Deaf ballerina (*Dfb*), a new spontaneous mouse mutation with phenotype and map position similar to wheels and whirligig (*Chd7*^{Whi}).

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Mutation (allele) symbol: *Dfb*

Mutation (allele) name: Deaf ballerina

Strain of origin: BALB/cJ

Current strain name: BALB-*Dfb*/J

Stock #006274 (jaxmice.jax.org)

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

Origin and Description
The dominantly inherited spontaneous mouse mutation deaf ballerina (*Dfb*) was discovered in an inbred colony of BALB/cJ mice at the Jackson Laboratory in 2002. In order to improve fecundity, mutant mice were successively outcrossed to the BALB/cByJ inbred strain. Mutant mice display circling and head tossing behavior typical of mice with inner ear defects. Hearing was assessed by auditory brainstem response (ABR)[1]. Analysis of hearing from four mutant mice (*Dfb*/+) and three controls (+/+) revealed that the mutant mice were deaf (ABR thresholds > 50 dB above normal) while the littermate controls retained good hearing (normal ABR thresholds). All 13 *Dfb* mutants examined were missing a corpus callosum in the brain. *Dfb*/+ males are viable and fertile, although under gross examination their testes appeared smaller than those of +/+ littermates. Female (*Dfb*/+) mutants are viable but do not breed. Female infertility precluded an investigation of homozygous viability or lethality. Heterozygotes of both sexes are slightly smaller with less fat than control littermates.

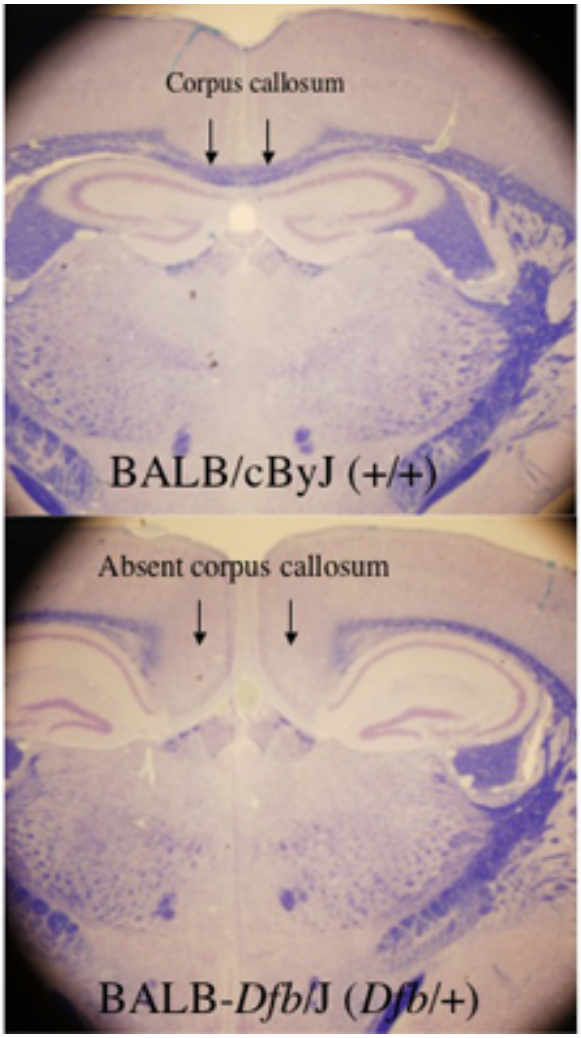
Genetic Analysis
To determine the mode of inheritance of deaf ballerina, a mutant mouse (*Dfb*/+) was mated with a normal (+/+) C57BL/6J mouse. Approximately 50% of the F1 generation was mutant, indicating a dominant mode of inheritance. A linkage-cross was then established by mating the F1 generation mutants (*Dfb*/+) to wild-type (+/+) BALB/cByJ mice. All backcross (N2) progeny were saved (N=183) and evaluated independently for circling behavior, hearing threshold and the presence of a corpus callosum. ABR tests revealed that all circling mice had severe hearing loss with thresholds 20-40 dB above those of non-circling mice; however, this impairment was less severe than that observed in *Dfb*/+ mice on the pure BALB background. Approximately 85% (58/68) of the

mutants from the linkage cross were missing the corpus callosum, again showing reduced penetrance of the phenotype in the backcross progeny.

Using our standard mapping practice[2], we genetically mapped *Dfb* to proximal Chromosome 4, in the region where the mouse *Chd7* gene is located (8.6 Mb, Ensembl, NCBI m36 mouse assembly), between the centromere and microsattelite marker *D4MIT181* (9.1 Mb).

Pathology

Coronal sections of the brain stained with luxol-fast blue/cresyl violet (LFB/CV) showed an absence of the corpus callosum in all of the inbred strain mutants and the majority (~85%) of the mutant mice from the linkage cross. Approximately 3% of the control linkage mice were missing the corpus callosum, which is consistent with the frequency of BALB/cByJ strain mice missing this structure. No other midline defects were detected.



Ophthalmic examination of the eye revealed some pigment loss in the retina of mutant mice, but this may be due to or exacerbated by the albino coat color of the BALB/cByJ mouse. An electroretinogram (ERG) gave a normal response to a light flash indicating normal retinal function.

Discussion

The phenotype and dominant inheritance of *Dfb* mice are similar to those of mice with mutations in the *Chd7* gene (Bosman et al., 2005), including dizzy (*Dz*), eddy (*Edy*), and cyclone (*Cy*) and the unproven alleles wheels (*Whl*) and wheels-like (*Whll*). The absent corpus callosum and the extreme degree of hearing loss of *Dfb* mutants, however, have not been previously described in *Chd7* mouse mutants. Mutations in the *CHD7* gene in humans are known to underlie CHARGE syndrome (Vissers et al., 2004). Although heart, eye and nasal abnormalities were not detected in *Dfb* mice, this could be due to differences in inbred strain backgrounds or to the highly variable phenotypes associated with *Chd7* mutations in mice and with human CHARGE syndrome.

Acknowledgments

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References

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[1] **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.

[2] **Standard Mapping Protocol**

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.

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