A new spontaneous mutation in the *Ush1c* gene named deaf circler 3 Jackson

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Mutation (allele) symbol: *dfcr-3J*

Mutation (allele) name: Deaf circler 3 Jackson

Gene symbol: *Ush1c*

Strain of origin: STOCK Tg(CAG-Bgeo/GFP)21Lbe/J

Current strain name: STOCK *Ush1c*$^{dfcr\text{-}3J}$/J

Stock #006104 (jaxmice.jax.org)

Phenotype categories: neurological/behavioral: motor capabilities/coordination/ movement anomalies/deafness/circling

Origin and Description:
The recessively inherited, spontaneous mutation named deaf circler 3 Jackson (*dfcr-3J*) was originally discovered by Lori Knowles in the STOCK Tg(ACTB-Bgeo/GFP)21Lbe colony of mice (Stock #003920). Mutant mice display head bobbing and rapid circling behaviors, which are commonly indicative of vestibular dysfunction and often accompanied by hearing loss. The mutant mice were crossed to C57BL/6J mice, and the Tg mutation was deleted from the colony by selective mating (confirmed by genotyping), creating the new strain designated STOCK *Ush1c*$^{dfcr\text{-}3J}$/J. The colony is currently maintained by brother/sister mating of a homozygous male and heterozygous female.

Genetic Analysis:
An intercross was performed with CAST/EiJ mice, and 60 mutant F2 animals were analyzed. Using our standard mapping protocol[1], the mutation was mapped to the region on Chromosome 7 where the *Ush1c* gene is located. Due to the mapped proximity of the *Ush1c* gene, a complementation test for allelism was performed between mice heterozygous for the new mutation and mice heterozygous for the deaf circler (*dfcr*) mutation of the *Ush1c* gene, also known to cause circling behavior and deafness. The test-cross produced 3 litters with a total of 30 mice, 4 of which had mutant phenotypes, thus confirming allelism.

Pathology:
A routine pathological screen[2] of a 46-week-old mutant female mouse and a heterozygous male littermate revealed no gross structural abnormalities in the mutant except for dilated renal tubules that are likely due to the advanced age of the animal. Examination of whole mounts of inner ears isolated from 2 mutant females and one littermate control showed no

gross structural abnormalities or abnormalities of the otoconia in the utricle or saccule. A detailed examination of inner ears for hair cell abnormalities was not undertaken.

The auditory brainstem response (ABR)[3] was used to assess the hearing of two mutant mice and a littermate control at 6 weeks of age. The mutant mice were deaf showing no response at the highest stimulus presented (100 dB SPL), while the littermate controls exhibited good hearing. A clinical eye exam revealed no abnormalities.

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[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

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

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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]**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.

[3]**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.