Jackson shaker 2 Jackson, a new spontaneous mouse mutation in the *Ush1g* gene.

Leona Gagnon, Leah Davis* and Kenneth R Johnson
Source of Support: This research was supported by NIH/NCRR grant RR01183 to the Mouse Mutant Resource (M.T. Davisson, PI) and NIH/NIDCD grant DC04301 (K.R. Johnson, PI).
*Summer Student internship program supported by the National Science Foundation, the Barbara H. Sanford Endowed Scholarship Fund, and the Horace W. Goldsmith Foundation.

Mutation (allele) symbol: *js-2J*
Mutation (allele) name: Jackson shaker 2 Jackson
Gene symbol: *Ush1g*
Strain of origin: B6.Cg-T^{2J} +/+*qk*
Current strain name: B6(Cg)-$Ush1g^{js\text{-}2J}$/J
Stock #: 006111 (jaxmice.jax.org)
Phenotype categories: neurological/behavioral: motor capabilities/coordination/ movement anomalies/deafness/head bobbing

Origin and Description
The recessively inherited spontaneous mouse mutation Jackson shaker 2 Jackson (*js-2J*) was identified in 2002 in a research colony (B6.Cg-T^{2J} +/+*qk)* of Eva Eicher's. Mutant mice display head tossing and circling behavior indicative of vestibular dysfunction and hearing loss. Mutant mice were crossed to C57BL/6J and intercrossed. Mice that displayed the new circling behavior but not the *T* or *qk* phenotypes were selected for breeding, thus producing the current colony designated B6(Cg)-$Ush1g^{js\text{-}2J}$/J. The colony is now maintained by brother/sister mating of a heterozygous female and a circling mutant male.

A routine pathological screen[1] revealed no gross abnormalities in mutant mice as compared with a control littermate with the exception of hair cell loss observed in the inner ear. Four mutant mice and two littermate controls were assessed for hearing by auditory brainstem response (ABR). All mutants (12-54 days old) were deaf (no response at the highest stimulus presented, 100dB) while their control littermates retained good hearing (normal ABR threshold). A clinical eye exam revealed no abnormalities.

Genetic Analysis
An intercross was performed with CAST/Ei and 54 F2 animals were analyzed. Using our standard mapping protocol[2], the mutation was mapped to a region of chromosome 11 where the *Ush1g* gene is located.

A complementation test was performed between mice heterozygous for the new mutation in the *Ush1g* gene and Jackson shaker (*js)*, a mouse mutation previously identified in the

Ush1g gene. The test-cross produced two litters with a total of 14 mice, five of which were mutant, thus confirming allelism.

To characterize the new mutation at the DNA level, PCR primers were designed to amplify each exon of the *Ush1g* gene including the splice donor and acceptor recognition sequences. Each PCR product was sequenced at The Jackson Laboratory core sequencing facility. Each exon's sequence was then compared to the mouse sequence publicly available, and a 155 base pair deletion was detected within exon 2 of the *Ush1g* gene (Fig. 1). The deletion induces a frame-shift coding for 51 altered amino acids and then a premature stop codon (Fig. 2).

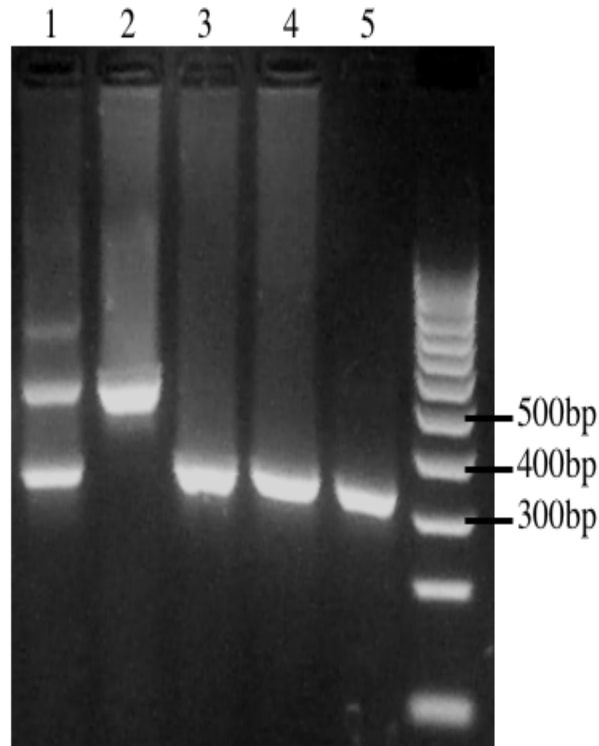

Fig.1, Genotyping the *js-2J* mutation using primer sets 2p1F(CTCCCCTCCACAATATTCCAA) and 2p1R(TCAGGGTGCTGGACGTGA). A 155 bp deletion is contained within this region of exon 2 of the *Ush1g* gene. Lane1, heterozygous (+/*js-2J)* PCR products of 324 and 479bp, lane 2 wildtype (+/+) pcr product of 479bp, lanes 3-5 homozygous (*js-2J*/*js-2J*) PCR product of 324bp.

	+/+ cDNA sequence		*js-2J/js-2J* cDNA sequence
1	AGTCCTCCCC GTCCGGGGAC AATTCTCGGT GCCCCTTGCC	1	AGTCCTCCCC GTCCGGGGAC AATTCTCGGT GCCCCTTGCC
41	CGTCCCGTCC AACTTGGGCG CCATGAATGA CCAGTATCAC	41	CGTCCCGTCC AACTTGGGCG CCATGAATGA CCAGTATCAC
81	CGGGCCGCCA GGGATGGCTA TCTGGAACTC CTCAAGGAGG	81	CGGGCCGCCA GGGATGGCTA TCTGGAACTC CTCAAGGAGG
121	CCACCCGGAA GGAGCTGAAT GCCCCTGACG AGGATGGCAT	121	CCACCCGGAA GGAGCTGAAT GCCCCTGACG AGGATGGCAT
161	GACCCCGACT CTCTGGGCTG CCTACCATGG CAACCTGGAG	161	GACCCCGACT CTCTGGGCTG CCTACCATGG CAACCTGGAG
201	TCTCTGCGCC TCATTGTGAG CCGCGGGGGT GACCCGGATA	201	TCTCTGCGCC TCATTGTGAG CCGCGGGGGT GACCCGGATA
241	AGTGTGACAT CTGGGGAAAC ACACCCTTGC ATCTGGCAGC	241	AGTGTGACAT CTGGGGAAAC ACACCCTTGC ATCTGGCAGC
281	TTCCAATGGT CACCTGCACT GCCTGTCCTT CCTCGTCTCC	281	TTCCAATGGT CACCTGCACT GTGGGCAAGC TGAAGGACAA
321	TTCGGGGCCA ACATCTGGTG CCTGGACAAT GACTACCACA	321	GGCCTTCCGC GAGGCAGAGC GGCGCATCCG CGAGTGCGCC
361	CGCCACTGGA CATGGCCGCT ATGAAGGGCC ACATGGAGTG	361	AAGATGCAGC GCAAGCACCA CGAGCGCATG GAGCGGCGCT
401	CGTACGCTAT CTGGATTCCA TCGCGGCCAA GCAGAGCAGC	401	ACCGGCGCGA GCTGGCCGAG CGCTCCGACA CTCTCAGCTT
441	CTCAACCCCA AGCTGGTGGG CAAGCTGAAG GACAAGGCCT	441	CTCCAGCCTC ACGTCCAGCA CCCTGAGCCG ACGGCTGCAG
481	TCCGCGAGGC AGAGCGGCGC ATCCGCGAGT GCGCCAAGAT	481	CACATGACGC TGGGCAGCCA GCTGCCCTAC TCGCAGGCCA
521	GCAGCGCAAG CACCACGAGC GCATGGAGCG GCGCTACCGG	521	CGCTGCACGG TACGGCCAAG GGCAAGGCCA AGATCCAGAA
561	CGCGAGCTGG CCGAGCGCTC CGACACTCTC AGCTTCTCCA	561	GAAGCTGGAG AGGCGCAAGC AGGGGGGCGA GGGCACCTTC
601	GCCTCACGTC CAGCACCCTG AGCCGACGGC TGCAGCACAT	601	AAGGTCTCTG AGGACGGGCG CAAAAGCGTC CGATCGCTCT
641	GACGCTGGGC AGCCAGCTGC CCTACTCGCA GGCCACGCTG	641	CCGGCCTGCA GTTGGGTAGT GATGTGATGT TTGTGCGCCA
681	CACGGTACGG CCAAGGGCAA GGCCAAGATC CAGAAGAAGC	681	GGGCACCTAC GCCAACCCCA AGGAGTGGGG CCGTGCCCCA
721	TGGAGAGGCG CAAGCAGGGG GGCGAGGGCA CCTTCAAGGT	721	CTCAGGGACA TGTTCCTCTC GGACGAGGAC AGCGTCTCCC
761	CTCTGAGGAC GGGCGCAAAA GCGTCCGATC GCTCTCCGGC	761	GTGCCACACT GGCTGCCGAG CCTGCTCACT CGGAGGTCAG
801	CTGCAGTTGG GTAGTGATGT GATGTTTGTG CGCCAGGGCA	801	CACCGACTCA GGCCACGACT CTTTGTTTAC CCGCCCCGGC
841	CCTACGCCAA CCCCAAGGAG TGGGGCCGTG CCCCACTCAG	841	CTGGGTACCA TGGTGTTTCG AAGGAACTAT GTGAGCAGCG
881	GGACATGTTC CTCTCGGACG AGGACAGCGT CTCCCGTGCC	881	GGTTGCACGG GCTGGGCCGA GAGGACGGGG GTTTGGATGG
921	ACACTGGCTG CCGAGCCTGC TCACTCGGAG GTCAGCACCG	921	GGCAGGCACG CCGCGGGGTC GGCTGCATAG TTCCCCCAGC
961	ACTCAGGCCA CGACTCTTTG TTTACCCGCC CCGGCCTGGG	961	CTGGACGACG ACAGCCTAGG CAGTGCCAAC AGCTTGCAGG
1001	TACCATGGTG TTTCGAAGGA ACTATGTGAG CAGCGGGTTG	1001	ACCGCAGTTG CGGGGAAGAG CTGCCCTGGG ATGAGCTAGA
1041	CACGGGCTGG GCCGAGAGGA CGGGGGTTTG GATGGGGCAG	1041	CTTGGGCTTG GATGAGGACC TGGAGCCCGA GACCAGCCCC
1081	GCACGCCGCG GGGTCGGCTG CATAGTTCCC CCAGCCTGGA	1081	TTGGAGACCT TCCTGGCCTC GCTGCACATG GAAGACTTTG
1121	CGACGACAGC CTAGGCAGTG CCAACAGCTT GCAGGACCGC	1121	CCTCTCTCCT GCGGCACGAG AAGATTGACC TGGAGGCTCT
1161	AGTTGCGGGG AAGAGCTGCC CTGGGATGAG CTAGACTTGG	1161	GATGCTGTGC TCTGACCTGG ACCTGCGCAG CATCAGCGTG
1201	GCTTGGATGA GGACCTGGAG CCCGAGACCA GCCCCTTGGA	1201	CCCCTGGGGC CTCGGAAGAA GATCCTGGGG GCCGTGAGGA
1241	GACCTTCCTG GCCTCGCTGC ACATGGAAGA CTTTGCCTCT	1241	GGCGCAGGCA GGCTCTGGAG CGACCCCTGG CCCTGGAGGA
1281	CTCCTGCGGC ACGAGAAGAT TGACCTGGAG GCTCTGATGC	1281	CACGGAGCTA TGAAAGAGCT CCTCTCCTCA GACCAAATGA
1321	TGTGCTCTGA CCTGGACCTG CGCAGCATCA GCGTGCCCCT	1321	ATTGCAAGTT GCCACAACCC GCGGTGGGGC CAAGAGGTCC

Fig2. A partial cDNA sequence (accession number NM_176847) of wildtype (+/+) and mutant (*js-2J*/*js-2J*) mice. Nucleotides highlighted in red indicate the 155 bases which are deleted in Jackson shaker-2J mice. The deletion is completely contained within exon 2 of the *Ush1g* gene and induces a translational frame shift (yellow nucleotides in the *js-2J*/*js-2J* sequence) and a premature stop codon (red nucleotides in the *js-2J*/*js-2J* sequence.)

Acknowledgements

We thank Linda Washburn for identification of the original Jackson shaker 2 Jackson mutant mouse, Sandra Gray for mouse colony development, Heping Yu and Chantal Longo-Guess for ABR analysis, Coleen Marden and Rod Bronson for pathological screening and Norm Hawes for clinical eye evaluation.

[1]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.

[2]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.

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