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IMAGING DIAGNOSIS

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Novel MRI and histopathological findings in a young Bullmastiff cross dog with mitochondrial fission encephalopathy

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Abstract

A 9-month-old male Bullmastiff cross dog was presented with a history of progressive proprioceptive ataxia and behavior changes. Neuroanatomical localization was multifocal with forebrain and vestibulo-cerebellum involvement. MRI identified moderate diffuse cerebral sulci widening, dilation of the ventricular system, and rounded, well-defined, bilaterally symmetrical T2W, FLAIR, and T2* hyperintense intra-axial lesions affecting the olivary nuclei. Histopathological examination was indicative of a primary mitochondrial disorder. This was confirmed following genetic analysis which identified mitochondrial fission encephalopathy with a homozygous frameshift variant in the *MFF* gene. This case report documents diagnostic imaging and histopathological findings not previously reported in dogs affected with mitochondrial fission encephalopathy, suggesting a different selective regional vulnerability of the neurons.

KEYWORDS
canine, hypertrophy, neurodegenerative, olivary nuclei, precision medicine

1 | SIGNALMENT, HISTORY, AND CLINICAL FINDINGS

A 9-month-old, 26 kg, male entire Bullmastiff cross dog was presented with a 1-month history of progressive proprioceptive ataxia affecting all four limbs and abnormal behavior. The sire was a Bullmastiff, and the dam was a Bullmastiff/Staffordshire Bull Terrier cross. Both parents were reported to be clinically normal and no information regarding littermates was available. The dog was reported to be the smallest of the litter, slow to learn commands, and had experienced recurrent episodes of vacancy since 9 weeks of age. Biochemistry and complete blood cell count showed no abnormalities, and the patient was not on any medication.

A neurological examination was performed by an ECVN veterinary neurology resident in training (E.S.) under the supervision of an ECVN residency-trained experienced veterinary neurologist (J.M.) On neurological examination the patient had changes in mentation, experiencing multiple vacant episodes in which he was unresponsive to noise and touch stimuli. The dog was ambulatory with left-sided hemiparesis, left-sided vestibular ataxia, and circling in wide circles to the left. Postural reactions were hypermetric in all limbs. Cranial nerve deficits included an absent menace response in the left eye and reduced in the right eye, intermittent horizontal nystagmus (fast phase to the right), and bilateral ventral strabismus. The rest of the neurological and physical examination was unremarkable. The neurological findings were consistent with multifocal neuroanatomical localization affecting the

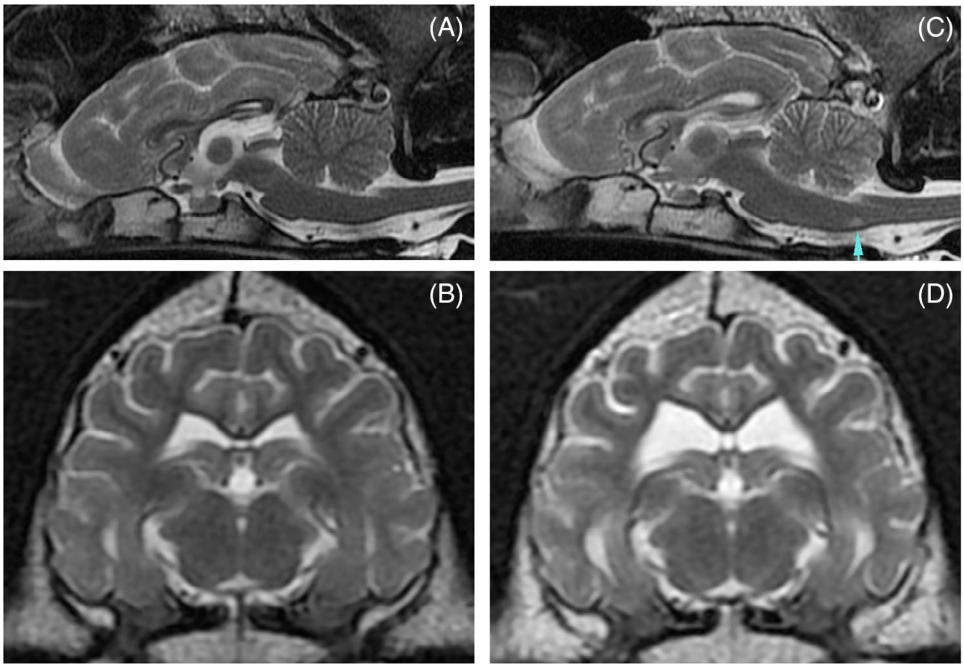

FIGURE 1 Sagittal T2W and transverse T2W images at first MRI (A, B), and same sequences from second MRI scan performed 6 months later (C, D). In all images, there is diffuse widening of cerebral and cerebellar sulci, and bilateral symmetrical enlargement of the ventricular system, more evident in the second scan images. A well-defined, T2W hyperintensity at the level of olivary nuclei can be seen on the sagittal image of the second MRI (C, blue arrow). Subtle hyperintensity of the substantia nigra is considered artefactual in this case, supported by a lack of histopathological changes in this area.

forebrain and vestibulo-cerebellum. Differential diagnoses considering the signalment and clinical findings were congenital malformation, a metabolic or neurodegenerative encephalopathy, or less likely an inflammatory and/or infectious process.

2 | IMAGING, DIAGNOSIS AND OUTCOME

The dog underwent general anesthesia and MRI of the brain was performed using a 1.5-T unit (Signa HDe MRI; HE Healthcare). Slice thickness ranged from 3 to 4 mm with 0.5 and 0.3 mm spacing, respectively. Sequences and imaging planes obtained included T2-weighted (T2W) in all three planes (sagittal; TE 118.48, TR 6100. Transverse; TE 10.7.12, TR 4420. Dorsal; TE 116.71, TR 5360) transverse T1-weighted (T1W) pre and postcontrast (Gadoteric acid, 279.32 mg/mL, Guerbet) (TE 12, TR 460), transverse fluid-attenuated inversion recovery (FLAIR) (TE 130.2, TR 10002) and transverse T2-weighted fast field echo (T2*) (TE 11.8, TR 460). Images were evaluated in digital format by an ECVDI board-certified veterinary radiologist (C.A.) and an ECVDI veterinary radiologist in training (A.O.). Images identified mild to moderate diffuse widening of the cerebral and cerebellar sulci. There was mild bilaterally symmetrical dilation of the ventricular system, most notable in the third ventricle (Figure 1). Two bilateral symmetrical focal pinpoint areas of T2W hyperintensity in comparison to the grey matter were visualized at the position of the olivary nuclei (Figure 2). Otherwise, the MRI study was normal with no abnormal contrast enhancement. A cisternal CSF sample was collected for analysis, which was normal, and tested PCR negative for *Toxoplasma gondii* and *Neospora caninum*. Urine did not show any abnormal organic acid levels. The findings were most consistent with a neurodegenerative or metabolic encephalopathy. The dog was discharged without treatment for monitoring progression at home.

Six months later the dog was re-examined due to progressive neurological signs and weight loss (1.3 kg since previous examination). The neurological examination was repeated by the same clinicians (E.S. and J.M.). The patient was ambulatory with symmetrical tetraparesis and proprioceptive ataxia affecting all limbs. Postural reactions remained hypermetric in all limbs. On cranial nerve examination, the dog had an absent menace response bilaterally, and persistent horizontal nystagmus (fast phase to the left). A repeat MRI of the brain was performed using the same 1.5T unit and identical sequences as previously described. Images were reviewed by the same ECVDI radiologist in training (A.O.) and different board-certified veterinary radiologist (C.A. [ECVDI]). Progression of the diffuse widening of the cerebral and cerebellar sulci was seen as well as increased bilateral symmetrical distension of the lateral ventricles (Figure 1). The focal T2W hyperintense lesions at the level of olivary nuclei had increased in size and appeared now as rounded, well-defined, intra-axial, bilateral symmetrical focal lesions. These lesions remained T2W, FLAIR, and T2* hyperintense compared to the grey matter, hypo to iso-intense on T1W images, with no contrast enhancement (Figure 2). A CT study of the thorax and abdomen was performed using a 16-slice unit (Bright Speed; GE Healthcare) for further investigation of the weight loss. Pre- and postcontrast (Omnipaque, 140 mg I/mL, GE Healthcare) images

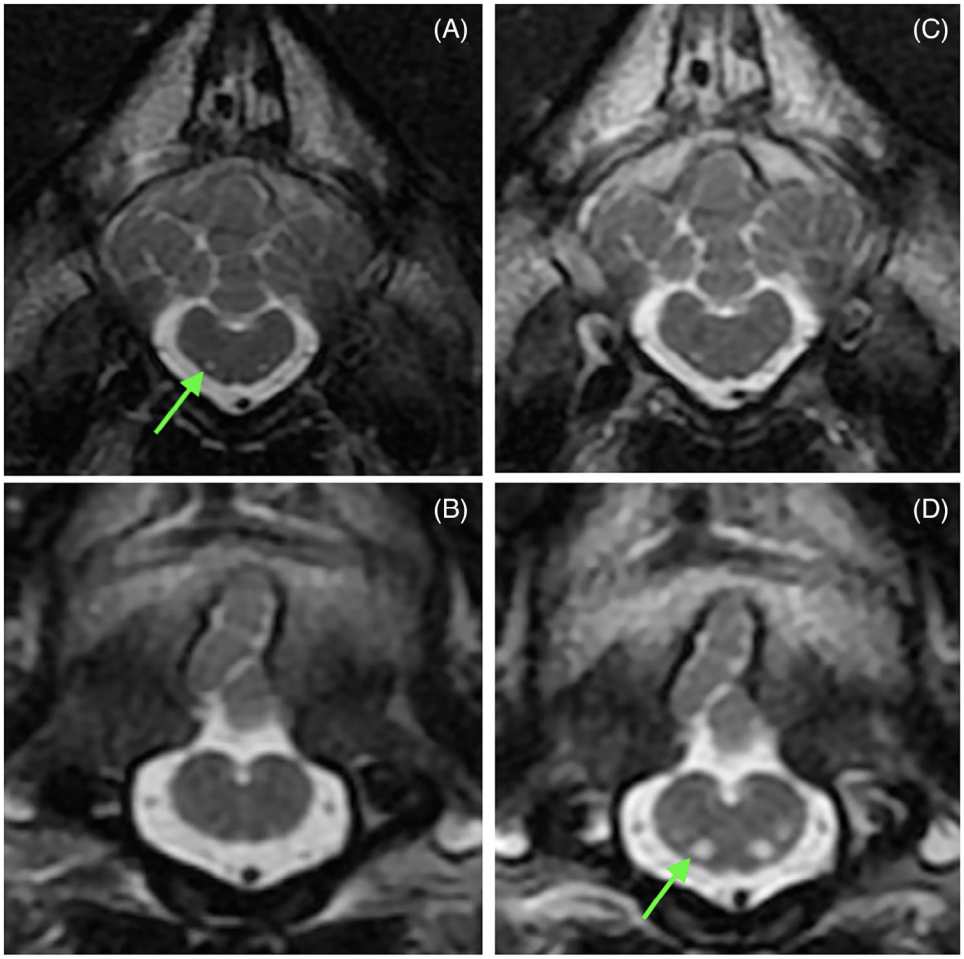

FIGURE 2 Transverse T2W images at the level of the olivary nuclei at first MRI (A, B) and second MRI performed 6 months later (C, D). More evident in the latter MR images there are small, rounded, and well-defined bilaterally symmetrical T2W hyperintense lesions in area of the olivary nuclei (green arrow). In panel D two subtle pinpoint areas of hyperintensity can be seen dorsolateral to the olivary nuclei, which are considered artefactual due to lack of corresponding histopathological changes.

were acquired in helical scan mode with 1.25 mm slices of the thorax (1.25 mm spacing, reconstructed into lung and soft tissue windows) and 2.5 mm slices of the abdomen (1.25 mm spacing, reconstructed into bone and soft tissue windows). The CT study was evaluated by the same radiologists reviewing the second MRI (A.O. and C.A.) and did not reveal any clinically significant abnormalities. Based on suspicion of a progressive metabolic encephalopathy the dog was discharged with nutritional supplementation "Aktivait" (VetPlus) for continued supportive care at home. One week later due to neurological deterioration, the patient was euthanized and the body submitted for necropsy. The necropsy and histopathological review were performed by a veterinary pathology resident in training and board-certified veterinary pathologist (K.B. [ECVP]). Macroscopically the leptomeninges of the myelencephalon appeared diffusely dark red and congested. Histopathologically, the olivary nuclei showed extensive disintegration and vacuolation of the neuropil with partial neuronal preservation (Figure 3). Surviving neurons displayed nuclear swelling and a moderate amount of Nissl substance within the perikaryon. Within the affected areas there was a prominent gliosis composed of reactive astrocytes and gitter cells. The cerebellum had multifocal patchy loss of Purkinje cells with the presence of spheroids within the white matter and torpedoes within the granular tissue with occasional Purkinje cells swollen or shrunken with a hypereosinophilic cytoplasm and pyknotic nucleus. Cerebellar nuclei showed milder degenerative changes than observed in olivary nuclei with mild to moderate gliosis, vacuolation of the neuropil, and multiple small- to medium-sized axonal swellings. Symmetrically, there was a mild to moderate vacuolization of vestibular nuclei associated with gliosis as well as in the central grey substance and cuneate nuclei. Cerebral cortical areas and deeper nuclei did not show overt histopathological changes on H&E-stained sections besides a mild impression of gliosis. These histological findings within the central nervous system were indicative of a mitochondriopathy. Within the heart, histologically, there was also a multifocal adipocyte replacement of cardiomyocytes throughout the right and left ventricles and moderate interstitial fibrosis of the left ventricle consistent with primary cardiomyopathy. Genomic DNA was isolated from formalin-fixed paraffin-embedded brain tissue samples using the Maxwell RSC DNA FFPE kit and a Maxwell RSC instrument (Promega). The DNA was genotyped for

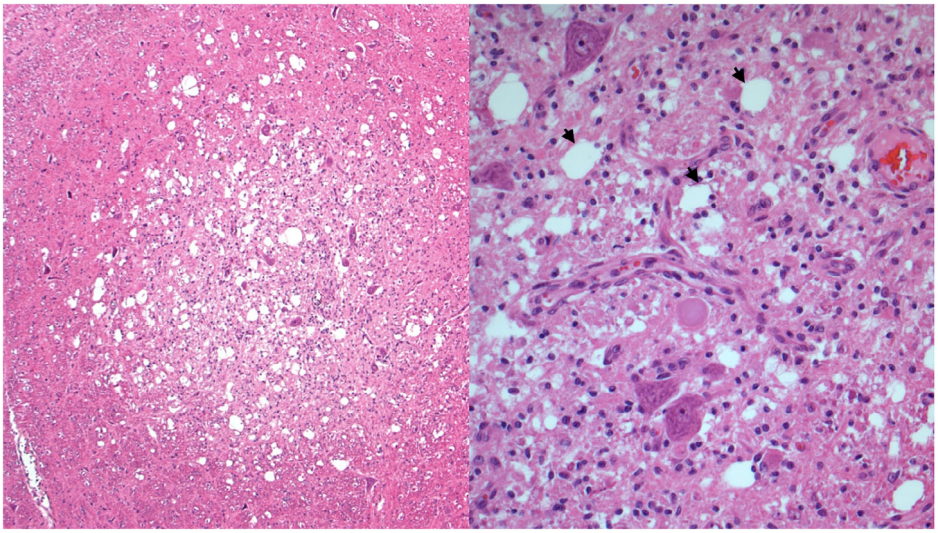

FIGURE 3 Formalin-fixed, paraffin-embedded, five-fold microscopic section of the olivary nuclei (left image) and 20-fold microscopic section of the same section (right image) with hematoxylin and eosin stain. Extensive disintegration and vacuolation of the neuropil (black arrows) with partial neuronal preservation can be seen.

the previously reported XM_0385740000.1:c471_475delinsCGCTCT frameshift variant in the *MFF* gene encoding the mitochondrial fission factor.[1] The dog was homozygous for the mutant allele and the final diagnosis was mitochondrial fission encephalopathy.

3 | DISCUSSION

Mitochondrial fission encephalopathy (MFE) is a newly termed autosomal recessive condition described in the Bullmastiff breed with a proposed causative homozygous frameshift variant in the *MFF* gene.[1] Loss of function in the *MFF* gene results in hyperfused mitochondria. It is proposed that this leads to the accumulation of damaged and abnormal mitochondria within cells.[2] Currently within the veterinary literature, Bullmastiffs found to carry the *MFF* variant in a homozygous state share a similar clinical phenotype characterized by chronic and progressive ataxia, impaired vision, and behavior abnormalities.[1,3,4] MRI of these individuals consistently displays bilateral symmetrical T2W hyperintense and T1W isointense foci localized to the cerebellar nuclei (Figure 4) and widening of the sulci of the cerebral cortex with enlargement of the ventricular system compatible with brain atrophy.[1,3] Microscopically, the hyperintense bilateral symmetrical foci within the cerebellar nuclei correlate with spongy vacuolar changes and gliosis.[1,4]

In the current case, there are several similarities with the Bullmastiffs previously reported regarding neurological examination and clinical history. However, this dog had no visible lesions in the cerebellar nuclei on MRI (Figure 4) and much milder degenerative changes on histopathological examination. Instead, this case showed prominent tissue disintegration and spongy vacuolar changes within the olivary nuclei resulting in a different regional distribution of the lesions previously reported in MFE. Bilateral symmetrical malacia of the olivary nuclei has been reported in dogs with other necrotizing encephalopathies, many of which have been linked to Leigh syndrome and other mitochondrial disorders in humans.[5–7] Humans with genetic variants directly or indirectly affecting the activity of mitochondria, will often show neuropathological changes within the olivary nuclei suggesting an increased vulnerability of the olivary-cerebellum to specific mitochondrial defects.[8–10] It is unclear why our case shows a different MRI pattern from the previously reported Bullmastiffs. Selective vulnerability of diverse neuron populations has been reported in many neurodegenerative diseases and it is hypothesized that cell-type specific properties may contribute to an enhanced susceptibility to mitochondrial defects.[11] In which case it could be theorized that the different genetic backgrounds in this case caused the change in regional selective vulnerability. Alternatively, as mitochondrial fission encephalopathy is a neurodegenerative disorder, it is postulated that the progression of the disease leads to changes within the olivary nuclei and could be part of the natural history of the disease. This is supported by the marked progression of the olivary nuclei lesions observed on the second MRI.

Interestingly, in humans, a condition called hypertrophic olivary degeneration (HOD) has been reported in various diseases including mitochondrial syndromes.[12] HOD is a rare form of multisynaptic and transneuronal degeneration of the inferior olivary nucleus (ION) in which hypertrophy is the result of the injury affecting function of the afferent fibers to the ION as part of the dentato-rubro-olivary pathway, so called Guillain–Mollaret triangle (GMT).[12] Histopathologically, HOD is characterized by vacuolar degeneration and increase in the number of astrocytes, resembling reported changes of the presented dog.[13] Noteworthily, diffusion tensor imaging (DTI) studies of GMT in patients with HOD can show signal changes in all anatomical components of GMT while conventional MRI only identifies lesions in ION.[14] The use of DTI imaging could have been useful in this present case, as it may have demonstrated early changes within the cerebellar nuclei, that were only observed histopathologically. Utilizing quantitative imaging

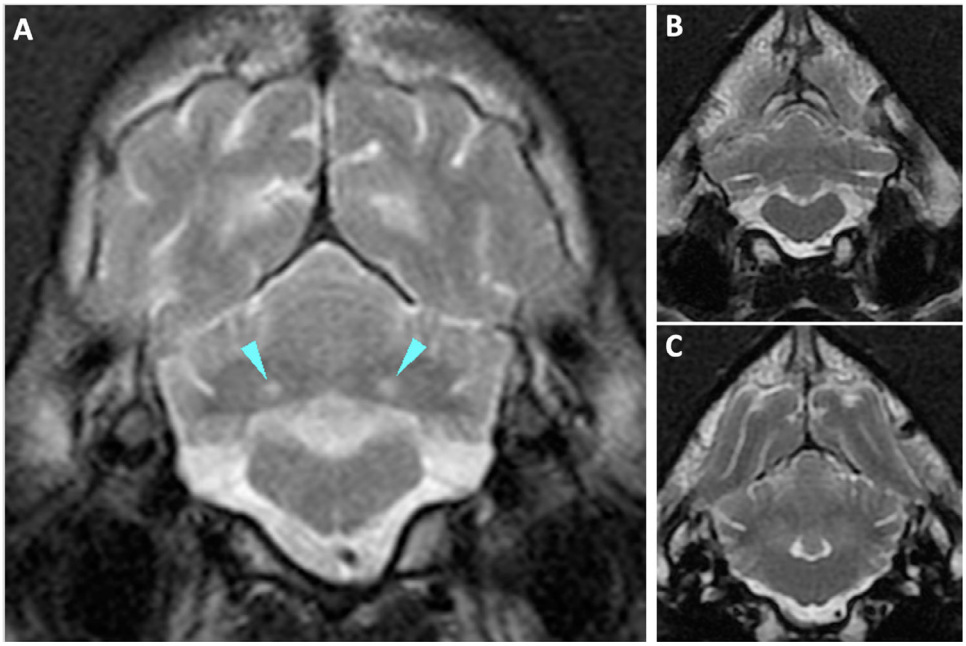

FIGURE 4 Magnetic resonance imaging of a previously reported Bullmastiff with genetically confirmed MFE (A), alongside the present case (B, C). A, T2W transverse image at the level of the cerebellum showing two bilateral symmetrical T2W intra-axial hyperintense lesions at the level of the cerebellar nuclei (arrowheads). B, C, T2W transverse images of the current case at similar levels of the cerebellum showing no lesions affecting the cerebellar nuclei.

studies in this way would be valuable in future research into suspected or confirmed MFE.

The histopathological findings of our case also identified degenerative and dysplastic changes affecting the myocardium suggestive of a primary cardiomyopathy. Similar histopathological findings have been seen in one dog with MFE and it has been reported that *Mff*−/− knockout mice also have severe dilated cardiomyopathy.[1,15] These changes support the use of multimodal imaging to evaluate the thorax and abdomen in patients with MFE as affected dogs could suffer additional cardiac changes.

This case report presents a young Bullmastiff cross dog with MFE resulting in MRI and histopathological findings that have not been previously reported in other Bullmastiffs with the same genetic variant. Olivary nuclei changes represent an additional MRI finding that can support a diagnosis of mitochondrial fission encephalopathy.

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b. Acquisition of data: Suiter, Baiker, Christen, Minguez
c. Analysis and interpretation of data: Suiter, Gutierrez-Quintana, Baiker, Minguez, Kaczmarska, Leeb, Christen, Ororbia, Anselmi

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a. Final Approval of the Completed Article: Suiter, Gutierrez-Quintana, Baiker, Minguez, Kaczmarska, Leeb, Christen, Ororbia, Anselmi

Category 4

a. Agreement to be accountable for all aspects of the work ensuring the questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved: Suiter, Gutierrez-Quintana, Baiker, Minguez, Kaczmarska, Leeb, Christen, Ororbia, Anselmi

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All data relevant to the case report is included within the article. Reports and images are available from the first author upon reasonable request.

PREVIOUS PRESENTATION OR PUBLICATION DISCLOSURE

Nothing to declare.

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