Stunted; A Short Face Mutation on Chromosome 19 in the Mouse

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Mutation Symbol: *stn*

Mutation Name: stunted

Strain of Origin: C57BL/6J

Current Strain Name: C57BL/6-*stn*/J

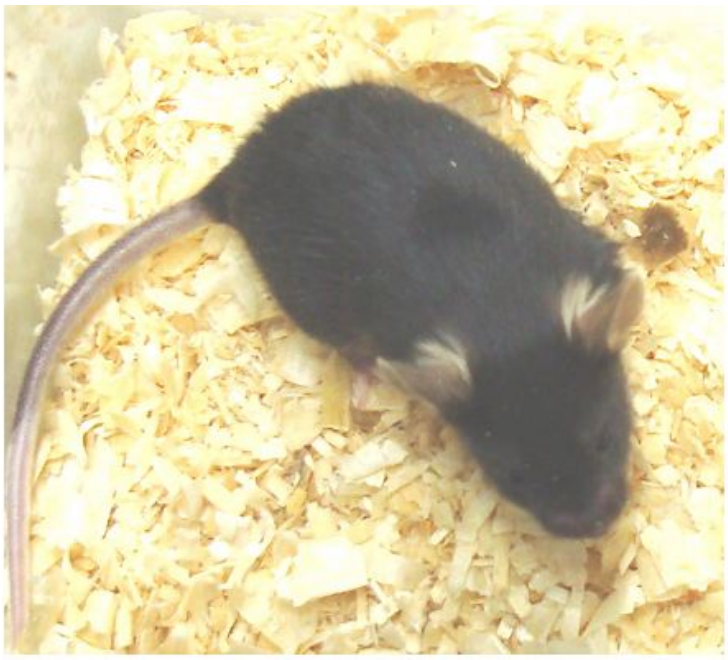
Stock #004507 (jaxmice.jax.org)

Phenotype Category: craniofacial, coat color

Origin and Description

The stunted mutation (*stn*) was discovered in a production colony of C57BL/6J mice at The Jackson Laboratory. Mutants have short faces. In addition, 95 % of mutants have a white belly spot; 38 of 40 mice screened had this coat color defect. About 14% of the colony developed hydrocephaly (24 out of 164 mice screened). Mice with hydrocephalus are not used for colony maintenance.

Stunted mutants are viable and fertile. The colony is maintained by homozygote x homozygote matings. There are no longer controls in the colony.



A 4-week-old male *stn*/*stn* mouse

Genetic Analysis

The *stn* mutation was proven to be inherited as an autosomal recessive mutation by mating mutants together and having 100% of offspring affected.

For linkage analysis, a backcross was used with a DBA/2J inbred mated to a C57BL/6-*stn*/J mutant. F1 hybrids were backcrossed to an *stn/stn*. The N2 progeny were visually phenotyped and spleens and tail tips were collected from both mutants and heterozygotes. Following our standard mapping protocol[1], the mutation maps to Chromosome 19 between markers *D19Mit13* and *D19Mit91*. This spans 33 cM to 47 cM or, more precisely, 31.9 Mb to 47.9Mb. An allele test was done with *Papss2*, which maps in this same region of Chromosome 19. *Papss2* is not allelic with *stn*. Allele tests were also done with diminutive (*dm*) and stubby (*stb*) and *stn* was not found to be allelic.

Pathology

Routine pathological screening was done on a 7-week-old male *stn/stn* and a 5 ½ -week-old male *stn/stn.* No lesions were found. Another male at 5 ½ weeks had severe hydrocephalus and rosettes in the retina.

An ophthalmoscope was used to view the eyes of three (2 male and 1 female) 3-month-old *stn/stn* mutants (eyemutant.jax.org/screening). One male and one female had retinal spots on the left eye. The other male had normal eyes. Six 5-week-old mutants were examined. Three females and one male were normal and two females had half rings around the optic nerve in the right eye.

Hearing was assessed by auditory brainstem response (ABR)[2] threshold analysis. The ABR results showed that two 3-month-old *stn/stn* males had normal hearing but two other 3-month-old *stn/stn* males had moderate or greater hearing loss. An ear cross section was also done on an *stn/stn*. The inner ear was okay, but it did have otitis media.

Discussion

Stunted mice have a shortened face and the high incidence white belly spots in mutants appear to be a part of the stunted phenotype. Whether hydrocephaly is a part of the stunted mutation or is segregating as a different mutation, is unknown. However, past attempts to breed hydrocephaly out of the colony resulted in a diminishment of the striking short face phenotype, indicating that hydrocephaly may be a part of the stunted phenotype.

Acknowledgements

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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

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

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.