A Neurological Mutation on Mouse Chromosome 14 named agitans-like.

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Mutation (allele) symbol: *agil*

Mutation (allele) name: agitans-like

Gene symbol: *agil*

Strain of origin: C3H/HeJ

Current strain name: C3H/HeJ-*agil*/GrsrJ

Stock #004780 (jaxmice.jax.org)

Phenotype categories: neurological

Abstract
A neurological mutation named agitans-like has been found at The Jackson Laboratory. This autosomal recessive mutation is recognized by 15 days of age by its shaky, unsteady, wobbly gait. Mutants die around 3 weeks of age. This mutation maps to Chromosome 14 between *D14Mit39* and *D14Mit115*which is near the neurological mouse mutation agitans (*ag*).

Origin and Description
This mutation was found in April 1999 in the strain C3H/HeJ at F233 by Faye Leonetti in a production colony at the Jackson Laboratory and brought to the Mouse Mutant Research (MMR) deviant search program. Homozygous mutants are easily recognized by 15 days of age by their wobbly gait and die about a week later. The strain is maintained by progeny testing.

Genetic Analysis
Tests for Allelism
A test for allelism with quaking (*qk*) produced 0 *qk*/16 born.

A test for allelism with wasted (*wst*) produced 0 *wst/* 24born.

A test for allelism with shiverer (*shi*) produced 0 *shi*/33 born.

Tests for allelism were done with *agil* and three other phenotypically similar new mutants in the MMR and no mutants were produced.

A test for allelism with wabbler lethal (*wl*) which is listed as syntenic on Chr 14 is currently in progress.

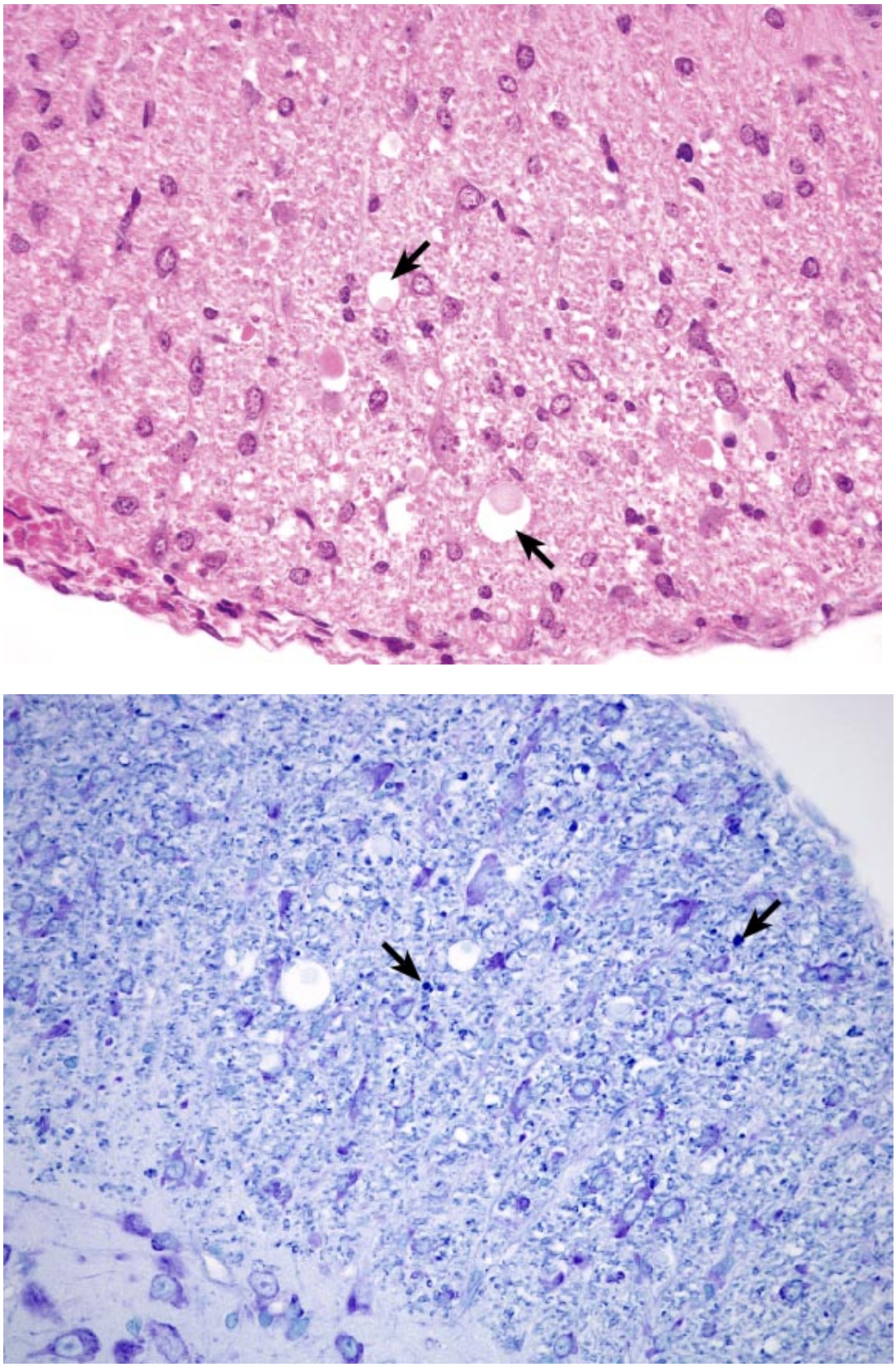
Linkage Cross for Mapping

A heterozygous *agil* male was crossed to two female C57BL/6J mice and produced F1 progeny. Using our standard MMR procedures[1], these F1s were intercrossed to produce 72 F2 affected animals, of which 21 were used to determine the chromosomal location of the agitans-like mutation. A genome-wide scan using a DNA pool of 21 affected animals and parental controls indicated linkage on Chromosome 14 with *D14Mit166.* The individual DNAs from the linkage cross were then typed for this marker and 5 additional Chr 14 markers. The recombination estimates with standard errors and best gene order are: centromere-*D14Mit5*- 2.4 +/- 2.4-*D14Mit39* - 2.4 +/-2.4-*agil*-2.4 +/- 2.4 - [*D14Mit115*, *D14Mit225*]-16.6 +/- 7.3 ? [*D14Mit265*, *D14Mit166*]. Gene order and recombination frequencies were calculated with the Map Manager computer program (Manley 1993). The complete Chr 14 linkage data for 21 F2 *agil*/*agil* mice has been deposited in the Mouse Genome Database, accession #J85257. Based on the Ensembl assembly for Chr 14, the chromosomal position for *agil* is between 59213353 bp (*D14Mit39*) and 63304173 bp (*D14Mit115*). A neurological mutant named agitans (ag) has a very similar phenotype to this new mutation,and maps near the hairless locus (*hr*) which is located at 61014601-61032224 bp. Agitans-like may be a remutation to agitans, but a direct test for allelism was not done because the agitans strain is not available here at The Jackson Laboratory.

Pathology

A pathological screen[2] of ten mutants and two controls, all at about 3 weeks of age, had slightly less deeply staining myelin in spinal cord stained with luxal fast blue. Three of ten mutants had a few scattered dystrophic axons and degenerating myelin sheaths (see histology below) in spinal cord white matter. These changes are consistent with early degeneration of spinal cord white matter. Hearing was assessed by ABR testing[3] on three homozygous mutants and six +/- controls. The controls all had good hearing. The mutants varied from being deaf to showing moderate hearing loss. One of the homozygous mutants had little hearing with a long latency/central pathway problem as observed in the mutants shiverer and quaking.

Weight measurements were made on four 21-day old male mice. Two homozygous mutants appearing near death weighed 6.1 grams and 5.4 grams as compared to two littermate controls that weighed 10.1 grams and 10.6 grams.



scattered dystrophic axons and degenerating myelin sheaths in spinal cord white matter from an *agil* homozygote

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References
Mouse Genome Database (MGD) Mouse Genome Informatics Project, The Jackson Laboratory, Bar, Harbor, Maine. World Wide Web (informatics.jax.org).

Manley KF (1993) A MacIntosh program for storage and analysis of experimental mapping data. Mamm Genome 4,303-313.

Footnotes
[1]**Standard Mapping Protocol used in The Mouse Mutant Resource**

Linkage crosses

To map new mouse mutations we use CAST/Ei, an inbred strain of *Mus musculus castaneus*, as our standard linkage testing strain. In some cases, because of breeding difficulties or reduced phenotypic penetrance, we use other strains. An intercross of F1 hybrids is usually used to analyze linkage of recessive mutations and a backcross to analyze linkage of dominant mutations. Our goal is to produce enough informative mice from each mapping cross to test the recombinational products from at least 100 meioses.

DNA isolation

DNA is extracted from the frozen tail tips of mutant (homozygous) F2 mice or backcross progeny by a standard hot sodium hydroxide and Tris (Hot SHOT) procedure (Truett, et al., 2000) or from spleens using standard phenol extraction methods.

Polymerase chain reaction

PCR primer pairs (MapPairs, from Research Genetics, Huntsville, Ala., or from Integrated DNA Technologies, Coralville, Ia.) are used to type MIT microsatellite markers positioned throughout the genome. PCR reactions contain 20 ng genomic DNA in 10 ul containing 50 mM KCL, 10 mM Tris-HCL (pH 9.0 at 250C), and 0.01% Triton-X-100, 2.25 mM MgCl2, 100 nM of each primer (forward and reverse), 100 uM of each of four deoxyribonucleoside triphosphates, and 0.5 Units of Taq DNA polymerase (Amplitaq from Applied Biosystems #N808-0145). Amplification consists of one cycle of denaturation at 940C for 3 minutes followed by 30 cycles, each consisting of 940C for 15 sec. denaturation, 550C for 2 minutes of annealing, and 720C for 2 minutes of extension. After the 30 cycles, the final product is extended for 7 minutes at 720C. The PCR products are run on 2.5% Metaphor agarose gels or 6% polyacrylamide (non-denaturing) gels. The gels are then stained with ethidium bromide, destained with distilled water, visualized on a UV light table, and photographed.

Pooled DNA Method (Taylor et al 1994).

In the case of an intercross, a pool of DNA prepared from 25-30 mutant F2 mice is compared with DNA from F1 hybrids. In the case of a backcross, a DNA pool from 25-30 mutant N2 mice is compared with a pool from 25-30 unaffected N2 mice. These DNA samples are typed by PCR for MIT markers located throughout the genome. For linked markers, the mutant strain allele will predominate in the DNA pool from mutant mice compared with controls. When a particular marker indicates linkage by analysis of the pooled sample, individual DNA samples are typed with that marker and additional markers in the same region to confirm linkage. Once a linkage is confirmed, additional DNAs from individual mice are typed to obtain a finer map position.

Linkage analysis

Gene order and recombination frequencies are calculated with the Map Manager computer program (Manley1993, 2001), a MacIntosh program for storage and analysis of genotyping data.

References

Manley KF (1993) A MacIntosh program for storage and analysis of experimental mapping data. Mamm Genome 4: 303-313.

Manly KF, Cudmore RH Jr, and Meer JM (2001) Map Manager QTX, cross-platform software for genetic mapping. Mamm Genome12: 930-932.

Taylor BA, Navin A, and Phillips SJ (1994) PCR-amplification of simple sequence repeat variants from pooled DNA samples for rapidly mapping new mutations of the mouse. Genomics 21: 626-32.

Truett GE, Heeger P, Mynatt RL, Truett AA, Walker JA, and Warman ML(2000) Preparation of PCR-quality mouse genomic DNA with hot sodium hydroxide and Tris (HotSHOT). Biotechniques 29:52-54

[2]**Standard Histology Protocol used in The Mouse Mutant Resource**

For fixation of tissues, mice were deeply anesthetized with tribromoethanol (avertin) until they no longer displayed a withdrawal reflex in the hind limbs and then perfused intracardially with Bouin's fixative following a flush of the vasculature with saline solution. After soaking in Bouin's for one week to demineralize bones, tissues were dissected. Six segments of spine with axial muscles and spinal cord in situ, representing cervical, thoracic and lumbar spinal segments, were dissected. The brain was removed and sliced into 6 cross sectional pieces at the levels of olfactory lobes, frontal cortex, striatum, thalamus, midbrain, rostral and caudal medulla with cerebellum. Midsagittal slices of hind leg through the knees were prepared. Slices of basal skull through the pituitary and inner ears were taken. Both eyes, salivary glands and submandibular lymph node, trachea plus thyroid and sometimes parathyroid were removed and cassetted. A longitudinal slice of skin from the back was removed. The thymus, slices of lung, and a longitudinal slice of heart were cassetted. Similarly slices of liver through gall bladder, kidney with adrenal attached, pancreas and spleen were prepared. The stomach was sliced longitudinally to include both squamous and glandular portions. Loops of small intestine from 3 levels and slices of large intestine and cecum were removed, as were slices of urinary bladder. The whole uterus, with ovaries attached, was taken. In males testes were sliced longitudinally. The accessory male organs including seminal vesicles, coagulating gland and prostate were removed en block. Altogether in most cases all tissue fit into a total of 10 cassettes. The cassettes were processed in an automatic tissue processor to dehydrate tissues which were then embedded in paraffin. Six micron sections were cut and stained with hematoxylin and eosin (H&E). Sections of brain and spinal cord in vertebral bones also were stained with luxol fast blue (LFB) for myelin and cresylecht violet (CV) for cellular detail.

[3]**ABR thresholds** in mice are determined using a semi-automated computer system (Intelligent Hearing Systems, Miami, Florida). Subdermal needle electrodes are inserted at the vertex and ventrolaterally to both ears of anesthetized mice. Specific auditory stimuli from 10-100 dB SPL are delivered binaurally through plastic tubes from high frequency transducers. ABR thresholds are obtained, in an acoustic chamber, for clicks and for 8, 16, and 32 kHz pure-tone pips.