clinical signs, histopathological changes, and pedigree data.

Results: Signs of cerebellar dysfunction were observed in puppies of both sexes from 5 different litters (1995–2009) of phenotypically normal parents. Clinical signs started before the age of 2 months and resulted in euthanasia of all puppies by the age of 13 weeks. Histopathology disclosed marked bilateral spongy degeneration of the cerebellar nuclei and vacuoles in the granular cell layer and foliate white matter of the cerebellum. In some puppies, discrete vacuoles in gray and white matter were present in other parts of the brain. Furthermore, spheroids and dilated myelin sheaths were observed. Pedigree data and segregation frequency support an autosomal recessive hereditary disorder.

Conclusions and Clinical Importance: Malinois suffer from a hereditary spongiform degeneration that predominates in the cerebellum and causes an early onset of clinical signs with unfavorable prognosis. Future efforts should increase awareness among veterinarians and breeders and aim to identify underlying metabolic mechanisms and the affected genes.

Key words: Belgian Shepherd; Central nervous system; Vacuoles.

Hereditary degenerative diseases of the central nervous system have been reported in purebred dogs over the last 2 decades.^{1–5} The cerebellum frequently is affected in neurodegenerative disorders.⁶ A common cerebellar disease is cerebellar cortical degeneration (abiotrophy), which has been recognized in a variety of breeds such as American Staffordshire Terriers, Scottish Terriers, Australian Kelpies, Old English Sheepdogs, English Bulldogs, and Gordon Setters.^{1,2,7–10} The disease is characterized by cerebellar atrophy, loss of Purkinje cells, depletion of granular cells, and atrophy of the molecular layer.^{1,11} In some breeds such as the Bavarian Mountain Dog and Brittany Spaniel, a selective loss has been described.^{12,13}

Disorders affecting primarily the cerebellar nuclei and cortex are less common and can be accompanied by neurodegenerative changes in other locations of the brain or spinal cord. This group of disorders tends to affect dogs at a young age and to have an unfavorable outcome. A familial cerebellar ataxia with hydrocephalus is described in Bull Mastiff puppies.^{14,15} Specific lesions are bilateral, symmetric, spongy, vacuolization, gliosis, and axonal

Abbreviations:

CT	computed tomography
GFAP	glial fibrillary acidic protein
MRI	magnetic resonance imaging
TE	echo time
TR	repetition time

degeneration within the cerebellar nuclei. In some puppies, the caudal colliculi and lateral vestibular nuclei also were affected. A presumed hereditary polioencephalomyelopathy is recognized in the Australian Cattle Dog and the Alaskan Husky.^{4,5} These encephalopathies show not only spongy but also cavitory lesions and are compared with Leigh's disease in humans. In the Australian Cattle dog, an extensive involvement of the spinal cord gray matter is present, whereas in the Alaskan Husky the most severe lesions are in the thalamus. A status spongiosus also is briefly reported in Saluki puppies and young Rottweilers.^{6,16,17} The latter is characterized by neuronal vacuolation of the cerebellar nuclei, Purkinje cells, and selected brain stem nuclei as well as spinal cord gray matter and visceral autonomic ganglia. A degenerative encephalomyelopathy in Kuvasz puppies is characterized by gliosis and spongy changes around the cerebellar nuclei and focal necrosis in the dorsolateral body of the caudate nucleus. The lesions are suggestive of an inherited metabolic disorder or a possible Amprolium toxicity, because puppies were treated with this medication to prevent coccidiosis.¹⁸ Furthermore, a congenital tremor with spongy degeneration of the central nervous system was recognized in 1991 in 2 Malinois crossbred puppies.¹⁹ A bilateral symmetric spongy state with predominant involvement of the gray matter was noted in the cerebellar nuclei and, less intensely in the cerebellar cortex, the nuclei of the brain stem, the cerebral cortex,

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and the lumbar and cervical intumescence of the spinal cord gray matter. In this report we describe Malinois puppies from different litters with ataxia and pronounced bilateral symmetric spongy degeneration that predominated in the cerebellar nuclei.

Material and Methods

Thirteen Malinois puppies from 5 different litters (1995–2009) with cerebellar ataxia were included in this study. Clinical history and signs, histopathology reports, imaging reports, and pedigree data of these puppies were analyzed retrospectively. Archived brain tissue was re-evaluated and further processed for glial fibrillary acidic protein (GFAP) immunohistochemistry and electron microscopy.

Pathology

In 10 puppies, necropsy and histopathological examinations of brain and different extraneural organ samples were performed. In 7 animals, the spinal cord and sciatic nerves also were examined. Two puppies from litter 2 and 1 puppy from litter 5 were not sent for necropsy, because they showed clinical signs similar to their affected litter mates. Brain, spinal cord, sciatic nerves, and organ samples were removed during necropsy and fixed in 4% buffered formaldehyde. Transverse sections of brain and spinal cord were embedded in paraffin, sectioned at 5 μ m, stained with hematoxylin and eosin, and evaluated by light microscopy.

In all animals, immunohistochemistry by the avidin-biotin complex method was applied to paraffin-embedded brain sections. A monoclonal antibody against N-protein of canine distemper virus^a was used to eliminate distemper infection, and in selected animals a rabbit anti-cow GFAP-antibody^b was used to assess astrocytes in the region of the cerebellar nuclei and the cerebellar cortex.

Because of the lack of glutaraldehyde fixed tissue, 1 mm³ samples of formalin-fixed cerebellar cortex (1 puppy of litter 2) and of paraffin-embedded cerebellar nuclei (1 puppy of litter 1) were used for ultrastructural examination. Tissues were postfixated in osmium tetroxide, dehydrated and embedded in glycid ether 100. Ultrathin sections stained with uranyl acetate and lead citrate were examined with a Zeiss EM 900 transmission electron microscope.^c

Neuroimaging

Brain imaging was performed in the 3 affected puppies of litter 5 and 1 healthy pup of the same litter at the age of 8 weeks with computed tomography (CT)^d and low-field magnetic resonance imaging^e (MRI) with a small multipurpose coil. In 1 affected puppy, the region of the lumbar spinal cord was imaged in addition to the brain. One puppy was reimaged with MRI immediately postmortem at 13 weeks of age. For the MRI study, T1—(repetition time [TR] 650 ms, echo time [TE] 20 ms, 3 mm; TR 30 ms, TE 9 ms, 1.1 mm, with and without contrast), proton density—(TR 1600 ms, TE 22 ms; 3.5 mm), and T2-weighted images (TR 1600 ms, TE 100 ms; 3.5 mm) were produced.

Pedigree Analysis

In order to test the hypothesis of an underlying hereditary disorder, pedigrees were searched for common ancestors, a pedigree map was created and the frequencies of healthy and affected puppies in the 5 litters were investigated. A simple Mendelian autosomal recessive disease predicts a 25% segregation frequency of affected puppies from carrier parents. This null hypothesis was tested by χ^2 -tests and a 95% confidence interval by overall pooled data of all 5 litters and nonpooled data of each litter (statistical package R, prop. test).

Results

Clinical Signs

A summary of patient data is given in Table 1.

Litter 1. Two of 8 puppies were affected in this litter and both puppies were males. An acute onset of clinical signs was noticed at 4 weeks of age. In both puppies, severe ataxia with hypermetria, stumbling, and falling were reported by the breeder and resulted in euthanasia within a week. At presentation before euthanasia, both puppies were in good physical condition, bright and alert. Both had severe intention tremor and cerebellar ataxia. They were unable to start walking without falling. No tremor was seen at rest.

Litter 2. This litter was produced by the same dam as litter 1 but with a different sire (Fig 1). Three of 7 puppies developed neurological signs. Onset of clinical signs ranged from 4 to 6 weeks of age and affected 2 male and 1 female puppies. Affected puppies again were in a good physical condition. In both male puppies, the breeder reported ataxia, wide-based stance, balance loss, and falling, comparable to what was observed in affected puppies from litter 1. Both puppies were euthanized by the local veterinarian without necropsy. Milder gait abnormalities were observed in the female puppy. The breeder also witnessed 3 episodes of falling associated with spastic extension of the limbs during eating. This female puppy was presented before euthanasia and necropsy at 9 weeks of age. It showed mild to moderate cerebellar ataxia characterized by a wide-based stance and hypermetria. No abnormal nystagmus was present but delayed postural reactions were noticed.

Litter 3. Two of 7 puppies were affected and both puppies were females. Onset of clinical signs was at 4 weeks of age. In both puppies, ataxia and stumbling were noticed and both puppies were euthanized at 6 weeks of age. At this time, they were able to walk with moderate ataxia and hypermetria. They showed a wide-based stance and muscle mass in the hind limbs appeared slightly decreased compared with that of their healthy littermates. These puppies were again in a good physical condition.

Litter 4. In this litter 3 of 8 puppies developed neurological signs at the age of 7 weeks. Ataxia and delayed postural reactions were reported by the local veterinarian. Because no improvement of signs occurred, the puppies were euthanized 1 week later and 1 male and 1 female puppy were referred for necropsy. The sex of the 3rd puppy is unknown.

Litter 5. This litter was produced by the same dam as litter 4, but with a different sire (Fig 1). Three of 10 puppies (2 males and 1 female) suffered from an onset of clinical signs at the age of 6 weeks. The local veterinarian reported cerebellar ataxia and hyperreflexia of hind limbs and euthanized the puppies at 13 weeks of age because of lack of improvement of clinical signs.

Neuroimaging

The CT and MRI scans performed at the age of 8 weeks did not detect obvious abnormalities of the brain.

Table 1. Patient data of 5 Malinois litters with hereditary cerebellar ataxia.

	Litter 1	Litter 2	Litter 3	Litter 4	Litter 5
General					
Sick puppies	2	3	2	3	3
Sex (F/M)	0/2	1/2	2/0	1/1/U	1/2
Litter size	8	7	7	8	10
Clinical details					
Onset (w)	4	4–6	4	7	6
Euthanasia (w)	5	6.5–9	6	8	13
Severity	+++	++ to +++	++	++	++
Histopathology					
Cerebellar vacuoles					
Cerebellar Nc	+++	+++	+++	+++	+++
Granular cell layer	++ to +++	++ to +++	++	++	++ to +++
Foliate white matter	++ to +++	++	++	++	++ to +++
Purkinje cell layer		(+)		(+)	
Scattered vacuoles					
Medulla oblongata		Lateral reticular nucleus	Lateral reticular nucleus	Medulla oblongata	Vestibular nuclei
Spinothalamic tract		Vestibular nuclei	nucleus	Vestibular nuclei	Olivary nucleus
		Caudate nucleus	Olivary nucleus	Caudate nucleus	Caudate nucleus
		Medial longitudinal fasciculus			Spinothalamic tract
Spheroids					
Spinothalamic tract		Medial longitudinal fasciculus	–	–	Spinothalamic tract
Spinal cord vacuoles (white matter)					
	NE	NE	+	+	++
Spinal cord spheroids (white matter)					
	NE	NE	(+)	–	+

U, unknown; F, female; M, male; w, weeks; NE, not evaluated; severity +++, strong ataxia causing immediate stumbling and falling when starting to walk; severity ++, moderate ataxia but ambulatory without immediate falling; histopathology assessed on a 5-point scale: —, no damage; (+), scattered lesions; +, mild lesions; ++, moderate lesions; +++, severe lesions.

Additionally, no obvious lesions were detectable in the MRI scan in 1 pup immediately after euthanasia at 13 weeks of age on dorsal, sagittal, and transverse images.

Pathology

On gross examination, the CNS of all puppies looked normal. Histologically, all animals showed marked to

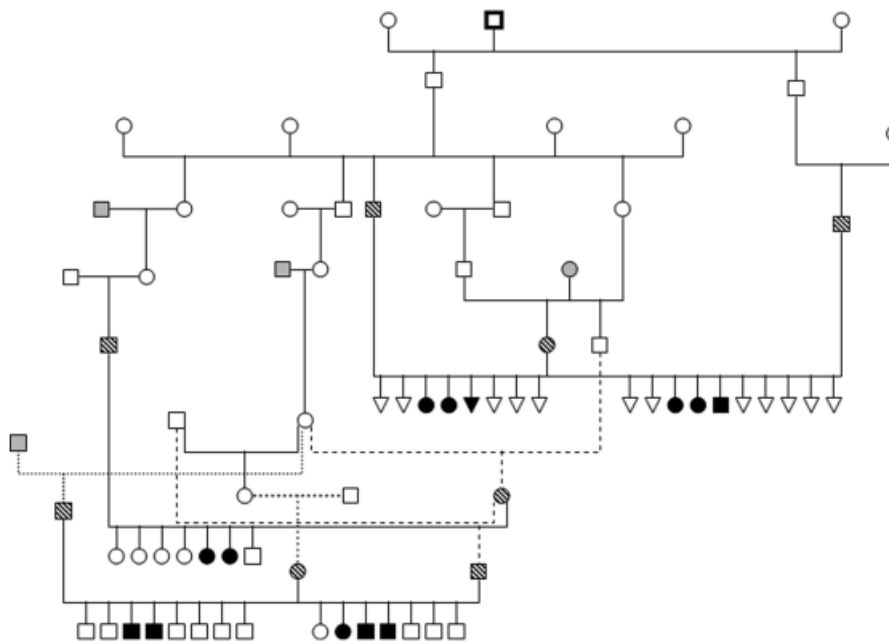


Fig 1. Pedigree map of 13 affected Malinois puppies: ancestors of affected animals were traced back to 1 common sire. Circles: female dogs; squares: male dogs; triangle: unknown gender; bold square: common sire; hatched circles or squares: carrier dogs that produced affected puppies; Solid black circles or squares: affected puppies; gray circles or squares: animals that also have the same common sire in their ancestry, full lineage not shown for easier overview of pedigree map.

severe bilaterally symmetrical vacuolization of the neuropil of the cerebellar nuclei (fastigial, interposital, and lateral nuclei) (Fig 2A, B). Mild to moderate vacuolization also was detected in the cerebellar granular cell layer and white matter of the cerebellar folia (Fig 2C). Scattered vacuoles in white and gray matter were seen throughout the medulla, pons, and midbrain. Scattered spheroids and scant dilated myelin sheaths with myelinophages were detectable in the white matter as well. Neuronal degeneration was evident to a very slight extent in the vestibular nuclei and the red nucleus of 1 puppy. The white matter of the spinal cord showed sporadic mildly dilated myelin sheaths and scattered spheroids, whereas no alterations were detectable in the gray matter (Fig 2D). The cerebral cortex did not have any lesions. A detailed summary of the detected changes in each litter is given in Table 1 by a 5-point scale to assess the severity of the damage. No lesions were seen in nerves and organ samples.

Immunohistochemical analysis for distemper antigen detection was negative in all puppies. GFAP-immunohistochemistry showed no alterations or activation of astrocytes in the region of the cerebellar nuclei and the cerebellar granular cell layer.

Ultrastructural examination was limited by the lack of availability of appropriately fixed tissue. The formalin-fixed tissue of the cerebellar cortex showed small vacuoles in the myelin lamellae and larger spaces probably formed by coalescence of these small vacuoles. The formalin-fixed and paraffin-embedded tissue of the cerebellar nuclei could not be assessed because of artifacts caused by processing of the tissue.

Pedigree Analysis

Affected puppies were produced by 3 different dams and 5 different sires. All parents were phenotypically normal. In all affected litters, 2 or 3 puppies developed neurological signs. Total litter size ranged from 7 to 10 puppies per litter. Male and female puppies were affected (Table 1). Pedigree analysis could trace back all litters to 1 common sire (Fig 1).

The observed overall segregation frequency in the 5 litters was 0.325 (13/40). Testing the segregation frequencies for the 25% null hypothesis, the overall χ^2 analysis ($\chi^2 = 0.833$, $df = 1$, $P = .36$, 95% confidence interval 0.19, 0.49) and the nonpooled χ^2 analysis ($\chi^2 = 2.04$, $df = 5$, $P = .844$) did not reject the null hypothesis and strongly supported a simple autosomal recessive inheritance.

Discussion

In this study, we report that Malinois suffer from a hereditary cerebellar ataxia and describe the clinical and histopathological findings in 13 affected puppies from 5 different litters and phenotypically normal parents. The main features of this disorder are signs of cerebellar disease starting before the age of 2 months and bilateral symmetric spongy degeneration of the cerebellar nuclei with more discrete vacuoles in the granular cell layer and white matter of the folia. These features were accompanied in some but not all puppies by additional vacuoles in brain stem nuclei and the white matter of the brain stem and spinal cord. In the latter locations, scattered spheroids and dilated myelin sheaths with myelinophages also were seen.

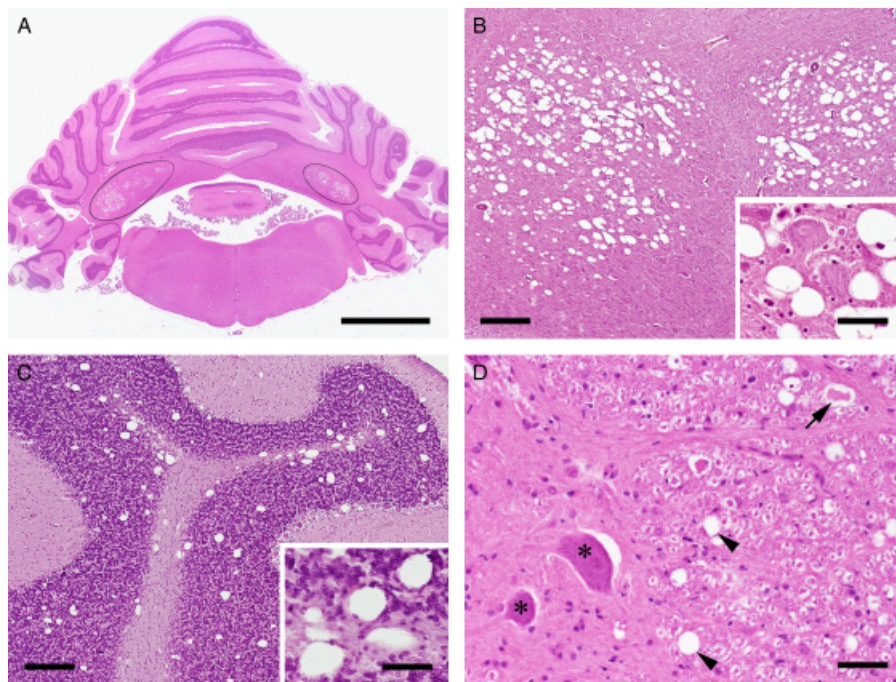


Fig 2. Histopathologic examination of Malinois puppies with cerebellar ataxia. Hematoxylin-eosin stain: (A) spongy appearance of cerebellar nuclei (encircled), bar = 5 mm; (B) large vacuoles in the lateral and interposital nuclei, bar = 80 μ m, insert: detail of (B), normal neurons laying adjacent to vacuoles, bar = 400 μ m; (C) vacuoles in granular cell layer and foliate white matter, bar = 80 μ m, insert: detail of (C), vacuoles in granular cell layer and foliate white matter, bar = 400 μ m; (D) spheroids (arrow) and dilated myelin sheaths (arrowheads) in the white matter of the spinal cord (C7), normal motor neurons (asterisks) in the ventral horn of the gray matter. Bar = 400 μ m.

Cachin and Vandeveld reported 2 Malinois mixbreed puppies with congenital tremor in 1991. These puppies showed some clinical and histopathological similarities to our puppies and might have suffered from the same disease. In the Cachin and Vandeveld¹⁹ report, clinical signs were evident by the age of 3 weeks. On physical examination, the puppies, including 1 littermate without neurologic signs, were described as thin with dull hair coat. In our litters, physical condition and hair coat were normal in all affected puppies, and we suspect that the physical condition in the other report was independent of the neurologic disease itself. The major finding in those 2 sick puppies was a severe, coarse tremor that was accentuated during voluntary movement and excitement, but resolved during rest and sleep. The authors stated that this tremor was more severe than a typical intention tremor of cerebellar disease. Furthermore, wide-based stance, a stilted gait, and hypermetria were described.¹⁹ In one of our litters, a strong intention tremor accentuated by voluntary movement was reported, typical of cerebellar disease. A wide-based stance, stilted gait, and hypermetria also were seen in our puppies with variation in the acuteness and severity of neurological signs. The earliest onset of clinical signs was noticed by breeders by 4 weeks of age and latest onset of clinical signs was reported to be 7 weeks. Some puppies showed an abrupt onset and severe clinical phenotype. They suffered from severe intention tremor and were more or less unable to start walking without immediate stumbling and falling. Puppies with a milder clinical phenotype were ambulatory, but ataxic, hypermetric, and showed a wide-based stance and episodes of falling with spasticity during eating. Time from onset of clinical signs to euthanasia ranged from <1 week to 7 weeks, with the majority of affected puppies surviving <3 weeks and being euthanized at the age of 9 weeks. Puppies lived until the age of 13 weeks in only 1 litter (Table 1). From this retrospective study, it is difficult to state whether clinical signs are progressive or static in the affected puppies. In the Cachin and Vandeveld¹⁹ report, euthanasia was also performed at the age of 9 and 13 weeks and clinical signs did not progress. The histopathological examination of the previous 2 Malinois mix puppies reported a bilateral spongy degeneration of the entire neuraxis, affecting predominately the gray matter.¹⁹ We also observed a bilateral symmetrical spongy degeneration of the neuraxis. However, there were some variations from the previous report. First, we did not detect a spongy state of the cerebral cortex and the spinal cord gray matter in any of our puppies. Second, the cerebellum always was the most severely and consistently affected anatomical brain region in our dogs. Spongy lesions in other parts of the CNS were less consistent and more discrete. And third, we found scattered spheroids in the white matter of brain and spinal cord, whereas Cachin and Vandeveld did not find any axonal lesions. The spheroids most probably originate from fragmentation of the distal part of the axon, because neuronal lesions were not detectable.

Comparing our findings to reports in other breeds, it seems that the familial cerebellar ataxia of Bull Mastiffs

had the most similar features.^{4-6,14,15} Bull Mastiff puppies have a later onset of clinical signs (around 10 weeks of age). The reported ataxia, primarily in the pelvic limbs and mild hypermetria in the thoracic limbs, is similar to the clinical phenotype of the less severely affected Malinois puppies. Bull Mastiffs also suffer from bilateral spongy changes within the cerebellar nuclei and some of them had lesions outside of the cerebellum in the lateral vestibular nuclei as well as in the caudal colliculi.^{14,15} However, Malinois puppies did not have hydrocephalus, bizarre or hysterical behavioral abnormalities, or visual problems. In the Bull Mastiff puppies, changes in the region of the cerebellar nuclei were visible on imaging of the brain at the age of 14 weeks. T2-weighted MRI images demonstrated these spongy changes best. On T2-weighted images, lesions were seen as approximately 3–4 mm paired focal and roughly circular areas of increased signal intensity.¹⁵ In comparison, we could not visualize the vacuolar degeneration on CT and MRI scans performed in 3 Malinois puppies at the age of 8 weeks and in the postmortem scan in 1 pup at 13 weeks. Reasons are most likely limited image resolution and less pronounced spongy changes in physically smaller and younger puppies. Detection of these vacuoles could most likely be improved with a high-field MRI and a thinner slice thickness than used in our study. Furthermore, the optimal imaging technique for detection of this disease likely would be contiguous T2-weighted transverse MR images, perpendicular to the brain stem, combined with exact dorsal images planned on the transverse series. MRI of the brain might help to distinguish this disorder from the classic cerebellar cortical abiotrophies by the absence of cerebellar atrophy.^{20,21}

The pathophysiological mechanisms resulting in the vacuoles in our Malinois puppies remain to be determined. A limitation of our study is that no tissue was originally processed for ultrastructural examination, and results of processed archived material have to be interpreted with caution. However, a metabolic or biochemical disorder with or without mitochondrial involvement seems very likely because in other dog breeds organic acidurias and mitochondrial defects have both been identified as underlying mechanisms of spongy lesions. In West Highland White Terriers or Staffordshire Bull Terriers, an L-2-hydroxyglutaric aciduria has been described and in the Bull Terrier the L-2-hydroxyglutarate dehydrogenase gene mutation has been discovered.^{22,23} Furthermore, a mutation of mitochondrial cytochrome b has been identified in Australian Cattle dogs and Shetland Sheepdogs with inherited spongiform leukoencephalomyelopathy.²⁴ Future studies will be required to identify the cause of this disease in the Malinois and establish whether there are similarities to previously identified canine disorders and whether a comparable hereditary disorder is present in humans. Cachin and Vandeveld have discussed certain similarities with Canavans disease in children, which is an autosomal recessive leukodystrophy caused by a mutation in the aspartoacylase gene.^{19,25} In the Bullmastiff report, a comparison with human Leighs disease (a disorder that belongs in the group of mitochondrial diseases) was drawn.^{15,26} In humans, however, a variety of different

autosomal recessive cerebellar ataxias have been identified.²⁷ New genetic tools in dogs already allow more precise comparisons with some human neurologic disorders and use of canine patients as spontaneous models.²²

Segregation patterns of this study support a simple, highly penetrant, autosomal recessive mode of inheritance. Affected puppies belonged to both sexes and all were produced by unaffected parents. However, because of small patient numbers, results should be considered provisional, and future analysis in larger patient cohorts might still provide evidence that more than a single gene is affected. Pedigree analysis could trace back all litters to 1 sire. In some puppies, this sire was found up to 7 generations back in the pedigree (Fig 1), demonstrating that the suspected genetic mutation has existed for at least 15 years and is maintained by phenotypically normal carrier dogs in the Malinois population. It is assumed that more affected puppies were born in the study time frame but were not presented to our institution. It is further postulated that affected puppies have been born in other countries as well, because all parents in this study were either imported dogs or originated from imported dogs. Thus, it is likely that the suspected defective gene is more widespread than currently known. The early onset of clinical signs might cause breeders and veterinarians to think first about some other congenital or infectious causes, especially if the condition is seen for the first time. Therefore, it is important to educate breeders, breeder clubs, and veterinarians about this disease and to focus on identifying the affected gene. Furthermore, awareness about this disease would be useful in other breeds that carry Malinois ancestors in their pedigree, such as the Belgian Tervueren and Hollandse Herder. For identifying potentially affected genes, collection of blood samples from presumptively healthy dogs, carrier dogs, and affected puppies would be the first required step. A full genome screen with linkage studies would most likely be required to identify the causative mutation.

In conclusion, this study describes an autosomal recessive cerebellar disease in Malinois with an early clinical onset and unfavorable prognosis. Future work is needed to estimate the prevalence of this disease within the Malinois population and to identify the genetic cause of the disease.

Footnotes

^a CDV; kindly provided by C. Örvell, Stockholm, Sweden

^b Dako, Glostrup, Denmark

^c Zeiss EM 900, Zeiss, Oberkochen, Germany

^d CT Pace High Speed, Fa. General Electric, Vienna, Austria

^e 0.23 T, Outlook, Gold Performance, Philips Medical Systems, Vienna, Austria

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