Twitter, *twit*, a new neurological mutation on mouse Chromosome 14

Susan A. Cook, Roderick T. Bronson, and Muriel T. Davisson

Source of Support: NIH/NCRR grant RR01183 (M. Davisson, PI) and NCI Cancer Center Core Grant CA34196

Mutation (allele) symbol: *twit*

Mutation (allele) name: twitter

Gene symbol: *twit*

Strain of origin: D.B6Ei/2Ei

Current strain name: D2.B6Ei-*twit*/GrsrJ

Stock #004416 (jaxmice.jax.org)

Phenotype categories: neurological: tremor; survival: postnatal lethality

Origin and Description

The recessive *twit* mutation arose spontaneously in the private congenic D.B6Ei/2Ei research colony at N10F17 in 1999. Homozygous mutants are recognizable by 2.5-3 weeks of age by their smaller body size, gaunt torso, frailty, and constant quivering most obvious from the abdominal-thoracic area to nose. Mutants also show poor ability to hold onto an edge with either their fore or hindlimbs. Adult mutants may chatter excessively. Some homozygous mutants die before weaning, but others survive through adulthood although they are unreliable breeders. Thus, the colony is maintained by transplantation of *twit*/*twit* ovaries into C3SnSmn.CB17-*Prkdc*^{scid}/J (Stock #001131) hosts and mating to +/*twit* males or, alternatively, by heterozygous sibling matings. Viability of the mutant class from heterozygous matings is ~18% (18 surviving mutants were visibly classified from a total of 101 progeny including 9 missing before being typed). DNA has been preserved in The Jackson Laboratory DNA Resource. Sperm from +/*twit* males will be cryopreserved as will embryos generated by IVF.

Genetic Analysis

To map the *twit* mutation we typed by PCR MIT microsatellite markers polymorphic between CAST/Ei and D.B6Ei/2Ei-*twit* in a standard linkage intercross. Spleen tip DNAs from 59 F2 mutants (118 meioses) yielded the following results in centimorgans +/- standard error: [*D14Mit201, D14Mit202*]-- 0.85 +/- 0.8--*D14Mit44*--0.85 +/- 0.84-[*D14Mit127, D14Mit174, twit*]--0.85 +/- 0.84--[*D14Mit175, D14Mit15*]--0.85 +/- 0.84-*D14Mit120*. The order of markers used in this cross was determined with Map Manager (Manly, 1993) and our linkage data are available from MGD accession number J:85479. The MGSC (2003) database as shown by Ensembl (2003) agrees with our mapping order and places the flanking markers *D14Mit44* and *D14Mit175* in the B band of Chr 14 at 22.9 Mb and 27.9 Mb, respectively.

Three plausible candidate genes involved with cholinergic neurotransmisson lie within our flanked segment. Mice homozygous for the targeted allele *Colq*^{tm1Jrs} of *Colq,* collagen-like tail subunit (single strand of homotrimer) of acetylcholinesterase, exhibit tremors and retarded growth (MGD 2003). Mutations in human *COLQ* cause EAD (Endplate Acetylcholinesterase Deficiency). This myasthenic syndrome presents respiratory and feeding difficulties, variable muscle weakness and opthalmoplegia (OMIM 603033 and 603034, 2003). Mice homozygous for the targeted allele *Chat*^{tm1Fhg} of *Chat*, choline acetyltransferase, show abnormal neuromuscular synapses and perinatal lethality. Mutations in human *CHAT* cause FIMG (Myasthenic Gravis, Familial Infantile). Symptoms include episodes of increased weakness, bulbar paralysis and apnea (OMIM 118490 and 254210, 2003). *Slc18a3*, solute carrier family 18, member 3, encodes the vesicular transporter of acetylcholine and is associated with innervation changes in the developing mouse esophagus (MGD 2003). The human *SLC18A3* sequence is reported to be contained within the first intron of *CHAT* and may be regulated by the same or a contiguous promoter (OMIM 600336, 2003). Mice with the *Colq*^{tm1Jrs} and *Chat*^{tm1Fhg} mutations were not available for complementation tests. However, allelism tests with two phenotypically similar mutations on Chr14 shimmy, (*shmy*) Stock # 002238 and wabbler-lethal (*wl*) Stock #000147 were negative. A *shmy/shmy* female x +/*twit* male yielded no visibly affected mice from a total of 12 progeny. Similarly, a +/*shmy* female x a +/*twit* male yielded no affected mice from a total of 17. A +/*wl* female x +/*twit* male yielded no affected mice from a total of 22. Allele test progeny were aged 3 weeks to 5 months in case hybrid vigor and/or modifying genes delayed phenotypic expression.

Pathology:
Routine histological screening[1] found no neurologic lesions to explain the rostral tremors. Myelination in *twit*/*twit* mutants is normal. Preliminary examination of the heart, lungs, pharynx, trachea and nasal passages found no explanation for the chattering as all tissues appeared normal without infection or inflammation.

Acknowledgements:
We thank Linda Washburn and Eva Eicher, Ph.D. for initial observations of the mutant mice and for donating them to the Mouse Mutant Resource. We also thank Coleen Marden for histological preparations and Pat Ward-Bailey for Web posting.

References:
MGD (2003), Mouse Genome Database, Mouse Genome Informatics Project, The Jackson Laboratory, Bar Harbor, ME. (www.informatics.jax.org)

MGSC 16.30.1, Mouse Genome Sequencing Consortium (2003) (www.ensembl.org/Mus_musculus/)

Manley KF (1993) A MacIntosh program for storage and analysis of experimental mapping data. Mamm Genome 4, 303-3313.

OMIM (2003) Online Mendelian Inheritance in Man (omim.org)

[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.