Lethal Wasting (*lew*), a New Mouse Mutation causing Prewean Lethality

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Mutation Symbol: *lew*

Mutation Name: Lethal Wasting

Gene symbol: *Vamp1*

Strain of origin: C3H/HeDiSnJ

Current strain name: C3H/HeDiSnJ-$Vamp1^{lew}$/GrsrJ

Stock #004626 (jaxmice.jax.org)

Abstract
A novel spontaneous mutation causing lethality, lethal wasting (*lew*) appeared in the inbred C3H/HeSnJ in 1985. Mice homozygous for this recessive mutation are recognized by their wasting pre-weaning lethal phenotype, they curl up, waste away and die by 21 days. Mutants do not live to breed but no lesions have been found pathologically. The colony is maintained by progeny test. We mapped this mutation to Chr 6. No candidate genes have been identified for this mutation.

Origin and Description
A new autosomal recessive mutation " lethal wasting" was found at The Jackson Laboratory in 1985 on an inbred C3H/HeSnJ background at F16+5 N1F7 in the C3H/HeSnJ colony. 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 mutant is characterized by a wasting phenotype. The mutant lays on its side and dies by 19-21 days of age. By the age of two weeks the mutant curls its front paws inward and holds its back legs tightly together as it lays on its side. Before death the animals breathing is very shallow and it is so weak that sometimes it is buried under the shavings in the box as if it were dead. Further examination shows it to be alive, but barely. At two weeks of age the mutants' body weights are less than their littermates by approximately 30%. This colony is maintained on its own inbred background by progeny test, as homozygous mutants are not able to breed. The lethal wasting mutation was mapped to Chromosome 6 using an intercross with CAST/Ei (*Mus musculus castaneus*).

Genetic Analyses
Breeding data from both the maintenance cross and the CAST/Ei mapping intercross show a recessive inheritance. The mating scheme of heterozygote x heterozygote produces approximately 22.3 % mutants, which is somewhat less than the expected 25%.

The CAST/Ei intercross produced all phenotypically normal offspring in the F1 generation. Homozygotes did appear in the F2 generation from this cross at about 12.7 % or up to 27.0 % if dead mice that are of unknown genotype (missing, found dead) are taken into account.

The progeny of an intercross to CAST/Ei were used to map this mutation to Chr 6. A female CAST/Ei was mated to a heterozygote mutant (homozygotes do not survive to breed). Each pair of mice was progeny tested to show the mutant phenotype and those pairs that tested were used for analysis. Genomic DNA was prepared from spleen and tail samples from 46 F2 mutant progeny by standard methods. DNA pools were made with DNAs from F2 mutant mice for mapping (Taylor et al, 1994). Selected microsatellite polymorphisms from each chromosome were typed by the polymerase chain reaction(PCR) using previously described methods (Ward-Bailey et al.1996) with primer pairs from Research Genetics. Amplified products were electrophoresed through 2.5% metaphor gels and visualized with ethidium bromide. PCR analysis of a DNA pool of equal aliquots from 18 F2 mutants from the CAST/Ei intercross revealed a deficiency of CAST/Ei products from genes on Chr 6 when typed with *D6Mit12*. DNAs from individual animals were then typed for three additional Chr 6 markers. Data was analyzed using Map manager (Manley, 1993). The recombination estimates with standard errors and the best gene order are centromere-*D6Mit11*-2.19 +/- 1.52-*D6Mit55*-7.76 +/-2.76- [*lew, D6Mit12*]- 3.29+/- 1.85 - D6Mit111. We detect no recombination between *lew* and *D6Mit12* in the 46 *lew/lew* homozygotes typed . The complete Chr 6 linkage data for 46 F2 *lew/lew* mice have been deposited in the Mouse Genome Database, accession # J:81354.

The mutations opisthotonos (*opt*), cerebellar deficient folia (*cdf*), and deaf waddler (*dfw*), located in the same region of Chr 6, were tested for allelism with *lew*. All allelism tests were negative.

Pathology

Comparison of mutants and controls showed no lesions. Tissues for histopathological examination were prepared from 15 mutants at approximately 2 weeks of age. Tissues were fixed *in situ* in mice deeply anesthesized with tribromoethanol (Avertin) by intracardiac perfusion with Bouin's solution following a flush of psysiological saliine. After demineralization, multiple cross sections of thoracic and lumbar vertebrae with spinal cord were prepared. Brains were sectioned coronally from the cerebrum through the cerebellum. Cross and longitudinal sections of lumbar muscles, fore-and hind limb muscles, and samples of each of the somatic organs (liver, spleen, pancreas, stomach,small intestine, colon, cecum, lungs, thymus and heart) also were prepared. For light microscopy sections all tissues were embedded in paraffin and stained with hematoxylin and eosin (H&E). Brain and spinal cord sections also were stained with Luxol fast blue-cresylecht violet (LFB-CV) and Bodian's stain. The comparison of these mutants with control animals showed no lesions. Dr. Phil Wood screened these mice for blood serum levels and found no differences between mutants and controls. Eyes of both mutants and controls were examined using a slit lamp ophthalmoscope and found to have

normal irises and lens. Hearing was tested using auditory brain stem response (ABR) and found mutant mice too young before death to get a reliable reading.

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Addendum

This mutation was found to be a G to T transversion in the gene vesicle-associated membrane protein 1, which results in a premature stop codon. Please see Nystuen et al., Neurogenetics 2007, 8(1) p1.