# A new spontaneous mutation resulting in body tremor and a tilted head

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Mutation (allele) symbol: *tth*

Mutation (allele) name: tremor with tilted head

Gene symbol: *tth*

Strain of origin: C57BL/6JEi

Current strain name:C57BL/6JEi-*tth*/J

Stock #004667 (jaxmice.jax.org)

Phenotype categories: neurological/behavioral: motor

capabilities/coordination/movement anomalies

**Origin and Description**

The *tth* mutation arose spontaneously on the C57BL/6JEi inbred strain in 1997. Mutant mice are identified visibly at three weeks of age by the presence of a tremor while walking and a tilted head. When picked up by the tail mutant mice display increased agitation followed by an increase in tremor and hyperactivity. Most mutant mice lean to one side, and this is most noticeable by their tilted head. The tilted head is not completely penetrant as some mutants appear only to tremble. Progeny from the tremor-only mice yield both mice with tremor and mice with tremor and head tilt. The two phenotypic characteristics are inseparable. Female mutant mice breed and raise their pups successfully while male mutant mice do not breed. Sperm from mutants are 66-75% motile and appear to have normal morphology, thus male infertility is not likely due to defective sperm. Auditory brain stem response results reveal no significant differences in hearing thresholds between homozygous mutant animals and controls, and no inner ear abnormalities were detected (see Pathology).

**Genetic Analysis**

Linkage cross: A mutant female was crossed with a male DBA/2J (+/+). The resulting F1 progeny were backcrossed to female mutant mice from the colony and 41 N2 generation mice were analyzed for linkage. Genetic linkage and marker order were analyzed with the Map Manager software program (Manley, 1993), and the mutation was mapped to mouse Chromosome 15. The mutation lies distal to *D15Mit16* (8/39 recombinant mice LOD=3.1) and proximal to *D15Mit41* (3/33 recombinants LOD=5.6). This map position corresponds to a region identified by the Neuroscience Mutagenesis Facility as a mutant region for *nmf4* (see Stock #004085 at jaxmice.jax.org), a similar mouse mutant generated by ENU mutagenesis, and suggests that these two independent mutations may be allelic.

## Pathology

The Mouse Mutant Resource standard pathology screen[1] completed on one 8-month-old mutant female and two 12-month-old mutants, one female and one male, revealed no significant lesions. Inner ears were analyzed after dissection from mutant and control mice, which were deeply anesthetized with tribromoethanol by intracardiac perfusion with phosphate-buffered saline (PBS) followed by Bouin's fixative. Inner ears were then either (1) decalcified in Bouin's fixative for two weeks, embedded in paraffin, serial cross-sectioned at 4m and counter-stained with hematolylin and eosin (H&E) or (2) made into whole mounts by flushing the dissected ear with neutral-buffered formalin through a hole made at the cochlear apex, dehydrating in ethanol and clearing in methyl salicylate overnight.

## Acknowledgements

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## References

Manley KF (1993) A MacIntosh program for storage and analysis of experimental mapping data. Mamm Genome 4, 303-313.

MGD 2004, Mouse Genome Database, Mouse Genome Informatics Project, The Jackson Laboratory, Bar Harbor, ME. (www.informatics.jax.org)

NMF 2004, The Neuroscience Mutagenesis Facility, The Jackson Laboratory, Bar Harbor, ME.

[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.