Lethargic 4 Jackson; a new spontaneous mutation in the *Cacnb4* gene

Authors: Son Yong Karst, Sandra Gray, Patricia F. Ward-Bailey, Leah Rae Donahue, Kenneth R. Johnson, Muriel T. Davisson

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

Mutation (allele) symbol: $Cacnb4^{lh\text{-}4J}$

Mutation (allele) name: lethargic 4 Jackson

Gene symbol: *Cacnb4*

Strain of origin: C3Fe.SWV-Mbp^{shi}/J

Current strain name: C3Fe(SWV)-$Cacnb4^{lh\text{-}4J}$/GrsrJ

Stock #007499 only available as DNA from the Jackson Laboratory DNA Resource

Phenotype categories: neurological/behavioral: motor

capabilities/coordination/movement

Abstract
The new recessive lethargic 4 Jackson mutation arose spontaneously and has been identified as a remutation of the *Cacnb4* gene by its map position on Chromosome 2 and by a direct test for allelism.

Origin and Description
The lethargic 4 Jackson remutation arose in a production colony of C3Fe.SWV-Mbp^{shi}/J mice at the Jackson Laboratory in 2001 and was discovered by Cheryl Crabtree. Mice homozygous for the new lethargic 4 Jackson remutation exhibit the wobbly gait behavior and smaller body size that is characteristic of the original lethargic mutation ($Cacnb4^{1h}$), however seizures have not been reported in this new remutation. Females breed but males have not bred to date. Both sexes live a normal lifespan.

Genetic Analysis
Using our standard mapping procedures C3Fe(SWV)-$Cacnb4^{lh\text{-}4J}$/J mice were mated to CAST mice. The progeny produced by that mating were then intercrossed and they produced 50 F2 mutant mice that were utilized for linkage analysis.

The $Cacnb4^{lh}$ mutation maps to Chromosome 2 between *D2Mit7* (NCBIm 36 position 38.06 Mb) and *D2Mit61* (NCBIm 36 position 60.49 Mb) and is non-recombinant with *D2Mit157* (NCBIm 36 position 55.6 Mb). The original $Cacnb4^{lh}/J$ mutation is located at (NCBIm 36) position 52.2-52.4 Mb.

A direct test for allelism was set up by mating a heterogous female B6EiC3Sna/A-$Cacnb4^{lh}$/J (Stock#000504) mouse with a heterozygous male C3Fe(SWV)-$Cacnb4^{lh\text{-}4J}$/J

mouse. This mating produced 38 progeny of which 10 expressed the mutant phenotype, proving allelism.

Pathology

A pathological screen[1] of one female homozygote at 9 months of age showed no lesions and the myelin looked normal. One homozygous male at 32 weeks age had fewer than normal granule cells at base of folia and no other lesions. Another homozygous male at 32 weeks of age showed mild hydrocephalus and possibly Purkinje cell dendrites.

Hearing as accessed by auditory-evoked brainstem response testing[2] (ABR) of mutant mice was normal. The eyes of mutant mice were examined with an ophthalmoscope and displayed retinal degeneration which is clinically normal for the C3H mouse strains, the predominant inbred background in the C3Fe(SWV)-*Cacnb4*$^{lh\text{-}4J}$/J strain, and not caused by the *lh-4J* mutation.

Acknowledgements

The authors wish to thank Cheryl Crabtree for discovery of the mutant, Chantal Longo-Guess for hearing assessment, Norm Hawes and Ron Hurd for the eye examinations, and Coleen Marden for excellent pathological skills.

[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.

[2]Auditory-Evoked Brainstem Response (ABR) Thresholds

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. ABR thresholds of all mice and strains tested are entered in spreadsheet files for storage, easy access, and for the production of periodic progress reports. Click-evoked ABR waveforms, obtained at threshold (T) and at T+10, T+20 and T+30 dB or each mouse, are also stored for future reference. Mice of the CBA/CaJ strain are tested periodically as references for normal hearing, and for monitoring the reliability of the equipment and testing procedures.