Cerebellar Abiotrophy Characterized by Granular Cell Loss in a Brittany

L. M. TATALICK, S. L. MARKS, AND T. V. BASZLER

Key words: Abiotrophy; Brittany; cerebellum.

Cerebellar abiotrophy or degeneration has been reported in many breeds of dog: Kerry Blue Terrier, Gordon Setter, Rough-Coated Collie, Airedale Terrier, Finnish Harrier, Bernese Mountain Dog, Miniature Poodle, Brittany, Beagle, Samoyed, Clumber Spaniel, Akita, Fox Terrier, Cairn Terrier, Cocker Spaniel, Labrador Retriever, Golden Retriever, Great Dane, and Border Collie. Cerebellar abiotrophy is characterized by signs of progressive cerebellar disease and premature degeneration of cerebellar neurons. In some cases of cerebellar abiotrophy, a genetic basis has been documented. In the majority of cases, the predominant microscopic lesion involves loss or degeneration of Purkinje cells. Variable and less severe lesions occasionally occur in the granular layer, molecular layer or extrapyramidal neurons. This report documents an unusual case in a Brittany of cerebellar abiotrophy characterized by a marked paucity of granular neurons, a decreased width of the molecular and granular layers, and normal numbers of Purkinje cells.

A 3-year-old intact male Brittany was referred to the Washington State University Veterinary Teaching Hospital for ataxia and head tremors that had progressed over the previous 6 months. Physical examination revealed ataxia and an abnormal gait characterized by forelimb hypermetria and bilateral symmetric hopping ("bunny hopping") of the pelvic limbs. Neurologic examination revealed truncal ataxia and intention tremors. Results from a complete blood count and biochemical profile were normal. Based on the progressive

![Fig. 1. Brain; affected Brittany. Notice the small size of the cerebellum. Bar = 1 cm.](#)
nature of cerebellar disease, the owner requested euthanasia and necropsy of the dog.

The necropsy was performed immediately following euthanasia. Gross changes were limited to the brain. The weight of the entire brain, 71.6 g, was within normal limits for a 13.2-kg dog; however, the cerebellum was symmetrically smaller than normal and represented only 5.8% of the total brain weight (normal = 10%) (Fig. 1). All tissues were fixed promptly in 10% neutral buffered formalin. Sections of nervous tissue were trimmed as follows. Six sequential sections of cerebrum were taken from the frontal lobe to the occipital lobe. Three sections of the brain stem...
at the level of the caudal colliculi, the middle cerebellar peduncle, and the obex were evaluated. The cerebellum was sectioned longitudinally through the caudal vermis, uvula, nodulus, lingula, and rostral vermis and transversely through both the right and left cerebellar hemispheres. Two sections of cervical spinal cord, two sections of thoracic cord, and one section of lumbar cord were also trimmed. All tissue sections were dehydrated in graded ethanol, cleared with xylene, and embedded in paraffin. Three-micrometer sections were cut and stained with hematoxylin and eosin. Selected cerebellar sections were stained with Luxol fast blue and periodic acid-Schiff or Bielchowsky stains to evaluate myelination and to evaluate the morphologic appearance of axons. In other cerebellar sections, astrocytes were identified by an indirect avidin biotin peroxidase complex method using rabbit anti-anti-glial fibrillary acid protein (GFAP) antisera (Dako Corp, #Z334) and Mayer's hematoxylin counterstain. Similarly processed and stained sections of brain from an age-matched female Brittany were used as a control.

Histologically, the cerebellar folia were uniformly smaller than normal. There was a marked thinning of both the granular layer and the molecular layer with a marked paucity of granule cells (Figs. 2, 3). The Purkinje cells had normal morphology and distribution (Figs. 4, 5). There was a marked increase in astrocytic fibers in the granular layer as revealed by GFAP immunohistochemical evaluation (Figs. 6, 7). Although some increase in astrocyte fibers may have resulted from condensation of the granular layer, the number of astrocytic fibers is far greater than the total number of astrocytes in the granular layer of the control dog. Astrogliosis is also supported by the presence of occasional gemistocytes and binucleate astrocytes. Rare cell bodies in the cerebellar nuclei and cerebellar peduncles were angular, with deeply cosinophilic granular cytoplasm and nuclear pyknosis. The brain stem (including the caudal olivary nucleus, vestibular nuclei, substantia nigra, and reticular formation), the cerebrum (including the caudate nucleus), and the spinal cord were otherwise normal.

Morphometrically, the mean widths of the granular layer and the molecular layer were calculated by randomly measuring 50 independent widths for each layer. Purkinje cell numbers were evaluated by 50 independent, random counts of the number of Purkinje cells present in a 400-μm length of cerebellar folia. The mean width of the molecular layer (188.6 μm) and the granular cell layer (131.2 μm) in the affected dog were significantly different from the unaffected control values (480.8 μm and 296.0 μm, respectively), based on a two-sample t-test with P < 0.05. The ratio of the mean width of the molecular layer to the mean width of the granular layer was comparable in the affected dog (1.4375) and the control dog (1.624). There was no significant difference in the mean number of Purkinje cells between the affected dog (mean = 19.68, standard error = 1.053) and the control (mean = 21.2, standard error = 1.195), as determined by a two-sample t-test with P = 0.3421.

A hereditary basis for cerebellar atrophy has been well documented in Gordon Setters3,5,11 and Kerry Blue Terriers.2,10 Unfortunately, the parents and siblings of this Brittany were not available for clinical evaluation or genetic analysis, and a hereditary cause of this case of atrophy cannot be documented.

This case report describes an unusual form of cerebellar atrophy in a Brittany characterized by marked and diffuse depletion of the granular and molecular layers of the cerebellum with normal morphology and numbers of Purkinje cells. Histologically, these findings are different from cerebellar degenerations previously reported in the Brittany and other dog breeds. These degenerations are characterized predominantly by regional Purkinje cell loss with or without concurrent loss of granule neurons.1,2,5,7-11 Cerebellar degeneration has been described in the Rough-Coated Collie in which granule neuron loss was the predominant early lesion, with subsequent Purkinje cell loss as the disease progressed.7 However, there were individual Purkinje cell degenerative changes and subjective loss of cells even early in the disease process, and there was loss of neurons in the cerebellar roof nuclei, lateral vestibular nucleus, and inferior olivary nucleus in all but the earliest stages.2 The cerebellar changes observed in the present case differ from those of previous cases because there were no Purkinje cell degenerative changes or quantitative Purkinje cell loss 6 months past the onset of clinical disease. The period of clinical signs in this dog (6 months) probably was sufficient to allow for prominent Purkinje cell loss if Purkinje cell loss occurs secondary to granule neuron degeneration and loss. One hypothesis is that the marked loss of neurons in the granular layer is due to an innate metabolic error of granule cells and that concomitant decreased thickness of the molecular layer is expected based upon the decreased excitatory input from granule cell parallel fibers.2,4 Likewise, minimal changes would be expected in Purkinje cells because their major excitatory input comes from brain stem nuclei, with only minor excitation evoked from granule neuron synapses.6,8

References

8 Hsu SM, Raine L, Fanger H: Use of avidin-biotin peroxidase complex (ABC) in immunoperoxidase tech-

Downloaded from vet.sagepub.com by guest on September 27, 2016
Hypomyelination in the central nervous system has been described in calves, piglets, lambs, puppies, hamsters, rats, and mice. Strictly speaking, the term hypomyelination signifies defective myelin sheath formation, but the myelin present is of normal biochemical composition. In affected areas, axons are either thinly myelinated or nonmyelinated. The term dysmyelination signifies a similar situation, except that the myelin present is biochemically abnormal. In this study, hypomyelination is used to include both conditions because the critical morphologic expression of either is an inadequate quantity of myelin. Clinically, many animals with hypomyelination present in the neonatal period with whole body tremors, ataxia, and occasional seizures; hence, the common clinical designation of the syndrome as congenital tremors.1,2

Case records and a limited supply of paraffin-embedded tissues and electron micrograph negatives for this study were retrieved from the archives of the Anatomy Department of the New York State College of Veterinary Medicine (NYSCVM) at Cornell University (circa 1983). A litter of three Siamese kittens was apparently normal at birth. One kitten died of unknown causes at 2 days of age (no necropsy examination was performed). The two surviving kittens (one female and one of undetermined sex) were clinically normal until 4 weeks of age, when they developed progressively intense whole body (action) tremors accompanied by episodes of frenzied behavior with indiscriminant biting. Two previous litters of five kittens from the same queen and tom were normal. The two affected kittens were noted to the veterinary neurology service of the NYSCVM. Neurologic examination of both kittens revealed a quiet (normal) state at rest, but with activity, clinical signs as described above became evident. Differential diagnoses of storage disease or hypomyelination were considered.

The Siamese kittens (Nos. 1, 2) were euthanatized with intravenous barbiturate (Socumb®, Anthony Products, Arcadia, CA) at 6 weeks of age; a 6-week-old healthy female domestic shorthair kitten (No. 3) served as a control. Tissues collected at necropsy included brain, spinal cord, heart, lung, liver, kidney, adrenal gland, spinal (peripheral) nerve rootlets and attached ganglia, spleen, skin, and gastrointestinal tract samples. Samples of these tissue specimens were fixed promptly in 10% neutral buffered formalin and embedded in paraffin. For the present study, six paraffin-embedded blocks of CNS tissues were retrieved for each kitten (no formalin-fixed tissues were still available). These blocks contained transverse sections of telencephalon (frontal, parietal, temporal, and occipital cerebral cortex and basal ganglia), diencephalon (thalamus and hypothalamus), mesencephalon (midbrain at the levels of the rostral and caudal colliculi), dorsal metencephalon (lateral cerebellar hemispheres and vermis), ventral metencephalon (at the level of the fibers of cranial nerves VII and VIII and the middle cerebellar peduncles), myelencephalon (medulla just caudal to the obex), and a few transverse and sagittal sections of cervical, thoracic, and caudal lumbar spinal cord. These blocks were sectioned at 4 μm and stained with hematoxylin and eosin (HE), Holmes' silver, and luxol fast blue-cresyl violet (LFB) stains.

Sequential sections were also stained using an indirect immunoperoxidase streptavidin biotin technique (Zymed Streptavidin–Biotin System®, Zymed Laboratories, San Francisco, CA). The method of Wasserman et al.1 was used with the following primary antibodies: glial fibrillary acidic protein (GFAP, Biomedica Co., Foster City, CA), myelin basic protein (MBP, a gift from Dr. C. del Cerro, Rochester, NY), myelin-associated glycoprotein (MAG, stained with anti-leu 7, Becton Dickinson Co., Mountain View, CA), and proteolipid protein (PLP, a gift from Drs. J.-M. Matthieu, Switzerland, and T. V. van Wachendt, Germany). The GFAP stain was performed with rabbit anti-GFAP at a 1:320 dilution, the MBP technique with rabbit anti-MBP at a 1:516 dilution, the MAG stain with mouse anti-leu 7 at a 1:100 dilution, and the PLP technique with rabbit anti-PLP at a 1:500 dilution. No trypsinization of tissues was required, and appropriate serum controls were run.