Snell's waltzer 4 Jackson, a new spontaneous mutation in the *Myo6* gene

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Source of Support: This research was supported by NIDCD/NIH grant DC04301 (K R Johnson) and NCRR/NIH grant RR01183 (M T Davisson)

Mutation (allele) symbol: $Myo6^{sv\text{-}4J}$

Mutation (allele) name: Snell's waltzer 4 Jackson

Gene symbol: *Myo6*

Strain of origin: 129S1/SvImJ

Current strain name: 129S1/SvImJ-$Myo6^{sv\text{-}4J}$/J

Stock #008456

Phenotype categories: neurological/behavioral: motor

capabilities/coordination/movement anomalies/deafness/circling

Origin and Description

The recessively inherited spontaneous mouse mutation Snell's waltzer 4 Jackson (*sv-4J*) was identified by Rachel Miller in 2004 in an inbred 129S1/SvImJ colony. Mutant mice display head tossing and circling behavior commonly indicative of vestibular dysfunction and probable hearing loss. The hearing of 3 mutant mice and 5 heterozygous littermate controls was assessed by measuring their auditory-evoked brain stem response (ABR) thresholds at 6-7 weeks of age. All mutant mice were deaf showing no response to the highest stimulus presented (100 dB SPL), 2 of the heterozygous littermates had moderate hearing loss (~15 dB threshold elevations) while the other 3 exhibited good hearing (indicated by normal ABR thresholds). A routine pathological screen of a mutant female mouse and a heterozygous male littermate revealed no gross structural abnormalities in the mutant. Examination of whole mounts of inner ears isolated from 2 mutant males and one littermate control also showed no gross structural abnormalities or abnormalities of the otoconia in the utricle or saccule.

Genetic Analysis

An intercross was performed with CAST/EiJ mice and 70 mutant F2 animals were analyzed. Using our standard mapping practice[1] the mutation was mapped to a region of Chromosome 9 between markers *D9Mit177* (78 Mb position, NCBI Build 36) and *D9Mit196* (86 Mb). The myosin 6 gene (*Myo6*) is located within this region (80 Mb) and was identified as a good candidate gene because mutations in this gene, including the original Snell's waltzer mutation) have been previously shown to cause head tossing, circling behavior and hearing loss. A complementation test was set up between a female heterozygous for the new mutation and a male homozygous for the $Myo6^{sv\text{-}2J}$ mutation. The mating produced 2 litters with a total of 13 animals of which 6 were mutant, thus confirming allelism.

Acknowledgements

We thank Rachel Miller for the identification of the original Snell's waltzer 4 Jackson mutant mouse, Sandra Gray for mouse colony management, Leona Gagnon for mapping assistance, Heping Yu for ABR analysis, and Coleen Marden and Rod Bronson for pathological screening.

[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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