Twitcher 5 Jackson: a new remutation in the *Galc* gene.

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Mutation (allele) symbol: $Galc^{twi\text{-}5J}$

Mutation (allele) name: twitcher 5 Jackson

Gene symbol: *Galc*

Strain of origin: BXD32/TyJ

Current strain name: BXD32/TyJ-$Galc^{twi\text{-}5J}$/J

Stock #003613 (jaxmice.jax.org)

Phenotype categories: neurological

Origin and Description

A new autosomal recessive remutation to $Galc^{twi}$ arose spontaneously and was discovered by Martha Buzzell and Ben Taylor in a production colony of BXD32/TyJ mice at the Jackson Laboratory. Mice homozygous for the new $Galc^{twi\text{-}5J}$ remutation can be recognized at about 14-16 days of age by a moderate tremor and a smaller body size. Clinical weakness and wasting follows and death occurs usually about 21 days, but no more than 30 days.

The original twitcher ($Galc^{twi}$) phenotype was described on a mixed C57BL/6J and CE/J background. Clinical symptoms were first observed by day 30 and homozygous mice did not survive beyond three months of age. (Duchen LW, et. al., 1980) Subsequent backcrosses to C57BL/6J and the generation of a full congenic strain (> 10 backcrosses) reduced the onset of symptoms.

The gene underlying the *twi* mutation encodes galactosylceramidase (GALC), which is the enzyme responsible for the initial step of galactosylceramide (or galactocerebroside) degradation. Galactocerebroside is one of the most abundant and unique lipid constituents of the myelin sheath, and the twitcher mouse is a useful mutant in which to study myelination and myelin metabolism.

This strain is maintained by breeding the female hosts of homozygous ovarian transplants to the sibling males of the ovary donors and then intercrossing the heterozygous offspring. Heterozygous mice have normal life spans and are good breeders.

Genetic Analysis

This new remutation has recessive inheritance as shown by the results of mating hosts of homozygous ovarian transplanted mice to an unrelated male CAST/EiJ mouse. This mating produced only unaffected F1 progeny proving that the new mutation has recessive

inheritance. These unaffected F1 hybrids were intercrossed and generated affected F2 animals for linkage analysis.

Using our standard mapping protocols[1] this new mutation was mapped to Chromosome 12 where the original *Galc*^{twi} mutation is located, between *D12Mit6* (NCBI36 position 92.1Mb) and *D12Mit262* (NCBI36 position 111.3Mb).

We also performed a direct test for allelism to confirm this new mutation to be a re-mutation of twitcher. A *Galc*^{twi} heterozygous female was mated to a new mutant heterozygous male mouse, and this mating produced 15 offspring in 2 litters, of which 5 progeny were born with the *Galc*^{twi} phenotype proving allelism.

Pathology

A routine pathological screen[2] of 2 and 4 week old mutants showed that they had acutely necrotic neurons in ca3 of hippocampus and in deeper layers of cerebral cortex. These neurons have brightly eosinophilic cytoplasm and shrunken nuclei. Mutants of all ages examined had peripheral nerves with deficient myelin. This suggested that myelination of peripheral nerves is deficient rather than myelin being lost because of degeneration.

The eyes of two homozygous mice at 25 days of age were examined with an ophthalmoscope and found to be normal eyes.

Hearing as assessed by auditory brain stem response testing (ABR) of one homozygous mutant mouse at 25 days of age and one age-matched control showed both animals to have moderate hearing impairment. The hearing loss is likely due to the BXD32/TyJ strain background.

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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

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-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.