Totterer: a new mutation in the myelin protein zero (*Mpz)* gene.

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Mutation (allele) symbol: *Mpz*^{ttrr}

Mutation (allele) name: totterer

Gene symbol: *Mpz*

Strain of origin: B6.129P2-*Nos3*^{tm1Unc}/J

Current strain name: B6.Cg-*Mpz*^{ttrr}/GrsrJ

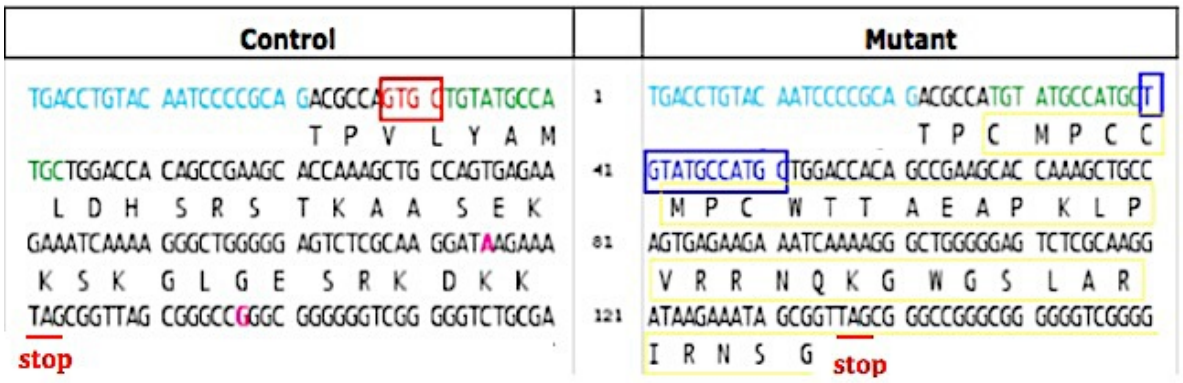
Stock #010494 (jaxmice.jax.org)

Phenotype categories: Neuromuscular

Abstract
A new spontaneous recessive mutation has been discovered and characterized at The Jackson Laboratory. Mice affected by the new totterer mutation have a neuromuscular phenotype causing a shaky gait. PCR and sequence analysis determined that this is a mutation in the myelin protein zero (*Mpz*) gene.

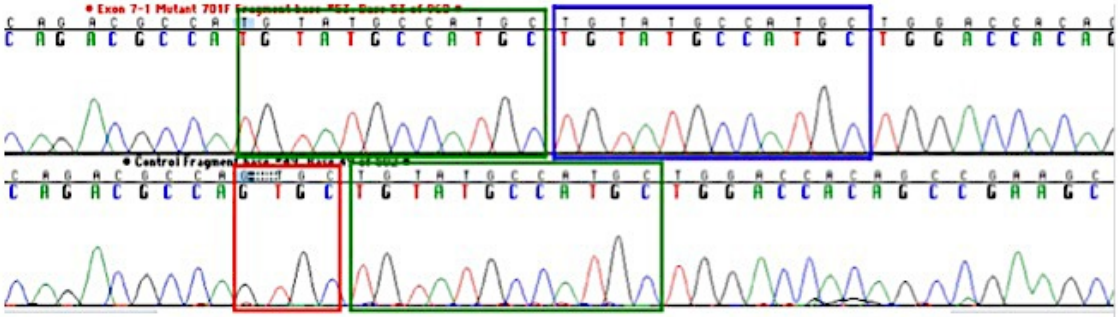
Origin and Description
The new spontaneous recessive totterer mutation arose in a production colony of B6.129P2-*Nos3*^{tm1Unc}/J mice at The Jackson Laboratory and was discovered by Victoria Gerrish. These mutant mice maintain normal weight and exhibit mild tremors first seen at about 2 weeks of age. The phenotype of the mutant mice slowly becomes more severe at six to eight weeks of age. Affected mice rapidly develop worsening muscle weakness characterized by a shaking, limb grasping, hunched, sprawled, and tottering walk. Affected animals have a shortened lifespan dying at irregular intervals. Female and male mutant mice may breed once or twice in their lifespan, but these animals often fail to breed. Female mutant mice are poor mothers due to the phenotype. This colony is also maintained by ovarian transplantation.

Genetic Analysis
Following our standard mapping protocols, a mouse homozygous for the *ttrr* mutation was mated with a CAST/EiJ mouse. This mating produced normal appearing F1 progeny. The F1 hybrid mice were then intercrossed to produce affected F2 mice for linkage analysis. This mutation maps to Chromosome 1 between *D1Mit114* (NCBI 37 position170.8mb) and *D1Mit150* (NCBI 37position 176.5mb). Based on phenotypic similarities to the *Mpztm1Msch* knockoutalle and map location, the *Mpz* gene was considered a candidate gene for this new mutation. Sequence analysis revealed a mutation in exon 8.



A. Protein coding region of Exon 8 of *Mpz*. The control DNA sequence translation is shown on the left, and the *ttrr* mutant DNA and its translation on the right. The four nucleotides deleted in the mutant sequence are enclosed by a red box in the control sequence, and the 12 green nucleotides duplicated in the mutant sequence are enclosed by a blue box in the mutant sequence. The mutation is predicted to change the reading frame resulting in multiple amino acid changes at the protein terminus (enclosed in yellow box).

A PCR product from genomic DNA was used to sequence the *ttrr* mutation. Primers were generated that produce a 517 base pair product flanking exon 8 of the *Mpz* wildtype allele; primer exon 8 Left (AAGTCCGGGACAGCAGTG) and primer exon 8 Right (GAGTACAAGACTTGGAAAGGAAGG). Sequence analysis of mutant DNA identified a four bp deletion and adjacent 12 bp duplication which alter the reading frame in the extracellular N-terminal region of the mutant MPZ protein.



B. Comparison of DNA sequence chromatograms of the homozygous *ttrr* (top) and +/+ control (bottom). The red and blue boxed region correspond to the red and blue boxed regions shown in A.

Pathology

A routine pathological examination[1] of eleven homozygous mutant mice revealed the following pathology consistent with central and peripheral demyelination:

One homozygous mutant at age 18 weeks had hypomyelination of peripheral nerves. Two homozygous mice at age 18 weeks showed degeneration and paucity of peripheral myelin and also showed degeneration of the dorsal columns of the spinal cord.

Two homozygous mice at age 20 weeks showed thin peripheral myelin and degeneration of the dorsal columns of the spinal cord.
Two homozygous mice at age 23 weeks had hypomyelination of peripheral nerves.
One homozygous mouse at 26 weeks of age showed deficient peripheral myelin and myelin degeneration.
One homozygous mouse age at 28 weeks showed many nerves having too many Schwann cell nuclei, hyperproliferation of Schwann cells, poorly developed myelin, and preneoplasia. These abnormalities could develop nerve sheath tumors.
One homozygous mouse at age 32 weeks showed deficient peripheral myelin and large axons.
One homozygous mouse at age 33 weeks showed peripheral neuropathy with large axons with thin myelin sheaths and degenerating myelin in central part of cranial nerve 5.

The eyes of one homozygous mouse at 12 weeks of age were tested by an electroretinograph (ERG) and found to be normal.

Hearing as assessed by auditory-evoked brainstem response testing of one homozygous mouse at 12 weeks of age showed normal hearing.

Acknowledgements
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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.