Cerebellar Cortical Degeneration in Adult American Staffordshire Terriers

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Adult-onset cerebellar cortical degeneration recently has been reported in American Staffordshire Terriers. We describe the clinical and histopathologic features of this disease and examine its mode of inheritance in 63 affected dogs. The age at which neurologic deficits first were recognized varied from 18 months to 9 years, with the majority of dogs presented to veterinarians between 4 and 6 years of age. Time from onset of clinical signs to euthanasia varied from 6 months to 6.5 years, with the majority of affected dogs surviving from 2 to 4 years. Initial neurologic findings included stumbling, truncal sway, and ataxia exacerbated by lifting the head up and negotiating stairs. Signs progressed to obvious ataxia characterized by dysmetria, nystagmus, coarse intention tremor, variable loss of menace reaction, marked truncal sway, and falling with transient opisthotonus. With continued progression, dogs became unable to walk without falling repeatedly. Cerebellar atrophy was visible on magnetic resonance images and on gross pathology. Histopathologic findings included marked loss of Purkinje neurons with thinning of the molecular and granular layers and increased cellularity of the cerebellar nuclei. The closest common ancestor of the dogs was born in the 1950s and inheritance was most consistent with an autosomal recessive mode of transmission with a prevalence estimated at 1 in 400 dogs. This inherited disease is comparable to the group of diseases known as spinocerebellar ataxias in humans. Many spinocerebellar ataxias in humans are caused by nucleotide repeats, and this genetic aberration merits investigation as a potential cause of the disease in American Staffordshire Terriers.

Key words: Cerebellar abiotrophy; Dog; Hereditary ataxia; Purkinje neuron.

Materials and Methods

Affected dogs underwent complete physical and neurologic examinations. With owner consent, a CBC, serum biochemistry, urinalysis, serum vitamin E and lactate concentrations, and thyroid testing (including free and total thyroxine and triiodothyroxine concentrations and thyroid-stimulating hormone concentration), were performed. The vitamin E concentrations and thyroid tests were performed by the Endocrinology section of the Michigan State University Diagnostic Center for Population and Animal Health. Dogs that received a full diagnostic evaluation were anesthetized (anesthesia protocol varied according to the veterinarian) and brain imaging was performed by using either magnetic resonance (MR) imaging or computed tomographic (CT) scanning. Cerebrospinal fluid (CSF) was sampled from the cerebello-medullary cistern and routine analysis was performed. When possible, the dog’s progress was followed every 3–6 months by repeat examination or by telephone communication with the owner.

In dogs that were euthanized, the brain was removed and the whole brain and cerebellum were weighed. The brains then were placed in 10% buffered formalin and after fixation were sectioned and embedded in paraffin. The brains from 3 control dogs, 1 American Staffordshire Terrier (13 years old), 1 small mixed-breed dog (14 years old), and 1 Beagle (4 years old), were removed and processed in the same way for comparison.

Six-micrometer-thick sections were cut and stained with hematoxylin and eosin, Luxol fast blue to evaluate myelin, and Bielschowsky silver stain to evaluate axons. Sections also were stained immunohistochemically with glial fibrillary acidic protein. To do this, the sections were dewaxed by passing them through xylene and sequentially decreasing concentrations of alcohol. Endogenous peroxide activity was quenched by incubation in 3% hydrogen peroxide for 10 minutes. A protein block then was performed with 10% goat serum for 30 minutes, after which polyclonal rabbit anti-goat glial fibrillary acidic protein antibody was applied at a concentration of 1:300 for 30 minutes at room temperature. After washing, the secondary antibody, biotinylated goat anti-rabbit antibody, was applied for 20 minutes at room temperature. After another wash, peroxidase-labeled streptavidin was applied for 20 minutes and then the product was visualized after a final wash with 3,3’-diaminobenzidine tetrahydrochloride. Dogs that were euthanized at university veterinary schools underwent complete postmortem examination, but brains of dogs that were euthanized elsewhere were removed, placed in 10% buffered formalin, and shipped to North Carolina State University (NCSU); the rest of the body was...
not subjected to postmortem examination. Counts of Purkinje neurons were performed by randomly selecting a field using the 10× objective and aligning it so that the junction between the molecular and granular layers ran across the full diameter of the field of vision. The number of Purkinje neurons was counted in 10 such fields in affected and control dogs. Fields from both cerebellar hemispheres and the vermis were included. The number of neurons counted was compared between the affected and control dogs by using Student’s t-test; P values <.05 were considered significant. In 1 dog, a section of the cerebellum was fixed in 4% glutaraldehyde and processed for electron microscopy. After fixation, 1-mm-thick pieces of tissue were processed for transmission electron microscopy by immersion in 1% osmium tetroxide for 1 hour at room temperature. After washing, the samples were dehydrated through ethanol culminating in 2 changes in 100% acetone. Tissues then were placed in a mixture of Spurr resin and acetone for 30 minutes and then immersed in 100% resin for 2 hours with 2 changes in resin. Finally, they were embedded in fresh resin in molds and polymerized at 70°C for 12 hours. Semithin sections were cut with glass knives and stained with 1% toluidine blue-O in 1% sodium borate. Areas of interest were identified on the semithin sections and trimmed for ultrathin sectioning with a diamond knife. Ultrathin sections were stained with methanolic uranyl acetate followed by lead citrate and examined with a transmission electron microscope.

Pedigrees of affected dogs were obtained if available, and the owners of siblings of affected dogs were contacted to determine the number of affected dogs per litter. The age of recognition of signs was compared between offspring and parents to determine whether any evidence of clinical anticipation was noted. The number of American Staffordshire Terriers registered with the American Kennel Club (AKC) from 1990 to 1998 was compared with the number of known cases of disease in the same geographic area over the same time period. The hypothesis of a major genetic determinant and its likely mode of inheritance were examined by using a logistic regressive model as described by Bonney.21 A general class D regressive model was used that estimated frequency of the deleterious gene, genotype-specific baseline parameters, and transmission probabilities. This logistic model was used to define the relationship between phenotype (affected status) and risk factors (covariates) such as genotype. Maximum-likelihood methods were used to compare the different logistic models. This model did not correct for ascertainment bias, it assumed the presence of Hardy-Weinberg equilibrium. The basics of this model have been implemented in the Segreg program of the Statistical Analysis of Genetic Etiology 4.3 package. To assess the nature of familial aggregation of affected status, segregation analysis was conducted. Parameter estimates corresponding to the maximum likelihood for each of the major modes of Mendelian inheritance (i.e., recessive, dominant, and codominant) were calculated by using the Segreg program. Values of −2 log-likelihood for each comparison were examined by using the chi-square (X^2) test. The most parsimonious model of inheritance was identified as having the lowest Akaike’s information criterion (AIC) defined as AIC = −2 ln(L) + 2 (number of independently adjusted parameters) where L = likelihood.22

Results

Sixty-three affected dogs were identified, 48 of which were evaluated by a board-certified neurologist or internist. The dogs came from all geographic areas of the United States, France, the Netherlands, Sweden, Romania, and Portugal. Of these 48 dogs, 5 were evaluated on repeated occasions over a period of up to 4 years. Fifteen dogs were not evaluated by a board-certified neurologist or internist. Eight of these dogs were evaluated by their regular veterinarian and details of the findings and videotaped neurologic examinations were evaluated by 1 of the authors (NO). Of these 8 dogs, diagnosis was confirmed at postmortem examination in 2 dogs, other conditions were ruled out by using MR imaging and CSF analysis in 1 dog, 2 were littermates of dogs with disease confirmed by postmortem examination, and 1 dog had the same parents as another dog with disease confirmed by postmortem examination. One dog had an affected sire, and the remaining dog has been followed for more than 18 months and has known carriers of the disease in both maternal and paternal sides of the pedigree. The 7 remaining dogs reported as affected by their owners (but not evaluated by a neurologist and with no videotape available) were directly related (siblings or parents) to dogs with confirmed disease. These dogs were not included in the pedigree analysis.

Diagnosis was confirmed by postmortem examination in 10 dogs, and an additional 24 dogs had cerebellar cortical atrophy on MR images (20 dogs) or other causes of their signs ruled out by CT scans (4 dogs) and CSF analysis (see below). Twenty-five close relatives (parents, offspring, and siblings) of the 34 dogs with confirmed diagnoses did not undergo a full evaluation and owners of 4 additional dogs declined further evaluation. These 4 dogs all had confirmed affected dogs in their pedigrees and had clinical signs and progression consistent with the disease. Because a definitive diagnosis was not reached in these 4 dogs, they were not included in the pedigree analysis.

Results of general physical examination were normal in all but 3 dogs, all of which had heart murmurs. One of these dogs had a cardiac defect with left-to-right shunting of blood and associated polycythemia, 1 had mild subaortic stenosis, and the remaining dog had mild tricuspid regurgitation and a ruptured right cranial cruciate ligament. Results of fundic examination were normal in all dogs. Owners 1st recognized neurologic signs in their dogs between 18 months and 9 years of age, but the majority of the dogs started to have problems that prompted concern from the owners between 4 and 6 years of age. Several owners commented that, in retrospect, their dogs had always seemed clumsy, but they felt that they were within normal limits at the time and it is unclear whether these dogs were showing neurologic deficits at that time. The neurologic signs progressed to the point where dogs were unable to walk over periods ranging from 6 months (1 dog) to 6.5 years, with the majority progressing over 2 to 4 years. Each of the 6 dogs that had a short course of signs (< 2 years) was descended from 3 or more generations of affected dogs with a longer clinical course. Many owners reported that clinical signs progressed in bursts with long intervening periods during which signs appeared to be static. The 1st signs noticed included stumbling, especially when negotiating steps, turning corners, walking uphill or downhill, and jumping up onto objects. Raising the head was commonly noted to elicit a body sway or stagger. Three owners reported that at this stage in the disease their dogs occasionally would develop opisthotonus causing recumbency for a brief period (a few seconds) or whole body jerks, and stiffening while asleep. Three owners reported that their dogs became unable to swim and 1 dog died from drowning.

As signs progressed the dogs developed ataxia characterized by hypermetria. In some dogs this was most marked
in the thoracic limbs, but in others the pelvic limbs were more severely affected. At rest they developed a wide-based stance and a pronounced truncal sway, and frequently would stand with 1 or more legs in an unusual position relative to their center of gravity. The dogs found it progressively more difficult to initiate movements (obvious when performing postural reactions), would stagger whenever a sudden movement had to be made, and would fall when shaking the head. Dogs did not develop a fine intention tremor, but sudden movements and excitement did result in overcompensation causing a degree of coarse intention tremor. Vertical, rotary, and horizontal nystagmus could be elicited in most dogs by rolling them onto their backs, and some owners noted that their dogs frequently had spontaneous nystagmus. Conscious proprioception was normal in all dogs, and motor strength and spinal reflexes were normal. Mentation and behavior remained essentially normal, but 2 owners reported that as their dogs became less confident, they were more likely to attack other dogs in the household if provoked. The menace reaction was variably present. Apart from intermittent head tilt that developed with sudden movements of the head, results of examination of the remaining cranial nerves were unremarkable. In all dogs, excitement, changes in terrain, and lifting the head worsened neurologic signs. A dog could appear normal when walked on a leash in a straight line, but became clearly ataxic when walked up a steep flight of steps or offered a treat held high above its head. All owners reported that the signs were much less obvious when the dog was at the veterinarian and apparently moving more carefully. As signs worsened, sudden movements could cause recumbency with opisthotonus.

Results of routine laboratory evaluation were unremarkable in all dogs tested except for the dog with polycythemia. MR imaging disclosed diffuse atrophy of the cerebellum in all 20 dogs imaged (Fig 1). The CT scans of 4 other dogs (Fig 2) and granular layer depletion also was observed in the cerebellar nuclei appeared more cellular than those of the controls, but no abnormal or reactive cells were observed. This finding was felt to reflect a loss of neurontal. Other areas of the brain and spinal cord and the rest of the body were histologically normal. Electron microscopy did not add any additional information on the cause of the Purkinje neuron necrosis.

Pedigrees were obtained for 52 dogs and these dogs had a closest common ancestor born in the 1950s. Figure 6 illustrates...
Fig 2. Appearance of the brain of (left) a control dog and (right) an affected American Staffordshire Terrier. The entire cerebellum is reduced in size in the affected dog. The cerebellum was 12% of the total weight of the brain in the dog on the left, and 7% in the dog on the right.

Fig 3. A transverse section of the cerebellum from an affected American Staffordshire Terrier stained with (left) hematoxylin and eosin and (right) Bielschowsky stain. No Purkinje neurons are present in the section on the left. The Bielschowsky stain highlights the baskets of processes that normally envelope Purkinje neurons, but in this dog, as a result of necrosis of Purkinje neurons, the baskets of processes are empty. The bar represents 300 μm.

a typical pedigree. Dogs of both sexes were affected, with a ratio of 1 female to 1.3 males. At least 2 littermates were affected in 4 litters, 3 littermates were affected in 3 litters, and 7 littermates were affected in 1 litter of 10 dogs. Both a parent and offspring were affected in 6 instances. Age of recognition of signs was compared in these parent-offspring pairs. The mean age of the offspring at 1st recognition of signs was 5.8 years, as compared to 7.4 years in the parents. In every instance, signs were recognized earlier in the offspring than in the parents and the mean age difference was 1.57 years. A total of 11,740 American Staffordshire Terriers were registered with the AKC from 1990 to 1998 and 32 affected dogs registered with the AKC were born in the same period. Therefore, the prevalence of the disease in the registered population of American Staffordshire Terriers was estimated at 1 in 400 dogs.

The pedigrees of 37 dogs in which full information on the status of affected dogs’ littermates was available were used in the statistical analysis of the mode of inheritance. After analysis of the pedigrees, the hypothesis of no major genetic determinant was rejected ($X^2 = 20.6, \text{ df } = 1, \text{ P } < .05$). The most parsimonious model of inheritance was a Mendelian recessive model with an estimated frequency of the deleterious allele within the collection of pedigrees of 39%. This recessive mode of inheritance is sufficient to explain the familial clustering of the phenotypes in the studied pedigrees.
Fig 4. Transverse sections from (A and C) an unaffected and (B and D) an affected American Staffordshire Terrier stained with (A and B) hematoxylin and eosin and (C and D) Bielschowsky stain. In (A), the Purkinje neurons lie between the molecular and granular layers and have long processes that extend into the overlying molecular layer. In (B), remnants of 2 Purkinje neurons are visible. The Bielschowsky stain reveals the usual appearance of the axons that terminate on the Purkinje neurons (C). In the affected dog, these axons appear to be truncated where they surround the surviving Purkinje neurons (D). The bar represents 150 μm.

Discussion

The recent recognition of inherited adult-onset cerebellar cortical degeneration in the American Staffordshire Terrier is of concern because it is an incapacitating disease for which no cure is known, and currently no definitive antemortem test is available beyond demonstrating cerebellar atrophy on MR images. The lack of findings on CT images most likely reflects the fact that CT does not provide good detail of structures in the caudal fossa and is not sensitive enough to detect cerebellar atrophy. This shortcoming is important because CT scanning is both more economical and more available than MR imaging but will not provide structural evidence of this degenerative disease and can only be used to rule out other cerebellar diseases such as neoplasia. However, in dogs with compatible signalment, history, and clinical signs, and with normal CT findings and noninflammatory CSF analysis, it is reasonable to make a presumptive diagnosis of cerebellar cortical degeneration.

The late onset of signs results in affected dogs being bred before they develop ataxia, potentially causing wide dissemination of the disease within the breed. It is likely that the mutation responsible for the disease already is widely dispersed within the population because the problem has been recognized in dogs from all geographic areas of America and in Europe, and affected dogs have a closest common ancestor dating back to the 1950s. The disease is comparable to the late-onset cerebellar degeneration described in Old English Sheepdogs and Gordon Setters with respect to clinical signs and progression. However, the extent and distribution of Purkinje neuron loss was much greater in American Staffordshire Terriers than in Old English Sheepdogs, and the disease appears to be more widespread within American Staffordshire Terriers than in the other 2 breeds.

Adult-onset spinocerebellar ataxias (SCA) or hereditary ataxias are a well-recognized group of devastating neurodegenerative diseases in humans with an estimated prevalence of 1 in 100,000 in certain populations. In this clinically and pathologically heterogeneous group of diseases, progressive loss of neuronal populations occurs within the cerebellum, specifically the Purkinje neurons and the granular cell layer. Loss of neuronal populations from other areas of the central nervous system also may occur, depending on the particular disease. This neuronal degeneration results in the insidious development of ataxia and a number of other neurologic signs (depending on the disease) that progress at varying rates until the patient is incapacitated. Our evaluation of the clinical and neuropathologic characteristics of affected American Staffordshire Terriers suggests that they bear a close resemblance to this group of neurodegenerative diseases in humans. Histopathologically, neuronal death was restricted to the cerebellar cortex in American Staffordshire Terriers, although in 1 dog the MR images were suggestive of some cerebral atrophy. Certainly, cognitive deficits can occur in humans with SCAs and
it is possible that neuronal loss occurs more diffusely than we have described.

Human SCAs can be inherited in an autosomal dominant or recessive fashion. Autosomal dominant cerebellar ataxias (ADCAs) are more common than their recessive counterparts, and are classified into 21 different types according to the gene or chromosomal locus associated with the disease. The traits common to ADCAs include adult onset of signs, clinical anticipation (increasing severity and decreasing age of onset with consecutive generations), and cerebellar atrophy on MR imaging. Nine of these disorders share an underlying genetic mutation mechanism of unstable expansions of trinucleotide (CAG or CTG) or pentanucleotide (AATTCT) repeats in untranslated or coding regions of the responsible genes. The gradual accumulation of these repeats in successive generations is believed to cause the phenomenon of clinical anticipation because the age of onset is inversely correlated to the length of the repeat. Despite advances in describing the genetic anomalies causing this group of diseases, it is estimated that 35–58% of cases remain genetically unclassified, depending on the population being evaluated.

Autosomal recessive cerebellar ataxias (ARCAs) are rare disorders in humans and their molecular characteristics

Fig 5. Low-power transverse sections stained with hematoxylin and eosin of (left) an unaffected dog and (right) an affected American Staffordshire Terrier. Thinning of the molecular and granular layers is present in the affected dog. Both images were taken at the same magnification. The bar represents 1 mm.

Fig 6. A representative pedigree from 1 of the families of affected dogs. Circles represent female dogs and squares represent males, open symbols are known unaffected dogs, solid black symbols are known affected dogs, and gray symbols are dogs of unknown status.
have not been delineated to the same extent as those of the ADCAs.\textsuperscript{24} The most common ARCA is Friedreich's ataxia, which also is caused by a trinucleotide expansion (GAA).\textsuperscript{23} Other forms are much more rare and examples include spinocerebellar degeneration caused by primary vitamin E deficiency\textsuperscript{22} and abetalipoproteinemia.\textsuperscript{24} The identity and function of the mutated proteins is unknown in many forms of ADCA and ARCA with the notable exception of SCA6, SCA3, Friedreich's ataxia, and a recently described ADCA associated with a mutation in the fibroblast growth factor 14 gene.\textsuperscript{33} In SCA6, the trinucleotide repeat occurs in the region of the gene that encodes the α\textsubscript{1A} subunit of the neuronal P/Q calcium channel.\textsuperscript{29} In SCA3, a protein called ataxin 3 aggregates in neuronal nuclei, but its role is unclear.\textsuperscript{24} The protein implicated in Friedreich's ataxia is called frataxin.\textsuperscript{24} It is a nuclear-encoded mitochondrial protein thought to play a role in iron homeostasis.

Examination of our results suggests that cerebellar cortical degeneration in American Staffordshire Terriers is inherited as an autosomal recessive trait, but test breedings are needed to confirm this finding. Several assumptions were made in the complex segregation analysis: genetic homogeneity among the pedigrees was assumed, ascertainment bias was not accounted for, correlation between dams and sires was not corrected, a 1-locus disease model was used, and Hardy-Weinberg equilibrium was assumed. The hypothesis of genetic homogeneity can be formally tested, but the pedigrees used in this analysis were not large enough to obtain significance when using these tests. However, given the degree of inbreeding in these pedigrees, homogeneity is likely. A variety of ascertainment corrections is possible to allow for bias in the collection of the pedigrees. The application of pedigree analysis did not alter the segregation of risk factors implies that mate selection within these pedigrees is not based on affected status. Because of the late age of onset of this disease, this assumption generally is thought to be valid, but if the disease is inherited with a desirable characteristic that specifically is being bred for, the assumption may not be correct. A correction factor was tested and did not significantly alter outcome. A 1-locus disease model implies the presence of only 2 alleles (ie, A and B) to account for the genetic component of the phenotype. This 1-locus model does not correctly model a disease controlled by 2 or more disease genes (ie, heterogeneity), but because we have assumed homogeneity, the 1-locus model generally is valid. A Hardy-Weinberg equilibrium assumes random matings, and implies that gene frequency and genotype ratios in randomly breeding populations remain constant from generation to generation. In general, when 3 generations of dogs have not been bred directly to one other (eg, parent to offspring), as was the case in the pedigrees used for this analysis, this assumption is valid. Finally, the significance of the results was evaluated through the use of AIC. For large samples, this statistic is approximately distributed as a chi-square statistic with degrees of freedom equal to the difference in the number of estimated parameters. This relationship allows use of chi-square analysis to refute the hypothesis of no familial aggregation. However, the pedigrees were not large enough to use chi-square analysis to differentiate among the different models of Mendelian inheritance. Therefore, the values of AIC were used to identify the most parsimonious model of Mendelian inheritance (ie, recessive). No other formal test of the models can be performed.

The frequency of the deleterious allele was estimated at 39% in the population of dogs in this study, but this was a selected group of dogs from the whole American Staffordshire Terrier breed, and presumably the allele frequency is lower in the whole population. The prevalence of the disease in all registered dogs was estimated at 1 in 400. However, unlike the estimates of allele frequency, this estimate is most likely an underestimate because we cannot assume that we have identified all affected dogs.

Clinical anticipation may have occurred in affected dogs. In humans, clinical anticipation is present when the age of onset of signs in offspring is a mean of 10 years earlier than in their parents. By comparison, in 6 parent offspring pairs of affected dogs, age of onset was 1.57 years earlier in offspring than in parents. However, in 3 of these pairs, both dogs were owned by the same person, and it is probable that signs of the disease therefore were detected earlier in the course of the disease in the offspring, and a statistical analysis of this data was not completed in view of the suspected bias. A wide variation in the rate of progression of signs was observed (from 6 months to 6.5 years), and successive generations appeared to produce dogs with more severe, rapidly progressive signs. These features could be explained by a tri- or pentanucleotide repeat disease, but to date such diseases have only been described in humans. It seems extremely unlikely that primary vitamin E deficiency is the cause of the disease in American Staffordshire Terriers because all dogs tested had serum vitamin E concentrations that were within the reference limits or were high. Moreover, supplementation with vitamin E and other antioxidants did not appear to influence the course of their disease. Similarly, a metabolic cause of the disease was not identified based on the results of serum lactate concentrations, but more complete screening of organic acid excretion is in progress.

The appearance of this disease and the apparent increase in its prevalence are of great concern for the American Staffordshire Terrier breed. A genetic test that can identify carrier and affected dogs before breeding is needed. Comparison of the features of this disease with the human SCAs provides a number of candidate genes that could be evaluated. The suspicion that signs of the disease are becoming more severe, presenting earlier, and progressing at a more rapid rate suggests that a tri- or pentanucleotide repeat may be the genetic defect underlying this disease.

Footnotes

\begin{itemize}
  \item 10% goat serum, Biogenex, San Ramon, CA
  \item Polyclonal rabbit anti-goat glial fibrillary acidic protein, Ventana Medical Systems Inc, Tucson, AZ
  \item Biotinylated goat anti-mouse antibody, Biogenex, San Ramon, CA
  \item Peroxidase-labeled streptavidin, Biogenex, San Ramon, CA
  \item 3,3 Diaminobenzidine tetrahydrochloride, Biogenex, San Ramon, CA
  \item Segreg program, Statistical Analysis of Genetic Etiology 4.3 package, SAGE, Case Western Reserve University, Cleveland, OH
\end{itemize}
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