Waddles (*wdl*): A New Mouse Mutation Affecting Gait (movement-ataxia) on Chromosome 4

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mutation symbol: *wdl*

mutation name: waddles

Strain of origin: C57BLKS

Current strain name: C57BLKS/J-*wdl*/GrsrJ

Stock #004625 (JaxMice.jax.org)

Phenotype categories:neuromuscular

Abstract
Many mutations in mice that affect movements and their underlying systems have been characterized and mapped to various locations on the mouse genome. *tn* (teetering), *ax* (ataxia), *Lc* (lurcher), *Atp2b2* (deaf waddler), *pdnpl* (tiptoe walking), *hop* (hop sterile), *hyh* (hydrocephaly with hop gait) , *je* (jerker), *ji* (jittery), *wv* (weaver), *mdf* (muscle deficient) , *mea* (meander tail), *Smbp2<nmd>* (neuromuscular degeneration), *nclf* (neuronal ceroid lipofuscinosis), *Npc1<spm>* (sphingomyelinosis), *nr* (nervous), *pcd* (purkinje cell degeneration), *qv* (quivering)*,rb* (rabbit), *Reln<rl>* (reeler), *rora<sg>* (staggerer), *shm* (shambling),*shmy* (shimmy), *swe* (slow wave epilepsy), *stu* (stumbler), *tip* (tippy), *unc5h<rcm>* (rostral cerebellar formation), *wl* (wabbler-lethal), and *Wnt<sw>* (swaying) are some of the mutations that affect movement in mice. We have maintained a novel mouse mutation that may help to identify an unrecognized gene and may provide a useful model of a human genetic syndrome. Here we report on this new mutation that we mapped to mouse Chromosome 4 and designate *wdl* for "waddles." The *wdl* mutation affects the gait of mice from early in life before weaning and remains unchanged throughout their entire life. A previously reported mutation with a similar phenotype, waddler (*wd*), was mapped to the same Chr 4 region but is now believed to be extinct so allele testing is not possible.

Origin and Description
The *wdl* mutation was found at The Jackson Laboratory in 1995 in the production colony of C57BLKS at generation F104 and is maintained in the Mouse Mutant Resource. This is an autosomal recessive mutation with complete penetrance on the inbred background on which it arose. The homozygous mutant is characterized by a wobbly side-to-side gait which is noticeable by two weeks of age and remains phenotypically similar throughout life. Some, but not all, of the homozygous mutant mice are slightly smaller than normal littermates. Homozygotes do not clasp their hind legs when picked up by the tail. Both mutant and control mice swim in a straight line when placed in water. Heterozygotes are

indistinguishable from their normal littermates. Males and females are viable and fertile. The *wdl* mutation is maintained by homozygous x heterozygous matings and pairs have an average litter size of 6 pups. When two homozygotes are mated together all progeny produced are homozygous mutant type. **Update**: In 2005 waddles was found to be a mutation in the *Car8* gene.

Genetic Analysis

Breeding data from the maintenance colony and from the CAST/Ei mapping intercross show a recessive inheritance. The mating scheme of heterozygote x homozygote (females or males) produce roughly 50% mutant phenotypes as expected. In the CAST/Ei intercross homozygous *wdl/wdl* mice mated x CAST/Ei produced phenotypically normal F1 offspring. The mutant phenotype was expressed in the intercross F2 generation at about 20% (less than the expected 25%).

To map the chromosomal location of the *wdl* mutation, a male homozygous mutant was mated with a female CAST/Ei (*Mus musculus castaneus*). F1 progeny were intercrossed and only F2 mutant mice contributed to the mapping data. 86 genomic spleen and tail DNA samples were prepared by standard methods. Selected microsatellite polymorphisms from each chromosome were typed by polymerase chain reaction (PCR) using primer pairs from Research Genetics. DNA pools were made with F2 mutant mice for mapping (Taylor et al, 1994). Amplified products were electrophoresed through 2.5% Metaphor gels and visualized with ethidium bromide. PCR analysis of a DNA pool of equal aliquots from 24 F2 mutants from the CAST/Ei intercross revealed a deficiency of CAST/Ei specific products for Chromosome 4, compared with an F1 control. The recombination estimates with standard errors and the best gene order are centromere -*D4Mit149-1.79 +/- 1.02-wdl- 0.81 +/- 0.80*-[*D4Mit181, D4Mit99*]-7.65 +/- 4.16-*D4Mit97*-2.52 +/-2.47-*D4Mit39*-1.62+/- 1.60-D*4Mit5*. Data was analyzed using Map manager (Manley, 1993). The complete Chr 4 linkage data for the 86 F2 *wdl/wdl* mice have been deposited in the Mouse Genome Database, accession # J:81344

Pathology

Comparison of mutants and controls showed no abnormal lesions in the brain, spinal cord, and organs, except for one isolated case of hydrocephalus. Eyes were examined using a slit lamp (ophthalmoscope) and were found to be normal for that strain. Hearing was tested using auditory-evoked brain stem response[1] (ABR). Hearing was abnormal by 7 months of age in mutant mice, but also in control littermate heterozygotes, indicating that the hearing impairment was caused by the C57BLKS background strain. When the *wdl* mutation was backcrossed onto a C57BL6/J background, mutant mice had normal hearing at 7 months of age.

Conclusion

We have mapped a recessive mutant called waddles (*wdl*), with a characteristic wobbly loss-of-balance gait, to mouse Chromosome 4 using an intercross between CAST/Ei and C57BLKS. *wdl* mutant mice show a remarkedly similar phenotype to the previously reported waddler (*wd*) mutant. Because both mutations map to the same location on Chr 4, they may be alleles of the same gene; however, the waddler mutation is extinct so

allelism cannot be tested. No other mutations with similar phenotypes have been mapped in this region.

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Bibliography
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Mouse Genome Database(MGD), Mouse Genome Informatics Project, the Jackson Laboratory, Bar Harbor, Maine. (January, 2000).

[1]Auditory-Evoked Brainstem Response (ABR) Thresholds
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. ABR thresholds of all mice and strains tested are entered in spreadsheet files for storage, easy access, and for the production of periodic progress reports. Click-evoked ABR waveforms, obtained at threshold (T) and at T+10, T+20 and T+30 dB or each mouse, are also stored for future reference. Mice of the CBA/CaJ strain are tested periodically as references for normal hearing, and for monitoring the reliability of the equipment and testing procedures.