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A Line of Berlin Druckrey IV Rats Proposed as a New Model for Human Hereditary Ataxia

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Abstract. An experimental colony of Berlin Druckrey IV (BD IV) rats with inherited, congenital, gradually progressive incoordination and rear limb ataxia was evaluated for clinical signs, gross and microscopic nervous system lesions, and mode of inheritance of the gene defect. Clinical evaluation suggested a lesion in the midbrain or brainstem, with resulting lower motor neuron functional impairment. Gross alterations in affected rats were atrophy of thigh musculature by six months of age and thoracic kyphoscoliosis. Histological evaluation of the nervous system revealed central chromatolysis of neurons within the red nuclei in 20 out of 24 affected rats. Additionally, in six out of 24 affected rats chromatolytic neural cell bodies of this nucleus contained strongly eosinophilic, coarsely granular, cytoplasmic deposits. Special stains (osmium tetroxide, Kinyoun’s acid-fast and periodic acid-Schiff) indicated these deposits consisted of lipopolysaccharide. Additional lesions in ataxic rats included qualitative reduction in neuronal cell bodies of the inferior olivary nucleus (10 out of 26 rats) and cerebellar Purkinje cells (5 out of 27 rats). No reduction in the number of spinal cord lower motor neurons was detected. Analysis of intercross pedigrees that were established between ataxic BD IV females and either normal Long Evans or Fisher males indicated a likely autosomal recessive mode of inheritance. The authors propose that this disease accompanying a new variant of the BD IV rat (to be designated "shaker" rat) provides a new and unique research model for ataxia with features in common with some human hereditary ataxias.

The human hereditary ataxias comprise a diverse group of familial progressive degenerative disorders that affect the cerebellum and various other regions of the nervous system. Classification of these disorders has been difficult due to marked heterogeneity in clinical signs, age of onset, rate of disease progression, mode of inheritance, and gross and microscopic lesions (4,18,28,33,42).

Molecular biology and linkage analysis have provided a means for categorizing the various disorders. Within this group of syndromes are the autosomal dominant ataxias, recently redesignated spinocerebellar ataxias (SCA), which include Machado-Joseph disease (MJD) and olivopontocerebellar atrophy (OPCA) (27), and Friedreich’s ataxia (FA), which is an autosomal recessive disorder (6).

Twelve different chromosomal mutations causing SCA have been identified (15,21,22,27,29,31,32,39,43,46,47,55,57). Six of these mutations (at loci SCA-1, SCA-2, SCA-3, SCA-6, SCA-7, and SCA-12) are comprised of trinucleotide repeat (TNR) expansions that result in abnormal gene products which cause disease (22,27,29,31,41,43,47,55,58). It is believed that many of these repeat expansions develop as a result of slippage during crossing over in meiosis, with the addition of the necessary number of triplets to bridge the gap created in one DNA strand (23).

These unstable triplet repeats lead to a phenomenon known as anticipation. This phenomenon involves an inverse correlation between the TNR length and the age of onset of disease, and a direct correlation between TNR length and severity and rate of progression of disease. Individuals with the longest repeat expansions exhibit the most severe forms of these diseases, starting at a very young age. Anticipation is responsible for at least some of the clinical heterogeneity seen in forms of SCA (2,43).

Joseph’s disease, or MJD, occurs most frequently in people of Portuguese-Azorean ancestry, but is seen in other populations (53) and has now been recognized as the most frequent cause of dominant spinocerebellar ataxia (14). There are three subtypes of the disease, based upon the age of onset and clinical signs. Symptoms usually develop between the ages of 15 and 35. Disease results from cell loss in the striatum, the cerebellum, and the substantia nigra. Patients have a lurching gait that is due to spastic leg movement. Speech and swallowing are often affected, and patients may experience involuntary eye movements, double vision, bulging
of the eyes, and frequent urination (39). The affected gene in MJD maps to the same three centiMorgan interval on chromosome 14 (54) that harbors the SCA-3 gene (53), and the two conditions are thought to be due to alterations of the same gene (13).

Five to eight individuals per 100,000 are affected worldwide with OPCA (15). Degenerative changes usually develop in adults after the third decade of life, although the age of onset of the condition has been documented as early as two years of age (in those individuals with markedly expanded trinucleotide repeats) and as late as age 70 (15). Presenting clinical signs are weakness in the arms or legs and eventual ataxia. The disease becomes progressively worse with time. Many patients with OPCA develop difficulty swallowing and talking (15,42). Dementia and ophthalmoplegia may develop or the autonomic nervous system may be affected (42).

Typical gross changes in the brains of those afflicted with OPCA include variable atrophy of the cerebellum and basis pontis. Microscopic lesions include neuronal loss in the olivary nuclei, variable loss of olivocerebellar and transverse fibers in the pons, and a decrease in Purkinje cells. There are myelin deficits in cerebellar white matter. Neuronal loss and accompanying astroglisis can also involve other nuclei (putamen, substantia nigra), and in such instances the disease is referred to as multiple systems atrophy (MSA). In still other cases of OPCA, posterior columns of the spinal cord are affected (42).

FA is the most common type of autosomal recessive ataxia in man, and is the only known autosomal recessive disease that is due to an expanded trinucleotide repeat (6). Most FA patients (96%) have a homozygous expanded GAA repeat that impairs transcription of the frataxin gene X25 (6,36) on chromosome 9 (37,45). A second FA locus is now believed to harbor a mutation leading to disease in a small number of patients (4%) with no mutation at X25 (30).

The spinocerebellar tracts, dorsal columns, and pyramidal tracts are mainly affected in patients with FA before they reach adolescence. The cerebellum and medulla are involved to a lesser extent. Clinically distinctive features of this form of ataxia include cardiomyopathy, pes cavus, optic atrophy, deafness, and scoliosis (6).

Various animal models of naturally occurring neurological disease parallel some aspects of human hereditary ataxia. Species and breed or strain include the Kerry blue terrier (24), and homozygous weaver (9,48), staggerer, lurcher (51), tottering, ducky, ogilans, stumbler, and teetering mice (34). Also, transgenic mice that harbor CAG repeat expansions leading to Purkinje cell degeneration and ataxia have been developed (5). However, given the constellation of clinical signs, lesion distribution and modes of inheritance of disease, additional animal models are still needed to elucidate some basic disease mechanisms.

The BD IV rats used in this investigation originated from one of 10 different strains of Berlin Druckrey rats established by Druckrey, beginning in 1937 (11). Ten years ago a spontaneous neurological mutation referred to in this report as 'shaker rat' occurred in one animal in a colony of the rats at Texas A&M University. Offspring derived from the proband were similarly affected. During the ten-year period that the colony has been maintained, the phenotype of the affected rats has remained stable. The features of this neurodegenerative disease support the use of this mutation of the BD IV rat as a desirable model for the study of human ataxia.

Materials and Methods

Animals

Group I Rats. Three BD IV ataxic rats of different ages were selected for detailed evaluation of clinical signs. The three age groups represented were one month old, three month old, and six month old.

Group II Rats. Seven 10-to 15-week-old BD IV shaker female rats and seven similarly aged normal male rats (five Long Evans and two Fisher rats) were used to generate intercross pedigrees. These pedigrees were established to determine the mode of inheritance of the gene defect.

Group III Rats. The nervous system of 11 BD IV rats was examined for gross and microscopic lesions in a pilot study. The rats examined included six ataxic BD IV rats (4, 1-2 months of age and 2, 6-9 months of age) and five phenotypically normal BD IV rats (1, 1-2 months of age and 4, 6-9 months of age). The latter group of phenotypically normal rats consisted of three rats that were potential carriers for the trait and two noncarrier rats that originated from a line of BD IV rats that had never exhibited ataxia.

Group IV Rats. Twenty-five ataxic BD IV rats, 26 age-matched noncarrier BD IV rats, and three potential carrier BD IV rats were selected for evaluation for lesions at key areas of the nervous system that were targeted for more detailed study following examination of Group III affected rats. The rats ranged in age from one month old to 26 month old.

Group V Rats. One three-month-old affected BD IV rat and one six-month-old control BD IV rat were selected for ultrastructural evaluation of the red nucleus.

Clinical evaluation. Central and peripheral nervous system function was evaluated by standard clinical methods (40,60) in order to determine clinical signs, disease progression and localization of lesions. Detailed clinical evaluation of the three shaker rats of different ages (Group I rats) was conducted and compared with the other affected and unaffected rats in the colony.

Genetic transmission. Seven intercross pedigrees were generated from parental matings of 10-to 15-week-old shaker BD IV female rats with either normal Long Evans or Fisher male rats of similar age (Group II rats). Reciprocal pedigrees that involved reversing the sex of the different strains were not made since the shaker males were too ataxic to successfully breed. At the same time that these parental matings were made, normal BD IV females were bred with normal BD IV males to provide a pool of foster mothers that would be able to nurse the pups born to parental shaker mothers. Fostering of the young was necessary since ataxia interfered with the ability of shaker mothers to assist their young in nursing and because some affected BD IV shaker mothers did not exhibit adequate mammary gland development for successful nursing of their young.

The F1 young of the parental shaker BD IV female and Long Evans or Fisher male matings were born after a normal-length gestation period of approximately 22.5 days. The pups were weaned and housed in pairs.
of the same gender after they had nursed from their foster mothers for 21 days.

At 10 weeks of age, when the unaffected F1 siblings reached sexual maturity, they were housed as male-female pairs in separate cages to generate intercross F2 offspring. The F2 progeny were phenotyped at four weeks of age.

**Fixation, processing, and staining**

*Light microscopy.* Rats were deeply anesthetized with intramuscular injections of ketamine hydrochloride (90 mg/kg) and xylazine (5 mg/kg) (15). Once deep pain response was lost, the chest cavity was opened and a 23-gauge butterfly needle was inserted through the ventrolateral wall of the left ventricle into the aorta. A small slit was made in the wall of the right atrium to allow for escape of circulatory fluid during perfusion.

Approximately six to 12 milliliters of saline containing heparin (20 units per milliliter to prevent clots) were administered with a perfusion pump at the rate of 10 to 12 milliliters per minute. This perfusion was followed by perfusion of 200 to 500 milliliters of 10% neutral buffered-formalin (NBF), depending upon the weight of the rat (35). Tissues were post-fixed for at least 24 hours in 10% NBF.

Transverse and longitudinal sections of nervous system tissues were cut. These tissues were routinely processed and embedded in paraffin. Tissue sections were usually cut at six to seven microns thickness and stained with hematoxylin and eosin (H&E) (8) or special stains. The special stains employed were Fontana Masson (for argentaffin granules), Kinyoun's acid-fast stain (for ceroid), periodic acid-Schiff (PAS) (for carbohydrates), Congo red (for amyloid), toluidine blue (for metachromatic substances), rhodamine (for copper) and Prussian blue (for ferric iron). Tissue sections from 13 Group IV rats were cut at a thickness of 50 microns and stained with cresyl violet to observe the degree of neuronal density, and loss if it occurred.

*Electron microscopy.* Two rats selected for ultrastructural evaluation were perfused with heparinized saline followed by Karnovsky's fixative containing 2% glutaraldehyde. Brain and spinal cord tissue was removed, post-fixed for 12 hours in the perfusing solution, and then placed in phosphate-buffered saline (PBS) at pH 7.2. Cubes of tissue less than 1mm on edge from the region of the midbrain containing the red nucleus were further post-fixed in 1% osmium tetroxide and embedded in Epon. Semi-thin sections of these blocks were stained with toluidine blue for light microscopic and ultrastructural examination (35).

**Gross and microscopic lesions.** The nervous systems of 11 Group III rats were examined for gross and microscopic lesions. Brain tissue alone was evaluated from two of the older control rats. Tissues collected from the six affected and the other three control rats included brain, spinal cord, sciatic nerves, axillary nerves, intracranial segments of the trigeminal nerve, and the segment of the vestibulocochlear nerve within the tympanic bulla.

The forebrains and brainstems of all 11 rats were serially sectioned into two to three millimeter thick coronal slabs for processing. Transverse and longitudinal sections of the cervical, thoracic, lumbar, and sacral spinal cord and peripheral nerves were cut at a thickness of four to five millimeters. Light microscopic tissue sections were prepared and stained by routine methods.

Based upon microscopic lesions identified in affected Group III rats, key areas of the nervous systems of additional rats (Group IV rats) were evaluated. Twenty-five ataxic BD IV rats, twenty-six age-matched noncarrier BD IV rats, and three potential carrier BD IV rats were perfused with 10% NBF for this purpose. Subgroups of the Group IV rats were examined in the following manner: 1) Four-millimeter-thick transverse slabs of tissue from the selected areas of the brains (midbrain, cerebellum, caudal brainstem) and spinal cords (cervical, thoracic, lumbar, and lumbosacral) of six of the ataxic rats and seven of the age-matched noncarrier rats were processed, embedded, and sectioned at 50 microns thickness. 2) Single, sagittal, four millimeter-thick slabs of brain tissue that contained all affected structures within the forebrain and brainstem were taken from 11 ataxic rats, 15 noncarrier rats, and two potential carrier rats. 3) Transverse, four-millimeter-thick sections of the affected areas of the brain were taken from the remaining eight ataxic rats, four noncarrier rats, and one potential carrier rat. 4) Four-millimeter-thick transverse and longitudinal slices of the cerebral, throracic, lumbar, and lumbosacral spinal cord were removed from nine of the shaker rats and eight of the noncarrier control rats. These various sagittal, longitudinal, and transverse slices of tissue were processed, embedded, and sectioned at six microns thickness. Additionally, spinal ganglia from all levels of the spinal cord were removed from two 26-month-old and two ten-month-old shaker Group IV rats. The ganglia were processed, embedded, and sectioned at six microns thickness as well. Special stains were done on six-micron-thick, serial, sagittal sections from the brain of one shaker rat with brightly eosinophilic intracytoplasmic granules in neurons of the red nucleus.

**Results**

*Clinical evaluation.* All affected BD IV pups were incoordinated and ataxic by three to four weeks of age, when their unaffected littersmates were walking normally. All of the ataxic rats were affected to a similar degree at that age, and the condition progressed uniformly in all affected rats. The severity of the trait was not influenced by gender.

Affected pups fell on their sides or over backwards when they groomed themselves or when they tried to move forward. Flexion and adduction of the hind limbs were impaired, causing the rats to rely on short thrusting-type motions of the rear legs to move forward. These clinical signs gradually worsened as the rats aged, but paralysis did not develop.

Sensation remained intact in the hind limbs. Front limb function remained normal, and they were used to pull the rats forward. Older rats (six months of age and older) exhibited mild, possible seizure-like activity consisting of frequent, mild fasciculation of the facial muscles and mild episodes of front limb extension and tremor that culminated in front limb rigidity for a duration of 10 to 15 seconds. Cranial nerve function was normal, except for a suspected vestibular and/or cerebellar deficit, based upon the fact that the rats were unable to maintain their balance. The growth rate of the affected rats was subnormal. The rats had no problem finding, chewing, or swallowing food, but were slower in reaching it than normal rats. Longevity of the ataxic rats was not affected. They survived comparably to controls to more than 2.5 years of age, at which time they were euthanized.

*Genetic transmission.* None of the F1 offspring that were generated in the seven intercross pedigrees showed any clinical signs of incoordination or ataxia throughout their life spans. A total of 281 F2 progeny were produced from all of the intercross pedigrees. Fifty-seven (20%) of these 281 young exhibited clinical signs by four weeks of age. The phenotype of these affected hybrid progeny was the same as that of affected purebred BD IV rats. Twenty-eight male and 29 female F2 intercross progeny were affected. This pattern
of a nearly 3:1 ratio of unaffected: affected progeny in alternate generations, with equal representation of male and female offspring in the affected group, suggests an autosomal recessive mode of inheritance is most likely involved; however, the existence of multiple defective alleles or genes influencing trait expression could not be excluded.

Gross and microscopic lesions. Thoracic kyphoscoliosis was present in 22 of the 32 affected BD IV rats in Groups III, IV, and V that were necropsied, but in none of the BD IV control rats from those groups. This condition was observed in shaker rats as young as four weeks of age, as well as in the oldest shaker rat that was examined (26 months of age). Grossly visible atrophy of all the large thigh muscle groups was present in all affected rats by the time they reached six months of age. Thigh muscle mass remained normal throughout the life spans of the control rats.
No gross lesions were detected in the nervous systems of the affected rats. The most consistent microscopic lesion observed within the central nervous system of affected rats was central chromatolysis characterized by cell swelling and extreme nuclear eccentricity in approximately one-third of the giant neuronal cell bodies of the magnocellular portion of the red nuclei (Figure 1). This lesion was present in all of the animals (20 out of 24) in which the red nucleus was present in sections examined. The change was seen in rats ranging in age from one month to 26 months (the youngest and oldest affected rats examined). The red nuclei were present in tissue sections from 24 out of 26 noncarrier BD IV rats and 5 out of 6 potential carrier BD IV rats. No red nucleus lesions were observed in these controls.

In six affected rats, the perikarya of neurons in the red nucleus contained brightly eosinophilic granules that varied in size from fine, "dust-like" particles to large, dense, single, irregularly-shaped aggregates (Figure 2). The larger particles were partially surrounded by clear spaces, suggesting they were contained within cytoplasmic vacuoles (lysosomes). The youngest animal exhibiting the particles was a three-month-old female rat, which had the smallest granules. The other five rats, which varied in age from 11.5 to 26 months and included three females, one male, and one rat of unknown sex, had much larger cytoplasmic granules. Granules stained positive for Fontana Masson, Kinyoun's acid-fast stain and PAS. The granules were negative for Congo red, toluidine blue, rhodamine and Prussian blue. Identical granules were
within the cytoplasm of other neuronal cell bodies in the brainstem and cerebellum, but in noticeably lower amount.

A qualitative reduction in the number of neuronal cell bodies occurred in the inferior olivary nucleus in 10 out of 26 (40%) ataxic BD IV rats in which this nucleus was present in sections examined (Figure 3). However, the morphology of existing neuronal cell bodies was normal. Affected rats that exhibited this change ranged in age from one month to 26 months. The number and morphology of cell bodies in this nucleus were normal in 26 out of 26 noncarrier control BD IV rats. The nucleus, which was present in sections from four of six potential carrier BD IV rats, was normal (Figure 4).

In five (19%) of the cerebellums that were sampled from 27 ataxic BD IV rats, Purkinje cells were qualitatively reduced in number and were occasionally separated by long spaces along affected folia (Figure 5). The Purkinje cells present in these rats exhibited normal morphology. Affected rats with Purkinje cell loss varied in age from six months to 26 months. Spaces between Purkinje cells were smaller in the cerebellums from 26 noncarrier and 5 potential carrier control rats that were examined (Figure 6). Cerebellar nuclei appeared normal in the ataxic rats.

The spinal cord was examined from a total of 15 shaker BD IV rats and 11 control BD IV rats. No loss of lower motor neurons was detected in any of the affected or control rats. Spinal ganglia at several levels of the spinal cord from two ten-month-old shaker rats (one male and one female) and two 26-month-old shaker female rats had no microscopic lesions. Sciatic nerves, brachial nerves, acoustic nerves and trigeminal nerves from six shaker rats and five phenotypically normal rats also had no gross or microscopic abnormalities. One to three large, nonstatting vacuoles of varying size filled the cytoplasm of neurons within the sensory ganglion of the trigeminal nerve in two of the shaker BD IV rats (both four-week-old and one normal BD IV rat, nine-month-old). These vacuoles compressed cell nuclei and cytoplasm to the periphery of the cells.

Electron microscopy. Ultrastructurally, the intracytoplasmic cosinophilic granules of central chromatolytic neurons of the red nucleus as detected by light microscopy in affected rats were small, dense, irregularly-shaped osmiophilic particles. Also, many free polyribosomes were present in the cytoplasm of the neuronal cytoplasm (Figure 7). These changes were not observed in the red nucleus of a control rat.

Discussion

The lesions in the red nucleus in the majority of affected BD IV rats were compatible with the neurological signs that occurred and provide a lead in the search for a candidate gene for the condition. The red nucleus is a prominent cell group within the superior cerebellar peduncle and serves as a major premotor center for afferent and efferent nerve impulses that are integrated to provide control of normal motor function in the limbs. Four types of neurons, based upon size, are recognized in the red nucleus of the rat. Medium and small neurons are found in the rostral parvocellular region, while giant and large neurons are present in the caudal magnocellular portion of the nucleus (50).

The nucleus receives numerous afferents from various nerve cell centers, including projections from the cerebral cortex, hypothalamus, posterior thalamic nucleus, zona incerta, and lesions in cerebellar nuclei (50). Five main efferent pathways originating from the red nucleus have been demonstrated in the rat. Predominantly magnocellular neurons, but some parvocellular neurons as well, give rise to the rubrospinal tract which projects to the dorsolateral funiculus of the spinal cord and to the intermediate region of the ventral horn in the cervical and lumbar spinal cord. The parvocellular neurons send projections to the inferior olivary nuclei and receive retrograde input from these same nuclei in return. Collaterals of the rubrospinal pathway cross to create contralateral rubrobulbar fibers that ramify with brainstem centers such as the facial nuclei and spinal vestibular nuclei. Rubrocoerulear and rubrothalamic projections have been demonstrated as well. Rubroconical projections have been suggested to be present but are not well characterized (50).

Altered output resulting from a lesion involving the various efferent pathways of the red nucleus could explain many of the clinical signs detected in the ataxic BD IV rat. Motor deficits involving the rubrospinal pathway would account for the rear limb ataxia and eventual skeletal muscle atrophy of denervation, as well as the minor, frequent tremors in the forelegs. Loss of stimuli to the epaxial or hypaxial musculature from spinal cord ventral horn neurons may be responsible for the kyphoscoliosis that occurred in so many of the rats. Incoordination may have been the result of combined abnormal efferent stimuli to the spinal vestibular nuclei via the rubrobulbar fibers and/or abnormal input to the cerebellum via either rubrocoerulear projections or rubro-olivary projections and the olivary climbing fibers to the cerebellum. Episodic facial tremors in older rats may have resulted from rubrobulbar fiber deficits to the facial nuclei. Alternatively, the facial tremors and front leg tremors and rigidity may have been due to seizures that would be indicative of potential altered cerebral impulses.

The profound central chromatolyis in giant magnocellular neurons in the red nucleus and the milder lesions involving the inferior olivary nucleus and cerebellum suggest that initial damage to the red nucleus may be the basis for the disease process. Since this population of red nuclear neurons receives input from cerebellar nuclei, a primary defect in those nuclei is a consideration as well. No qualitative defect of the cerebellar nuclei was observed. Quantitative analysis may provide a clue as to the role, if any, that these nerve centers play in ataxia in the rats.

Although the cause of lesions involving the inferior olivary nucleus and Purkinje cells of the cerebellum could not be definitively determined, it is possible that disturbed, deficient
or absent rubro-olivary or climbing fiber innervation of the olivary nucleus and Purkinje cells, respectively, may have played a role. One mechanism considered was transsynaptic neuronal degeneration that has been proposed as being involved in other neuronal degenerations of the CNS (1,12,38). The earliest appearance of Purkinje cell loss in a nine-month-old rat temporally followed lesions in the red nucleus and inferior olivary nucleus that were seen in rats as young as one month of age, suggesting cerebellar damage occurred later in the disease process.

Alterations of affected neuronal cells of the red nucleus included the expected detachment of ribosomes from the endoplasmic reticulum that is associated with central chromatolysis and an unexpected occurrence of cytoplasmic granules that were detected in affected rats as young as three months of age. The results of special stains indicated that the granular, osmiophilic pigment was similar to both lipofuscin and ceroid pigment (20,52). Both of these pigments are composed of protein, lipid, small amounts of carbohydrate, and very low levels of mineral such as copper and iron. Although these two pigments are commonly seen as a consequence of aging in post-mitotic cell populations like neurons, they are also considered to be a feature of cell injury from other causes. For example, these pigments can accumulate within cells following oxidative damage of phospholipid membranes by free radicals. Such deposits of protein and lipid within lysosomes can overwhelm their catalytic machinery and precipitate in a form that resists subsequent processing (20,52). Lipofuscin and ceroid precipitates can also result from disruption of the proteolytic system or lipid recycling system within a cell. Factors that cause this disruption include an increase in the activity, or amount, of protease inhibitors, or abnormal "marking" of proteins for degradation within lysosomes by various mechanisms available to cells. Production of abnormal phospholipase A2 or abnormal compositions of cellular lipids also are possible causes for altered lipid metabolism within lysosomes (20).

The vacuolation of neurons within the Gasserian ganglia of two shaker BD IV rats and one noncarrier BD IV rat was considered to be an incidental lesion. Vacuoles within the cytoplasm of various neuronal aggregates have been reported previously in a variety of animal species and have proven to be incidental lesions or artifacts of fixation (17,26,56,59).

The pattern of inheritance determined from the intercross pedigrees involving BD IV ataxic females indicated a likely autosomal recessive gene trait, although interaction of multiple alleles or genes could not be ruled out. The clinical uniformity of the condition in affected intercross hybrid progeny, as well as affected purebred BD IV offspring, supports an autosomal recessive, single copy gene defect as the cause of disease.

It was not possible to establish a reciprocal intercross pedigree between shaker BD IV males and normal Long Evans or Fisher females because the shaker male rats were too weak in the rear limbs to breed. However, it was not anticipated that the results of a reverse pedigree would have differed from those previously observed. Imprinting was not a factor in inheritance of the trait based upon the nearly classic 3:1 ratio of unaffected: affected F2 intercross progeny and the large F2 litter sizes. These factors indicated that both F1 parents contributed equally to the defective gene effects detected in F2 progeny (16).

In conclusion, the slowly progressive clinical signs that spare cranial nerve function and impair only locomotion in ataxic BD IV rats are similar to the clinical signs that occur in the milder forms of human SCA (32,46). Also, lesions common to affected rats and the milder forms of OPCA include neuronal loss in the inferior olivary nucleus of Purkinje cells of the rats (42), but not the central chromatolysis of neurons in the red nucleus and cytoplasmic granules that are unique changes in affected BD IV rats. Additionally, the eventual rear limb muscle atrophy in the affected rats suggested there was a likely effect on lower motor neurons of the spinal cord as in ataxic human patients (42), although no evidence of such a lesion was detected.

One type of human inherited ataxia, Friedrich's ataxia (FA), has a mode of inheritance and some clinical features in common with the ataxic BD IV rat. Other clinically distinctive aspects of FA (cardiomyopathy, pes cavus, optic atrophy, and deafness) (6) are not present in the murine disease.

Mapping studies are now underway to locate the affected gene in BD IV shaker rats using DNA from 21 F2 offspring in the largest of the intercross pedigrees that was established. Once a candidate gene for the condition is identified, we will be able to sequence the gene and establish whether an expanded repeat or a novel gene mutation is responsible for the clinical signs the rats share with those suffering from some forms of human hereditary ataxia.

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