Agitans-like 2 Jackson: A remutation to agitans-like on Chromosome 14.

Authors: Belinda S. Harris, Patricia F. Ward Bailey, Kenneth R. Johnson, Roderick T. Bronson, Muriel T. Davission

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Mutation (allele) symbol: *agil*^{2J}

Mutation (allele) name: agitans-like 2 Jackson

Gene symbol: *agil*

Strain of origin: AKR/J

Current strain name: AKR/J-*agil*^{2J}/J

Stock #008657

Phenotype categories: neurological

Abstract
We have identified a mutation causing affected mice to have an abnormal gait that can be recognized at two weeks of age. A direct test for allelism confirmed this new mutation to be a remutation to agitans-like (*agil*).

Origin and Description
The agitans-like remutation was found by Bonnie Williams at The Jackson Laboratory in a production colony of AKR/J mice in 2006. Mice affected by this new mutation were initially identified by their wobbling gait. When picked up by the tail, affected mice pull in their back legs in toward their bodies. The homozygous mutant mice are smaller than their littermates, both sexes breed, and they live a normal lifespan.

Genetic Analysis
Using our standard mapping protocols, a linkage cross was set up by mating a C57BL/J female mouse to a male AKR/J mouse affected by this new mutation. No affected animals were seen in the F1 progeny produced by this cross thereby demonstrating recessive inheritance. The F1 progeny were then intercrossed and the affected homozygous F2 mice produced were utilized for linkage analysis. Use of MIT markers *D14Mit133* and *D14Mit101* positioned this mutation near the previously described agitans-like (*agil*) mutation. A direct test for allelism between two heterozygous female mice carrying the *agil* mutation and a male heterozygous for this new mutation produced four affected homozygotes out of thirteen progeny born, proving allelism.

Pathology
A routine pathological screen[1] of two female homozygotes at three weeks of age showed dystrophic axons in the spinal cord and brain stem. Two mutant males at three weeks of age had dystrophic axons and vacuoles in the spinal cord white matter, cerebellar

peduncles and eighth cranial nerve root.

Hearing as assessed by auditory brainstem response testing (ABR) on three *agil*^{2J} homozygotes at one month of age showed normal hearing. One control tested at the same age had normal hearing.

The eyes of these same three mice at one month of age were examined with an ophthalmoscope and were determined to be normal.

Discussion

We report a remutation to *agil* which has pathology and a mapping position similar to the original *agil* mutation. This new remutation has been named *agil2J* and is available from the Jackson Laboratory DNA Resource.

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[1]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.