Flying Squirrel; a new neurological mutation on Chromosome 10

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Mutation (allele) symbol: *fsq*

Mutation (allele) name: flying squirrel

Gene symbol: *fsq*

Strain of origin: KK.Cg-A^y/J

Current strain name: KK(Cg)-*fsq*/J

Stock #008296 (jaxmice.jax.org)

Phenotype categories: Neurological

Origin and Description

Mice affected by the new flying squirrel (*fsq*) mutation were discovered by Arcel Dullas in a production colony of KK.Cg-A^y/J mice at The Jackson Laboratory. Because this new mutant strain was difficult to maintain, in vitro fertilization was performed with sperm from a mutant male into KK.Cg-A^y/J donor females. The presumed heterozygous progeny from this fertilization were then mated together and generated mutant progeny to maintain the flying squirrel (*fsq*) colony.

The heteroygote mice that were produced from this mating had two genotypes and phenotypes: A^y/a *fsq*/+ (yellow coat color) and a/a *fsq*/+ (black coat color). The flying squirrel colony was generated using only a/a *fsq*/+ black coat color mice.

Flying squirrel (*fsq*) mutant mice can be recognized at about 2 months of age when their bodies become stiff overall, in both limbs and trunk, but especially on the ventral side of the body. They take on a rigid spread out posture when picked up and sometimes will clasp their legs inward. Flying squirrel (*fsq*) mutant mice walk with a slight stagger and older homozygous mutant mice can't right themselves and their entire body seems rigid. Homozygous flying squirrel (*fsq*) mice can live to adulthood and are not fertile. Heterozygotes also have a normal lifespan but do breed.

Genetic Analysis

This new mutation has recessive inheritance as shown by mating a homozygous *fsq*/*fsq* mouse to an unrelated male CAST/EiJ mouse. This mating produced unaffected F1 progeny proving the new mutation to be recessive. The unaffected F1 hybrids were intercrossed, and 43 affected F2 animals were generated for linkage analysis. Using The Mouse Mutant Resource standard mapping protocols[1] this new mutation was mapped to Chromosome 10, between *D10Mit54* (NCBI 36 position 43.3 Mb) and *D10Mit138* (NCBI 36 position 53.4 Mb) and is non- recombinant with *D10Mit89*, *D10Mit256* and

D10Mit218 (84 meioses tested). A search in Mouse Genome Informatics (MGI) for known genes causing neuromuscular phenotypes, that are located between our flanking markers, yielded none. Another search for genes with neurological phenotypes, located in our region of interest produced four genes, all of which had very different phenotypes compared to the flying squirrel (*fsq*) phenotype and therefore not likely candidate genes.

Pathology

Hearing as assessed by auditory brain stem response testing (ABR)[2] of two homozygous mice and one control at 11 weeks of age showed that the homozygous mice were deaf and the control mouse had elevated thresholds. Fourteen non-mutant mice of the KK.Cg-A^y/J background strain tested at 6-8 weeks of age exhibited hearing impairment (ABR thresholds 20-30 dB above normal) similar to that of the littermate control mouse tested at 11 weeks of age, but not as severe as that of the two mutant mice, which showed no response to 100 dB SPL stimuli when tested at 11 weeks of age.

The eyes of 3 female homozygous mice were examined with am ophthalmoscope and had suture cataracts plus haze and wavy retinal blood vessels at 2 months of age. Three control males (+/?) had normal lenses. Electroretinogram testing (ERG) of one homozygous mutant had poor cone and poor flicker; one heterozygote had late cone. Fluorescein staining showed homozygous mice had wavy retinal vessels and leakage along the vessel. Ten unaffected mice of the background strain KK.Cg-A^y/J were also tested and most of mice had curly vessels indicating that the wavy vessel phenotype is a background effect and not caused by the *fsq* mutation.

A routine pathological screen[3] of a homozygous mouse at 16 week of age, showed no muscle lesions; mild hydrocephalus and the ear sections were poor. A homozygous mouse at age of 10 weeks showed mineral in wall of bladder and the other homozygous mouse at age of 10 weeks had a few dystrophic axons in spinal cord; legs had no abnormal muscle. We also screened five homozygous mice at 16 weeks and 21 weeks and one had severe hydrocephalus, another one had moderately severe hydrocephalus and all had no other lesions.

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Footnotes

[1]**Standard Mapping Protocol used in The Mouse Mutant Resource**

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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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]**ABR thresholds** in mice are determined using a semi-automated computer system (Intelligent Hearing Systems, Miami, Florida). Subdermal needle electrodes are inserted at the vertex and ventrolaterally to both ears of anesthetized mice. Specific auditory stimuli from 10-100 dB SPL are delivered binaurally through plastic tubes from high frequency transducers. ABR thresholds are obtained, in an acoustic chamber, for clicks and for 8, 16, and 32 kHz pure-tone pips.

[3]**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.