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Gracile Axonal Dystrophy (GAD), a New Neurological Mutant in the Mouse (42656)

KAZUTO YAMAZAKI,* NOBORU WAKASUGI,* TAKESHI TOMITA,* TATEKI KIKUCHI,† MASAKUNI MUKOYAMA,‡ AND KAZUYA ANDO‡

**Laboratory of Animal Genetics, Faculty of Agriculture, Nagoya University, Nagoya, 464, Japan, and Divisions of †Animal Models for Human Disease and ‡Degenerative Neurological Disease, National Institute of Neuroscience, NCNP, 4-1-1, Ogawahigashi-machi, Kodaira, Tokyo, 187, Japan*

Abstract. A new neurological mutant has been found in the F_2 offspring of CBA/Nga and RFM/Nga mice. Affected mice exhibited ataxia beginning at about 80 days of age, followed by tremor, difficulty in moving, and muscular atrophy of the hind limbs. The neurological signs became progressively severe, and death occurred by 5 to 6 months of age. Since the animals could be distinguished from normal mice by the abnormal positions of the hind limbs when the mouse was hung by the tail after 1 month of age, they could be bred until onset of the signs. Pathological examination revealed neuroaxonal dystrophy and degeneration in the gracile nucleus of the medulla oblongata and the gracile fascicules of the spinal cord, which could be the main cause of the clinical signs. The mutation is inherited as an autosomal recessive trait. It was, therefore, named gracile axonal dystrophy (GAD) with the gene symbol *gad.* The mice could be a new pathological model for the study of neuroaxonal dystrophy.

Neuroaxonal dystrophy occurs in many pathological and physiological conditions, and it has often been thought to be a nonspecific feature (1, 2). It has, however, been observed as a characteristic lesion in the following conditions (1, 2): infantile neuroaxonal dystrophy (3–5) and Hallervorden–Spatz disease in humans (4, 6–8), vitamin E deficiency in rats (9–13), aging in many species (14–16) including humans (17–19), and intoxication in rats (20, 21). Neuroaxonal dystrophy in the gracile nucleus and fascicules has been noted, particularly in vitamin E deficiency and the aging process (1, 2).

A hereditary syndrome of neuroaxonal dystrophy has been described in Suffolk sheep (22) and cats (23). This is inherited as an autosomal recessive trait and has been used as an animal model for the study of infantile neuroaxonal dystrophy, but so far, the nature of neuroaxonal dystrophy, as well as that of infantile neuroaxonal dystrophy, remains to be clarified.

We have discovered a new neurological mutant in mice whose characteristic lesion is neuroaxonal dystrophy in the gracile nucleus and fascicules. The purpose of this paper is to present the results of genetic and preliminary pathology studies on the neurological mutation in mice.

Materials and Methods. *Mice.* In January 1984 abnormal shuffling gaits appeared in three 80-day-old mice, two females and one male, who were F_2 offspring of CBA/Nga and RFM/Nga inbred strain mice. One abnormal female mated to a normal sib male produced a normal male, which was backcrossed to RFM/Nga. Thereafter, brother–sister matings were conducted. Affected mice showing the clinical signs were recovered in the succeeding generations. The mutant strain was tentatively called Gad/Nga and was maintained by the matings of carriers *inter se.*

For genetic analysis of this mutation, C57BL/6Nga was used as a normal control strain. CBA/Nga, RFM/Nga, and C57BL/6Nga were formerly introduced from the National Institute of Genetics, Mishima, Japan, the National Institute of Radiological Science, Chiba, Japan, and Kyoto University, Japan, respectively. They have been maintained subsequently in the Laboratory of Animal Genetics, Faculty of Agriculture, Nagoya University, Japan.

The mice were housed in plastic or wooden cages, considered to be more comfortable for the diseased mice. A pellet diet and water were available *ad libitum,* and ground chicken feed was used as a supple-

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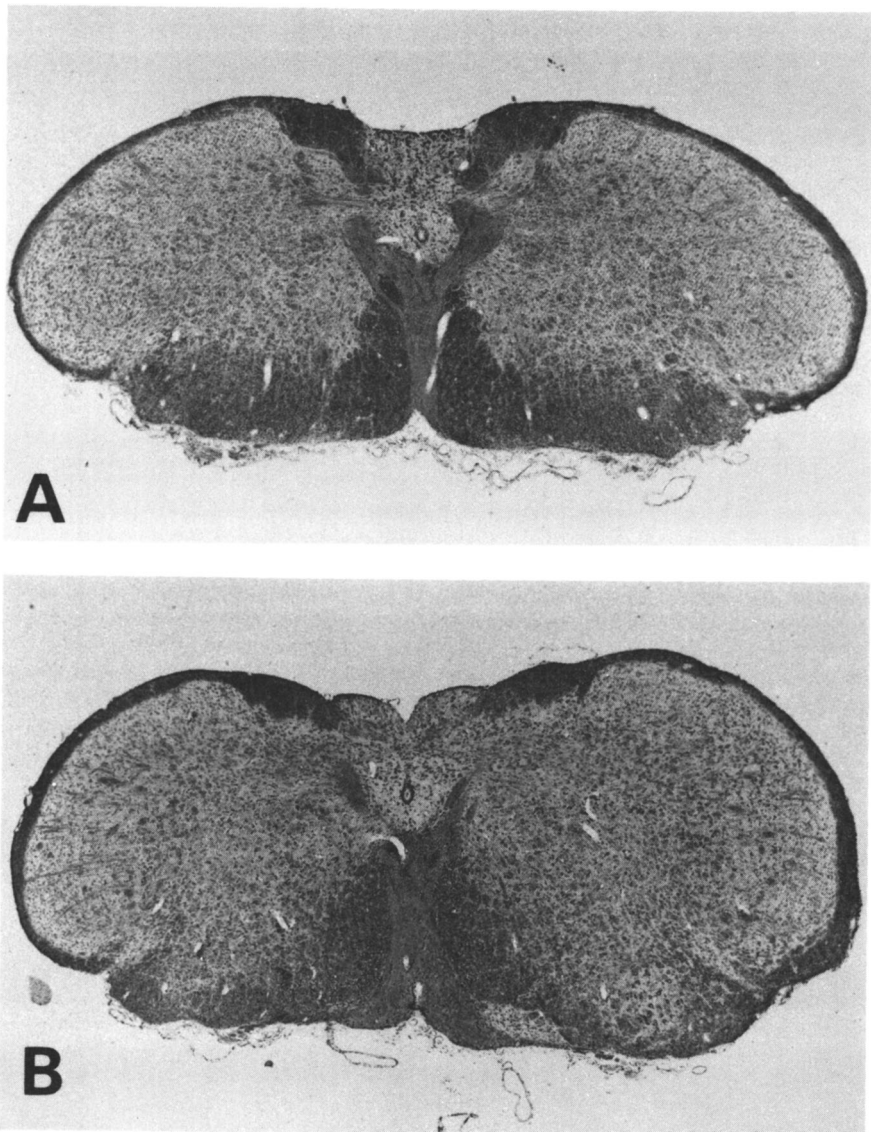

FIG. 1. (A) Cross-section of medulla in GAD mouse. Note the abnormal structure of the gracile nucleus. Klüver–Barrera, 38×. (B) Cross-section of medulla in control mouse. Klüver–Barrera, 38×. (C) Cross-section of cervical cord in GAD mouse. Note the gracile fascicules showing poor stainability, which meant loss of myelinated nerve fibers. Klüver–Barrera, 60×. (D) Cross-section of cervical cord in control mouse. Klüver–Barrera, 60×.

mental diet to ease feeding for the diseased animals. The room temperature was not controlled, but in the winter heating was provided to maintain the room temperature above 20°C. The housing was free of sendai virus and mouse hepatitis virus, and there were no deaths caused by infectious diseases.

Pathology examination. Eight affected mice at 130 days of age were killed by transcardiac perfusion of 10% buffered neutral formalin under deep anesthesia (pentobarbital-Na). Blocks of cerebrum, basal ganglia, brain stem, cerebellum, cervical, thoracic and lumbar cords, lumbar dorsal root ganglion and ventral root, sciatic nerve, and other visceral organs, as well as quadriceps femoris and anterior tibial muscle, were embedded in paraffin. Sections (6 μm) were ex-

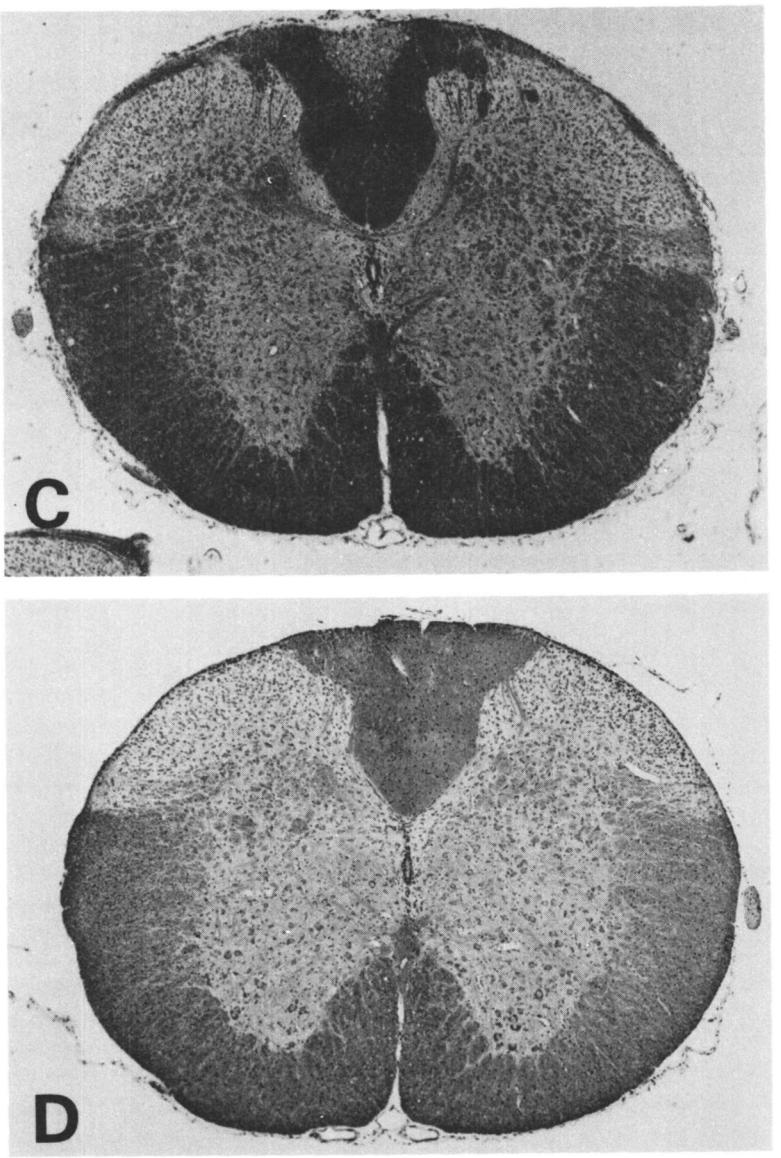

FIG. 1—*Continued.*

amined by light microscopy after staining with hematoxylin and eosin, luxol fast blue and cresyl violet (Klüver–Barrera) or by Bodian's methods.

Genetic analysis. Preliminary breeding data analyses of carrier × carrier and affected × affected matings within Gad/Nga were conducted for the appearance of normal and affected progenies. Reciprocal mating experiments were made between affected mice of Gad/Nga and C57BL/6 mice. Resulting F_1 offspring were crossed *inter se* to check the F_2 segregation ratio. Some of them were backcrossed to affected mice of Gad/Nga reciprocally to determine the N_2 segregation ratio.

Before mating, all animals were determined to be either normal or affected according to position of the hind limbs when the mouse was hung by the tail, as detailed below.

Results. *Description of clinical signs.* Affected animals became ataxic and began to drag the hind limbs at about 80 days of age. After that, tremor and muscular atrophy of

the hind limbs were observed, and body weights decreased gradually. The tail and abdominal fur tended to be wet with urine and feces. Affected males sometimes showed priapism.

Once the clinical signs appeared, the illness progressed rapidly. In terminal stages functional control of the hind limbs was completely lost, and locomotion was achieved by the forelimbs. Finally, muscular atrophy extended to the anterior part of the body, and affected mice became immobile, showing kyphoscoliosis. Death occurred by 5 to 6 months of age.

After 1 month of age, affected mice could be recognized by the abnormal positions of the hind limbs when the animal was suspended by the tail. At that time, they looked normal when observed in the cage, but when hung by the tail, they tended to stretch the hind limbs upward spasmodically and/or clasp them tightly. Affected males could be bred by about 80 days of age. First and second litters could be obtained from affected females, though their litter sizes appeared to be smaller than those of normal females.

Pathology examination. The brain, spinal cord, and peripheral nerves were normal on gross examination. The abnormalities observed most often by microscopic examination of affected mice were in the gracile nucleus of the the medulla oblongata and the gracile fascicules of the spinal cord, where nerve fibers degenerated and decreased in number (Fig. 1). Also many focally swollen axons, neuroaxonal dystrophy, and astrocytic proliferation were found (Fig. 2).

These changes, both loss of nerve fibers with gliosis and presence of swollen axons, were most evident in the gracile nucleus and upper portion of the cervical cord, but were also present in the lower portion of the cervical cord, in the thoracic cord, and to a lesser degree in the lumbar cord. No pathological change was found in nerve cells or fiber tracts in any other region of the brain, spinal cord, or peripheral nerves by the methods employed.

The muscles of the hind limbs showed atrophy from disuse. There was no evidence of necrosis or degeneration of the muscle fibers, and inflammatory cell infiltration was not observed. Visceral organs such as heart, lung, liver, kidney, spleen, and intestine appeared to be normal macro- and microscopically.

Genetics. The pedigree of Gad/Nga is shown in Fig. 3. The appearance pattern of affected animals in Fig. 3 could be explained by an autosomal recessive inheritance. Table I indicates results of test crosses within Gad/Nga. Matings of carrier × carrier produced offspring in a ratio of approximately 3 nor-

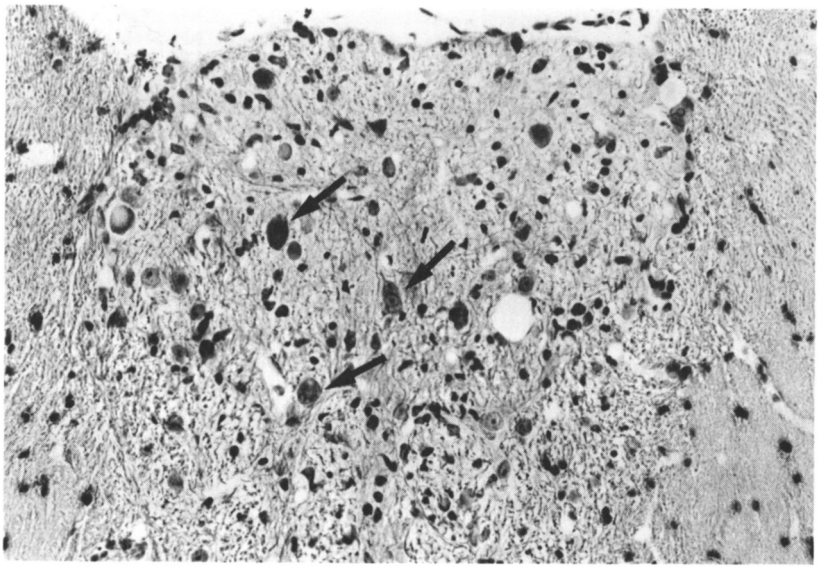

FIG. 2. The gracile nucleus of medulla in GAD mouse. Note focally swollen axons (arrows) and proliferation of glia cells. Bodian, 1050×.

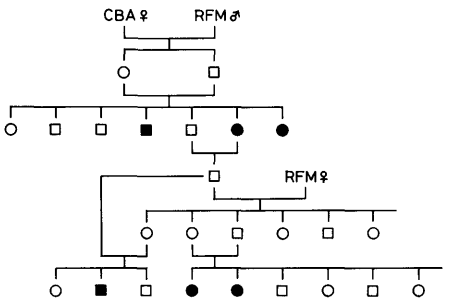

FIG. 3. The pedigree of Gad/Nga. Open squares and circles indicate normal males and females, and solid squares and circles indicate affected males and females, respectively.

mal and 1 affected. All offspring resulting from matings between affected mice showed the clinical signs. Results of test crosses between Gad/Nga and C57BL/6 are shown in Table II. None of the F_1 offspring were affected. The segregation ratios of F_2 and N_2 offspring were in agreement with expected ratios of 3:1 and 1:1, respectively. These results confirmed that the trait was controlled by a single autosomal recessive gene. The mutation has been named gracile axonal dystrophy (GAD) with the gene symbol *gad* from a characteristic axonal change of neuroaxonal dystrophy in the gracile nucleus and fascicules.

Discussion. Pathological and genetic studies on this new neurological mutant have shown that an autosomal recessive gene, *gad,* causes the lesion characterized by neuroaxonal dystrophy in the gracile nucleus of the medulla and the gracile fascicules of the spinal cord. In mice, neuroaxonal dystrophy has been described in the following neurological mutants: ataxia (*ax*) (24), shambling (*shm*) (25), and ducky (*du*) (26). However, neuroaxonal dystrophy in these mutants was not found to be a prominent lesion, nor did it localize as in GAD (*gad/gad*) mice.

Though neuroaxonal dystrophy was one particular axonal change in GAD mice, loss of nerve fibers was also noted in the gracile nucleus and fascicules. The relationship between these degenerative processes is not clear, since GAD mice examined were in the terminal stage. Neuroaxonal dystrophy could lead to loss of nerve fibers, but it was possible that these two changes were independent of each other.

GAD mice developed clinical signs characterized by ataxia, tremor, and difficulty in moving the hind limbs. Though no pathological change was found in the motor system, such as in the cerebellum, pyramidal tract, anterior horn cell, or anterior spinal root, the clinical signs were thought to be closely related to axonal changes in the gracile nucleus and fascicules, both of which compose the dorsal column pathway carrying sensory information from peripheral receptors (27). Ataxia caused by experimental lesions of the posterior columns was explained by loss of deep sensibilities (28). Muscular atrophy may appear secondarily from disuse in the lower part of the body of GAD mice.

Neuroaxonal dystrophy occurs characteristically in patients with infantile neuroaxonal dystrophy (3–5) and Hallervorden–Spatz disease (4, 6–8). These diseases have been considered to be controlled by an autosomal recessive gene. Infantile neuroaxonal dystrophy is characterized clinically by progressive neurological and mental disturbance, such as hyperkinesia, bulbar paralysis, rigidity, and dementia, following a period of normal development until about 2 years of age. The manifestation of Hallervorden–Spatz disease is a progressive extrapyramidal syndrome, exemplified chiefly by rigidity and dementia, beginning at approximately 7

TABLE I. RESULTS OF TEST CROSSES WITHIN Gad/Nga

Mating		No. of progeny	Phenotypes of progeny		Expected segregation ratio	χ^2
Dam	Sire		Normal	Affected		
Carrier (*gad/+*)	× carrier (*gad/+*)	309	227	82	3:1	0.389 $0.50 < P < 0.70$
Affected (*gad/gad*)	× affected (*gad/gad*)	5	0	5	0:1	

TABLE II. RESULTS OF TEST CROSSES BETWEEN Gad/Nga AND C57BL/6

Mating		No. of progeny	Phenotypes of progeny		Expected segregation ratio	χ^2
Dam	Sire		Normal	Affected		
Normal[a] (+/+)	× affected[b] (*gad/gad*)	20	20	0	1:0	
Affected[b] (*gad/gad*)	× normal[a] (+/+)	15	15	0	1:0	
Total		35	35	0	1:0	
Carrier[c] (*gad*/+)	× carrier[c] (*gad*/+)	66	52	14	3:1	0.505 $0.30 < P < 0.50$
Carrier[c] (*gad*/+)	× affected[b] (*gad/gad*)	81	38	43	1:1	0.309 $0.50 < P < 0.70$
Affected[b] (*gad/gad*)	× carrier[c] (*gad*/+)	31	18	13	1:1	0.806 $0.30 < P < 0.50$
Total		112	56	56	1:1	0 $P > 0.99$

[a] C57BL/6 used.
[b] Gad/Nga used.
[c] F_1 between C57BL/6 and Gad/Nga used.

to 9 years of age. For GAD mice a normal development also precedes the onset of the clinical signs. Although the murine symptoms occur later than those in humans, once the signs appear the disease progresses rapidly in both species.

Although some similarities exist between human diseases and GAD, one of the important pathological differences is the topographic distribution of neuroaxonal dystrophy. Neuroaxonal dystrophy is observed widely in the central nervous system in infantile neuroaxonal dystrophy, and mainly in the pallidum in Hallervorden–Spatz disease, whereas it is restricted to the gracile nucleus and fascicules in GAD. Another important difference is the pigment accumulation in the globus pallidus and substantia nigra in Hallervorden–Spatz disease and in some cases of infantile neuroaxonal dystrophy, but it is not observed in GAD. Nevertheless, Sacks *et al.* suggested that the primary lesion in Hallervorden–Spatz disease could be neuroaxonal dystrophy (8).

In the aging of many species (14), including humans (17–19), rats (15), and mice (16), neuroaxonal dystrophy in the nuclei of posterior funiculi, namely, gracile and cuneate nuclei, has been observed. In these cases the gracile nucleus was affected more severely than the cuneate nucleus.

Vitamin E-deficient rats manifest localization of neuroaxonal dystrophy and clinical signs similar to those of GAD mice (9–13). Furthermore, neuroaxonal dystrophy localized in the gracile nucleus has been found in patients with cystic fibrosis (mucoviscidosis) and congenital biliary atresia, resulting in fat malabsorption associated with a severe vitamin E deficiency (19, 29–31). Thus it is possible that the pathogenetic mechanism of neuroaxonal dystrophy in GAD is similar to that in aging or vitamin E deficiency.

The pathogenesis of neuroaxonal dystrophy is still unknown. It is, therefore, suggested that GAD mice offer new approaches to studying those phenomena causing neuroaxonal dystrophy in aging and vitamin E deficiency. Further, they could be a useful animal model for the study of infantile neuroaxonal dystrophy and Hallervorden–Spatz disease.

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