# Tiny wasting (*tnyw*); a new autosomal recessive mutation on Chromosome 3

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Mutation (allele) symbol: *tnyw*

Mutation (allele) name: tiny wasting

Strain of origin: B6,129-*Pemt2*$^{tmlJ}$

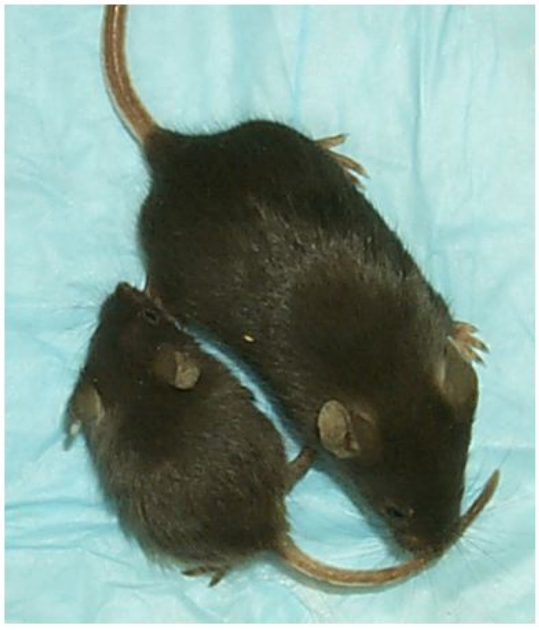
Current strain name: B6;129P2-*Pemt2*$^{tmlJ}$*-tnyw/*GrsrJ

Stock #005323 (jaxmice.jax.org)

Phenotype categories: size, neurological

**Abstract**
A new autosomal recessive mutation has been identified and named tiny wasting (*tnyw*). This mutation in the homozygous state causes affected mice to exhibit runting, an inability to coordinate movement, and premature death at around 3 weeks of age. This new mutation has been mapped to Chromosome 3.

**Origin and Description**
The *tnyw* mutation arose in a research colony of B6,129-*Pemt2*$^{tmlJ}$ mice at the Jackson Laboratory and was discovered by Leah Rae Donahue. This mutation causes a variable phenotype. All homozygotes have a reduced lifespan with most animals dying by 3 weeks of age. All mutants are smaller in size compared to their unaffected littermates.



A small and wasting mouse homozygous for the *tnyw* mutation is shown on the left and a littermate control is seen on the right. Both at 3 weeks of age.

Some mutants are unable to right themselves or have abnormal locomotion. Homozygotes can be identified by two weeks of age by their smaller wasting bodies. The *tnyw* colony is maintained by transplanting the ovaries of an affected female into a C3Smn.CB17-*Prkdc*$^{scid}$/J female mouse that is then mated to a *tnyw* heterozygote male mouse.

Two small mice affected by the *tnyw* mutation are shown on the left with a larger unaffected littermate control shown on the right. All two weeks of age.

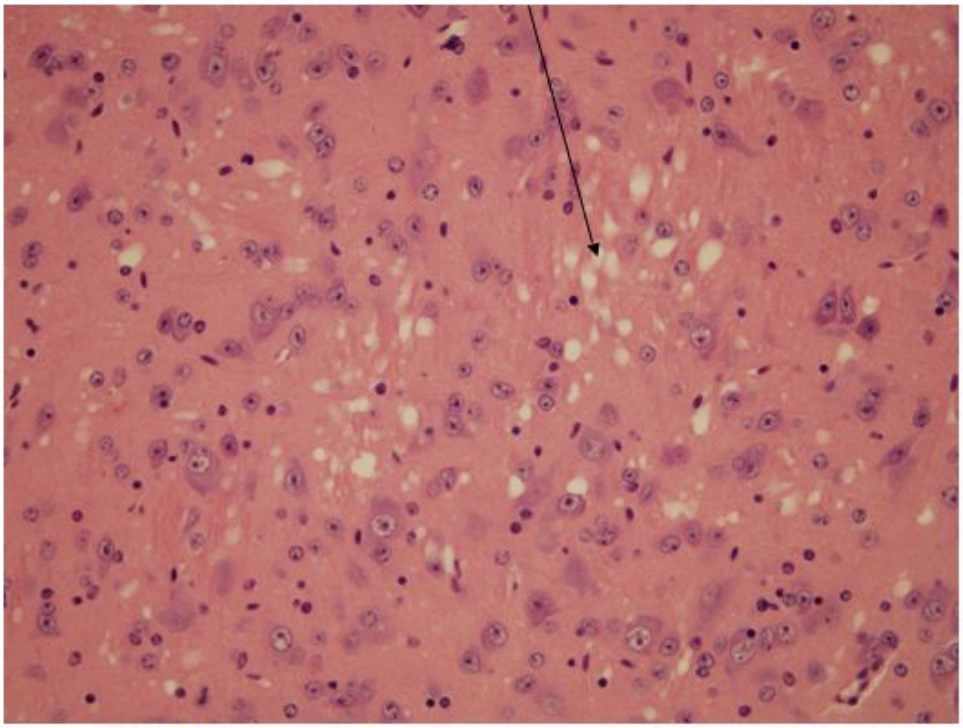
**Genetic Analysis**

In order to find the chromosomal location of the *tnyw* mutation a linkage cross was set up by mating an ovarian transplanted female mouse homozygous for the *tynw* mutation to a CAST/Ei male. The tested heterozygous F1 progeny from this cross were then intercrossed and in 7 matings produced 80 F2 mutant mice. The *tynw* mutation is inherited recessively as shown by traditional linkage cross analysis. No mutants were seen in the F1 generation of the linkage cross, and 25% of the F2 progeny produced were mutants, as expected.

Using our standard mapping protocols[1] we mapped this new mutation to Chromosome 3. Linkage was first found with microsatellite markers *D3Mit11* (NCBI 36 position 100.6 Mb) and *D3Mit29* (NCBI 36 position 90.7 Mb), both showing 2.4 % recombination. The mutation maps between *D3Mit11* and *D3Mit288* (NCBI 36 position 121.7 Mb) and is non-recombinant with *D3Mit346* (NCBI 36 position 116.1 Mb) and *D3Mit57* (NCBI 36 position 115.8 Mb).

**Pathology**

A routine pathological screen[2] of 44 *tnyw*/*tnyw* mutant mice from 16 days of age to 3.5 weeks of age was performed. There were vacuoles in the brain of all mutant mice. Sections of thyroid glands, pituitary glands, and somatic organs revealed no abnormalities.



A histopathological section of brain from a mouse homozygous for *tnyw* shows groups of small vacuoles scattered in the neuropil of the brain stem.

The eyes of 2 heterozygotes and 4 homozygotes were examined with an ophthalmoscope and the eyes of the heterozygotes were normal. The eyes of one of the 4 homozygotes had iris threads in the right eye and another mouse had a cataract in the right eye and the other two were normal. An abnormal lens was seen in four of the eyes. Slit lamp pictures show a suture cataract and lens is not round.

Hearing as assessed by auditory brainstem response testing on 2 mutants and 1 control revealed no hearing loss.


## Acknowledgements

The authors thank Norm Hawes for the eye examinations and Chantal Longo-Guess for the hearing assessments.


## Protocols

**[1]Standard Mapping Protocol used in the MMR**

**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.