A Spontaneous Point Mutation in the Mouse Gene Roundabout Homolog 3 Results in Impaired Balance

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Mutation (allele) symbol: *Robo3*^{m1J}

Mutation (allele) name: mutation 1 Jackson

Gene symbol: *Robo3*

Strain of origin: C57BL/6J

Current strain name: C57BL/6J-*Robo3*^{m1J}/GrsrJ

Stock #005544 (jaxmice.jax.org)

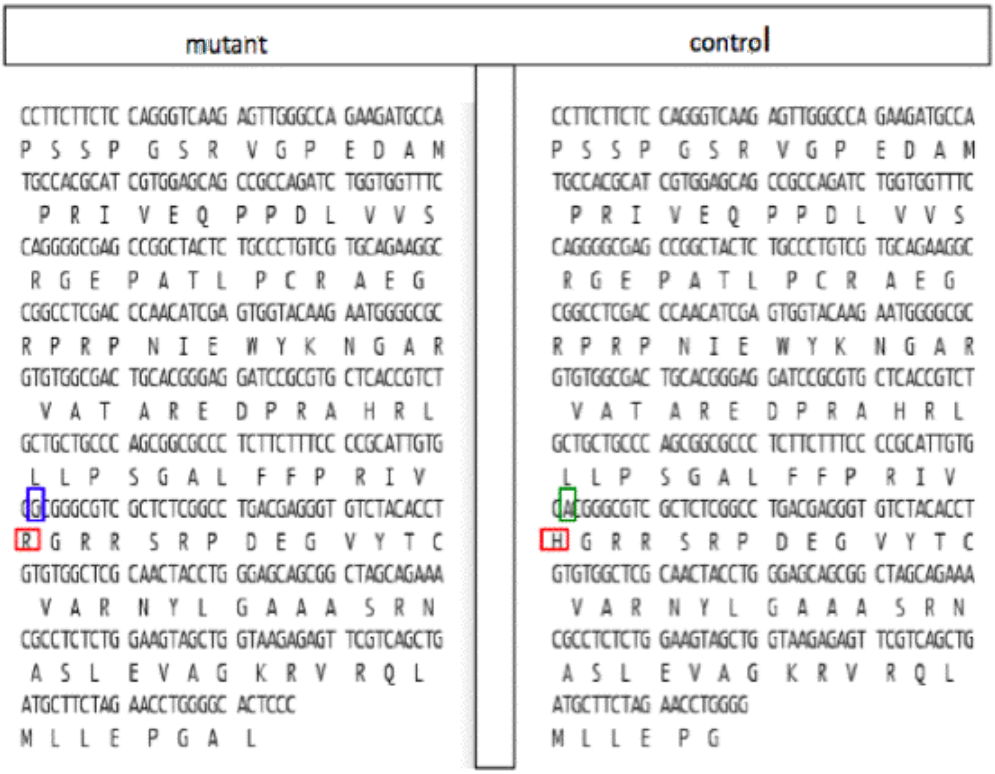
Phenotype categories: behavior, neurological

Abstract
A new recessive mutation that causes impaired balance has been characterized and identified as a point mutation of the gene roundabout homolog 3 (*Robo3*) by its map position on Chromosome 9, neurological phenotype, and Sanger sequencing based determination of mutation.

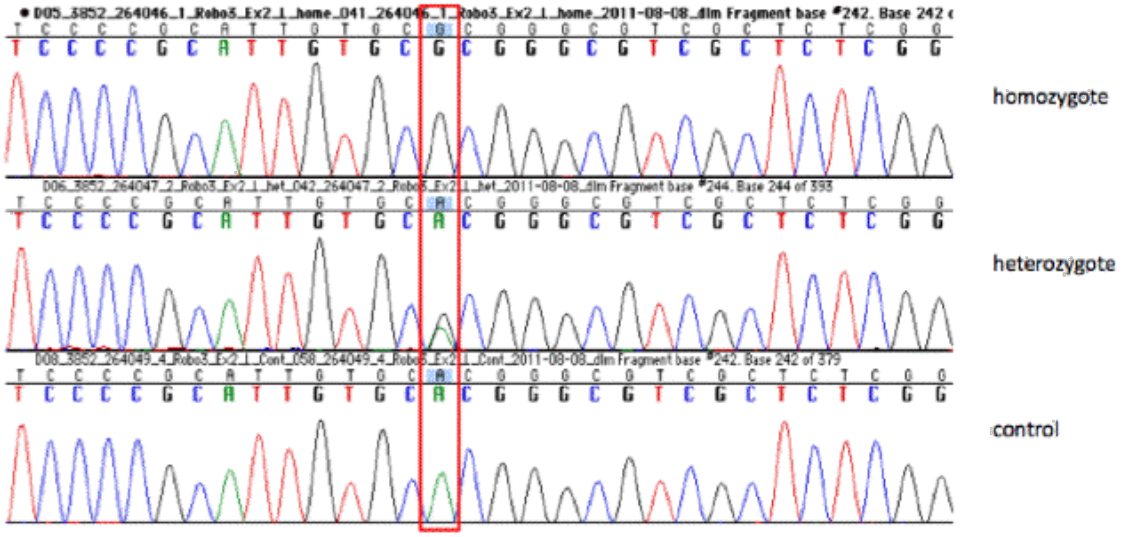
Origin and Description
A new spontaneous recessive mutation was discovered by Helen Tracey in a colony of C57BL/6J at The Jackson Laboratory in 2002. Mice homozygous for this new mutation are noticeable by wean age by their impaired balance. They lean to one side and have difficulty maintaining an upright position. Homozygous mice are fertile and live a normal life span.

Genetic Analysis
A homozygote was mated with BALB/cByJ producing only non-affected F1 progeny, proving this mutation to be recessive. Intercrossed F1 progeny generated affected F2 animals for linkage analysis. Using our standard mapping procedure this mutation was mapped to Chromosome 9, between *D9Mit247* (NCBI 37 position 36.9 Mb) and *D9Mit129* (NCBI 37 position 43.6 Mb).

Based on neurological phenotype and location within the mapped critical interval, roundabout homolog 3 (*Robo3*), which functions in axon guidance, was considered a plausible candidate gene. Sequence analysis revealed a single nucleotide substitution in exon 2 on Chromosome 9 in *Robo3*. Primers were generated that were predicted to produce from controls a 461 base pair product flanking *Robo3* wild type allele: primer Exon2 Left (CTCTTCAGAGCAAACCTGGTC) and primer Exon2 Right (CAACTTTCTGTTGGCCTTCC). Sequence analysis of mutant DNA identified a single nucleotide transition from A to G at position 37237171 in *Robo3.* This is a missense mutation and predicted to change amino acid from histidine to Arginine at protein position 130, which is within a putative immunoglobulin domain.



A portion of the protein coding region of *Robo3*. The control DNA sequence and its amino acid translation are shown on the right, and the *Robo3*^{m1J} mutant DNA and its translation on the left. A single nucleotide transition is enclosed by a blue box in the mutant sequence and a green box in the control sequence. The mutation that is predicted to change amino acid 130 from histidine to arginine is enclosed by a red box in the control and mutant sequence.



Comparison of DNA sequence chromatograms from a *Robo3*^{m1J} homozygote, heterozygote and wild-type control. The red boxed region corresponds to the green and blue boxed regions shown in the sequence figure.

Pathology

A routine pathological screen of one mutant at the age of 4 weeks showed macrophages in the lungs and central lobular congestion in the liver, but an electroretinogram (ERG) found the eyes to be normal. One mutant at 23 weeks of age and two mutants at 45 weeks of age were also assessed and found to have no significant lesions. Hearing, assessed by auditory brainstem response testing (ABR) of one mutant and one control mouse, showed normal hearing at 8 weeks of age. The eyes of one mutant and one heterozygote at 4 weeks of age were tested by electroretinography and found to be normal.

Discussion

Combinations of a floxed *Robo3* allele and specific Cre recombinase deleting transgenes has allowed characterization of the impact of localized ablation of ROBO3. A targeted deletion of *Robo3* results in complete perinatal lethality with defective axonal guidance that results in failure of commissural axons to cross the midline and failed left-right synchronization of the pre-Botzinger complex. The point mutation described here, which was identified from aberrant phenotype, results in a much less severe phenotype of impaired balance with normal viability and fecundity. This offers an alternative model for assessing a defect in a specific domain of this protein rather than its systemic or targeted ablation.

Acknowledgements

The authors thank Helen Tracey for discovery of the mutant, Roderick Bronson and Coleen Kane for pathological screening, Heping Yu for hearing assessment, and Norm Hawes and Ron Hurd for eye examinations.

Addendum

Prior to its characterization, this mutation was inadvertently transmitted from a recessive carrier in the C57BL/6J foundation stock into the strain B6.Cg-*Lep*^{ob}/J where it was isolated, backcrossed away from *Lep*^{ob} by breeding to pure C57BL/6J, and cryopreserved as Stock #013532.