Giant axonopathy (*gaxp*), a new spontaneous neuromuscular mutation on Chromosome 2 in the mouse

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Source of Support: This research was supported by NIH/NCRR grant RR01183 to the Mouse Mutant Resource (M.T. Davission, PI) and Cancer Center Core Grant CA34196.

Mutation symbol: *gaxp*

Mutation Name: giant axonopathy

Strain of origin: C3H/HeDiSnJ

Current strain of origin: C3H/HeSnJ-*Slc12a6*^{gaxp}/GrsrJ

Stock #004683 (jaxmice.jax.org)

Introduction

Giant axonopathy (*gaxp*) is a new autosomal recessive mutation that arose spontaneously in a mutant strain on the C3H/HeDiSnJ background in the Mouse Mutant Resource at The Jackson Laboratory. Homozygous mutants exhibit ataxia of hind legs with a slight side-to-side wobble as they walk. Histopathologic studies showed pale staining, vacuolated structures measuring up to 20 and 150 micra in deep cerebellar nuclei, pons, lateral vestibular nuclei and dorsal root and trigeminal ganglia. Ultrastructurally these structures were often bounded by a few layers of myelin, suggesting that they are swollen axons. We used an intercross between the C3H/HeDiSnJ-*gaxp* mutant and C57BL/6J to map the mutation to Chr 2. The most likely gene order places the mutation between *D2Mit128* and *D2Mit102* in 174 meioses tested.

Materials and Methods

Mice. All the mouse stocks used in this study were reared under modified barrier conditions at The Jackson Laboratory in the Mouse Mutant Resource (Davisson,1990). The *gaxp* mutation occurred in the C3H/HeDiSnJ-nm992 strain and originally was designated nm 2070. It is now maintained on the C3H/HeDiSnJ strain. For linkage analysis, C3H/HeDiSnJ-*gaxp/gaxp* mice were crossed to C57BL/6J to produce F1s. F1 hybrids from this initial cross were then intercrossed to produce F2 progeny. The F2 progeny were scored visually at 2 months of age. At that age when mutants are pulled backward by their tails in a cage with shavings they curl their hind legs towards their bodies, unlike normal mice which actively move their legs to resist being pulled backward. Spleens from 87 *gaxp/gaxp* mutant animals were collected and stored at -70C for subsequent DNA typing to map the mutation.

Histological techniques. Tissues for histopathological examination were prepared from 24 *gaxp/gaxp* and 5 +/? animals ranging in age from 2 to 12 months. Tissues were fixed *in situ* in mice deeply anesthetized with tribromoethanol (Avertin) by intracardiac

perfusion with Bouin's solution following a flush of physiological saline. After demineralization of bones in Bouin's solution for one week, midsagittal slices of hind legs and multiple cross sections of spine with spinal cord were cut. Six coronal slices of brain from olfactory lobes, caudally to cerebellum were also cut and representative slices of all somatic organs were prepared. Care was taken to ensure that neural tissue was not immersed for prolonged periods in ethanol, which is well known to produce artifactual vacuoles in white matter (Bronson, 2000). Tissues were embedded in paraffin and 6 micron sections were cut and stained with hematoxylin and eosin (H&E). Brain and spinal cord sections also were stained with luxol fast blue-cresylecht violet (LFB-CV).

Electron Microscopy: Two 5-month-old mutant mice were perfused with Karnovski's solution. Dorsal root ganglia were dissected out and embedded in epon. Thin sections were stained routinely and examined in an electron microscope.

Hearing test: Hearing was assessed by ABR (auditory brainstem response) threshold analysis (Zheng et al,1999) in two mutant mice.

Genetic analysis : Intercrosses with linkage testing stocks and with C57BL/6J were utilized to produce mutant mice. A genome scan was carried out to determine the chromosomal location of the *gaxp* mutation.

DNA isolation: DNA was extracted from the frozen spleens of 87 mutant (homozygous) F2 mice produced in the linkage cross by standard phenol/chloroform extraction procedures.

Polymerase chain reactions: PCR Primer pairs (MapPairs, Research Genetics, Huntsville,Ala.) of microsatellite markers *D2Mit386, D2Mit249, D2Mit128, D2Mit102, D2Mit484, D2Mit17* and *D2Mit51* were used to localize the new mutation on Chr 2. PCR analyses were carried out in 10 ul total volume reactions containing 20 ng genomic DNA, by previously described methods (Ward-Bailey et al. 1996).

Linkage analysis: Gene order and recombination frequencies were calculated with the Map Manager computer program (Manley 1993), a MacIntosh program for storage and analysis of experimental mapping data. The complete Chr 2 linkage data for 87 F2 *gaxp/gaxp* mice have been deposited in the Mouse Genome Database, accession number J:82451.

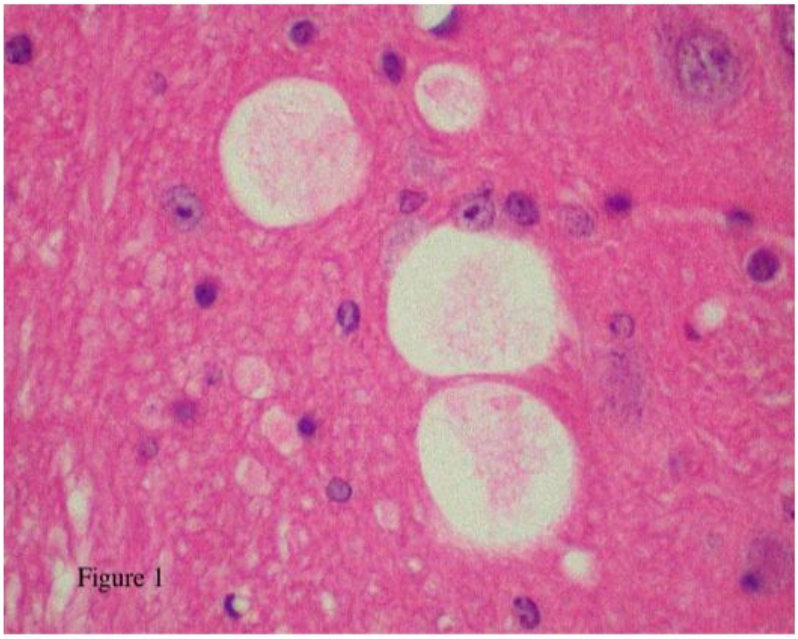
Results

Phenotypic characterization: Mutant mice are of normal size and both sexes are fertile. They exhibit a subtle clinical abnormality as they walk. They tend to lift their hind legs too high and have a slight side-to-side wobble. The most accurate way of diagnosing mutants is to drag them backwards by their tails.

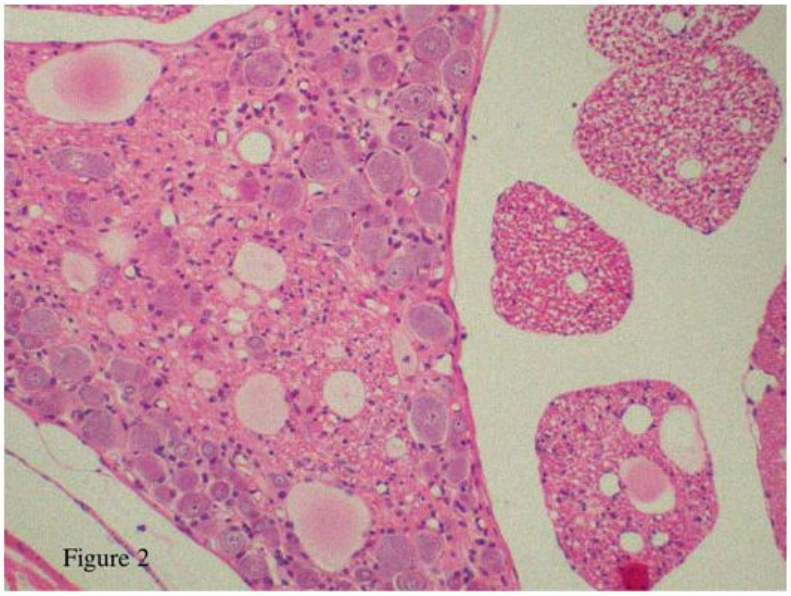
Hearing tests: Hearing is normal in these mutants.

Histopathology: Lesions were observed in white matter associated with dorsal root

ganglia, dorsal roots, deep cerebellar nuclei, lateral vestibular nuclei and pons. Other parts of the CNS and PNS were normal as were somatic organs, In H&E stained sections the lesions were large vacuoles ranging in size from 20 to 130 micra and containing flocculent pale eosinophilic material. The centers of some vacuoles contained more darkly staining eosinophilic material. Only rarely were vacuoles entirely empty, and none contained the pale basophilic artifactual material often observed in brains immersed in ethanol (Bronson, 2000).



Deep cerebellar nucleus from a 7-month-old *gaxp* homozygote showing several pale eosinophilic vacuoles. H&E 50X

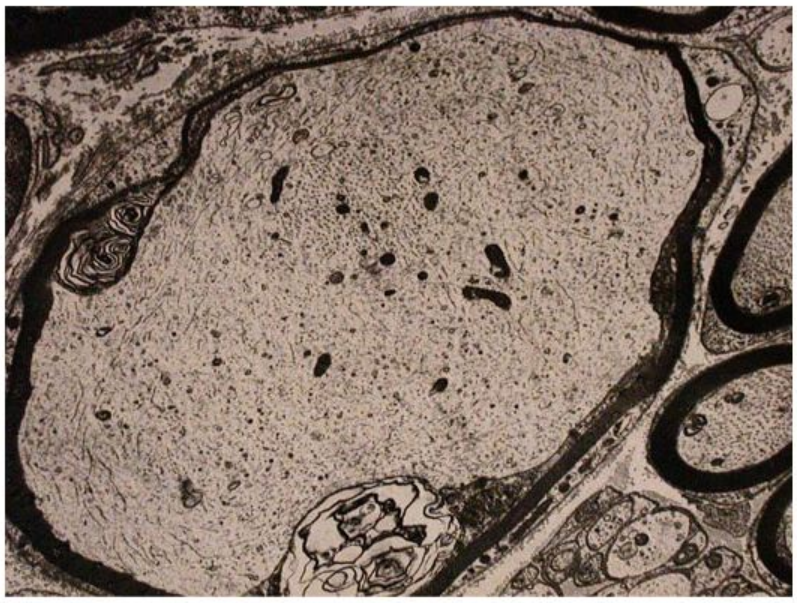


Dorsal root ganglion from the same mouse showing vacuoles of various sizes intermixed with nuclei of large neurons. H&E 25X

The lesions were not similar to typical dystrophic axons so commonly observed in diseased mouse brains (Bronson et al., 1990). Dystrophic axons stain much more intensely with eosin than do the vacuoles in *gaxp/gaxp* mice. In LFB/CV stained sections the vacuoles are empty or at most contain a small amount of blue staining material near of the center of the vacuole. At the level of light microscopy the LFB does not stain any

material around the vacuoles suggestive of myelin. Studies of mutant mice at different ages suggested that the numbers of affected axons increase with age and become much larger during the first year of life.

Electron microscopy: Ultrastructurally vacuoles appear to be greatly swollen axons surrounded by a few lamellae of myelin. In some places the myelin forms irregular myelin figures The cytoplasm of the axons contain widely separated mitochondra and loosely packed filamentous material, presumably neurofilaments, as well as membranous debris.



Ultrastructure of a dystrophic axon from dorsal root ganglion from a 5-month-old mutant with dispersed neurofilaments and a few mitochondria. A myelin sheath of variable thickness surrounds the axon. Several axons of normal size are also shown. X5000

Genetic Analysis: That *gaxp* is inherited as a recessive mutation was shown by inheritance and linkage cross data. Tests for allelism with anorexia (0 nm/50 progeny)and hotfoot<4J> (0 nm/17progeny) were negative. Mutation segregation ruled out Chr X linkage. A genome sweep of mutant F2 progeny from the C57BL/6J intercross was done by making a DNA pool from (21) mutant mice. (Taylor et al, 1994). PCR analysis of the DNA pool compared to the parental controls revealed a deficiency of C57Bl/6J specific products for Chr 2 with *D2Mit102* and *D2Mit51*. Individual DNA samples were then typed with typed these and five additional markers. The recombination estimates with standard errors and best gene order are centromere-*D2Mit386*-3.04 +/-1.3-*D2Mit249*-1.18 +/- 0.83-*D2Mit128*-1.75 +/- 1.00-*gaxp*-*D2Mit102*-1.17 +/- 0.82-[*D2Mit484*, *D2Mit17*]-18.82 +/- 4.0 – *D2Mit51*.

Discussion

Neuropathology: The swollen axons observed in *gaxp/gaxp* mice are a unique form of axonal dystrophy. Whereas the usual dystrophic axons are densely eosinophilic and are composed of densely packed organelles and filamentous material, the axons in these mutants contain only lightly packed organelles, suggesting that the swelling is due in part to uptake of water. Two inherited diseases of children characterized by dystrophic axons are infantile neuroaxonal dystrophy and giant axonal neuropathy. These are characterized histopathologically by accumulation of the dense form of dystrophic

axons. The genes for these human diseases have not been mapped.

Homologous human gene: A human disease gene, spinocerebellar ataxia type 5 (SCA5), is a non-life threatening heritable disease with onset between the ages of 10 and 68 years. The neuropathology of this disease has not been described. SCA5 maps to 11p11-q11, a region that is homologous to the map location of *gaxp*.

Candidate genes. A number of genes are known to link to the interval in which *gaxp* is located. Among these are brain derived neurotrophic factor precursor and voltage-gated potassium channel protein aKV1.4.

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