A spontaneous point mutation in the *Map3k11* gene causes dorsal stripes and necrotic tooth pulp

Authors: Son Yong Karst, Laura G. Reinholdt, David E. Bergstrom, and Leah Rae Donahue

Source of Support: This research was supported by grants RR01183 to the Mouse Mutant Resource (Leah Rae Donahue, PI) and Cancer Core Grant CA34196.

Mutation (allele) symbol: *Map3k11*^{m1J}

Mutation (allele) name: mutation 1 Jackson

Gene symbol: *Map3k11*

Strain of origin: STOCK-*Bdnf*^{tm3Jae}/J

Current strain name: STOCK *Map3k11*^{m1J}/GrsrJ

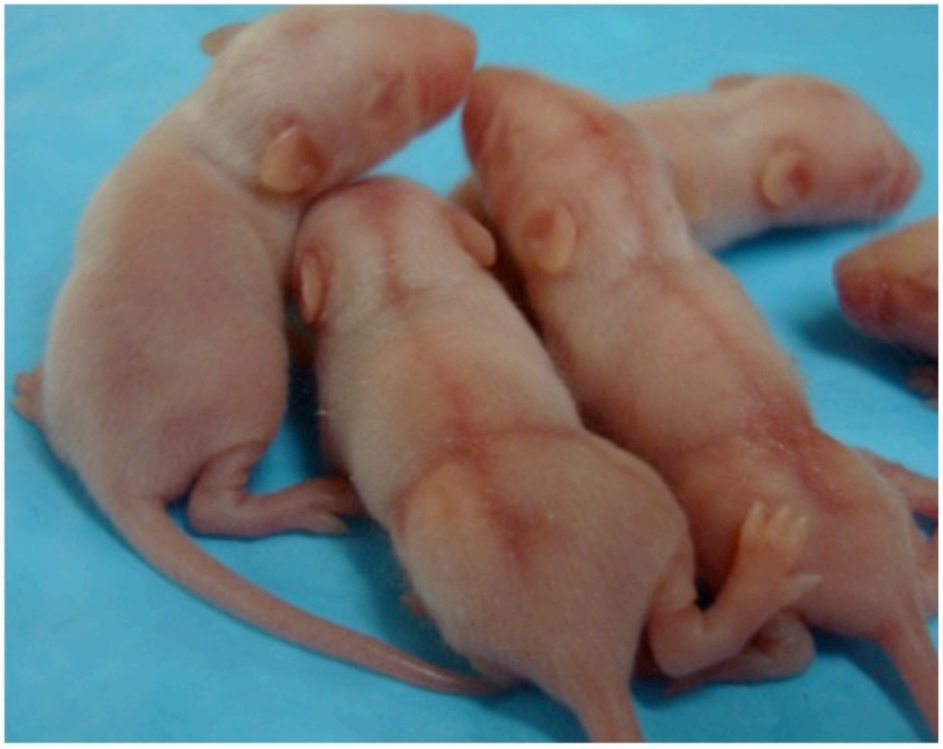
Stock #012874 (jaxmice.jax.org)

Phenotype categories: integument, teeth

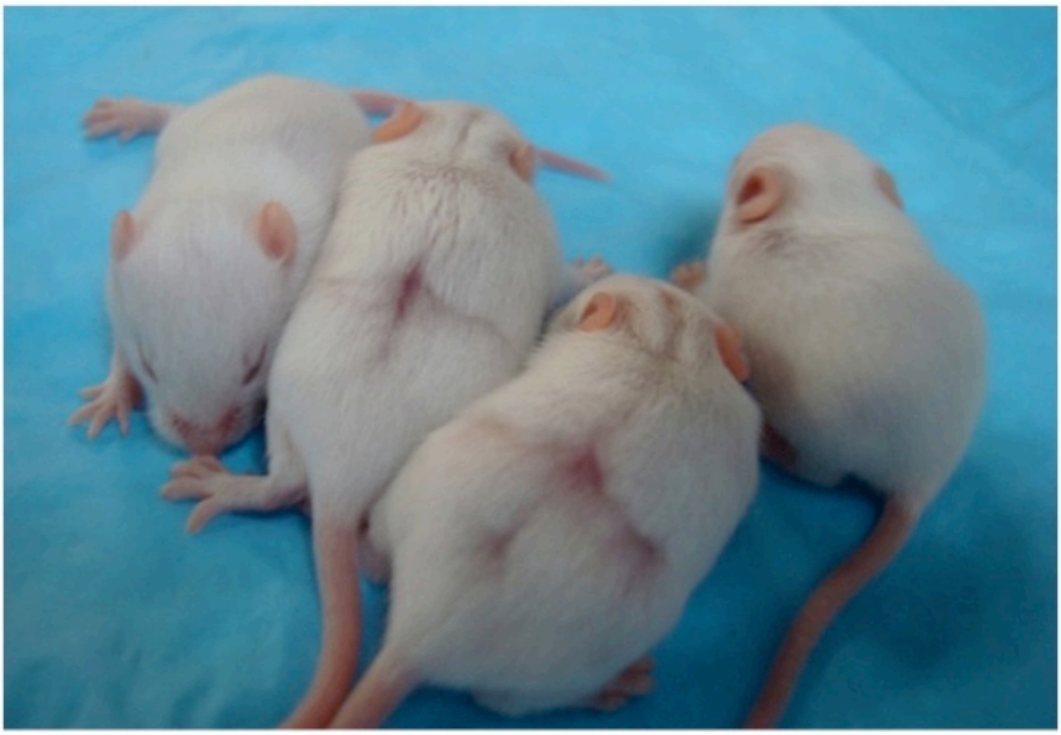
Abstract
A spontaneous recessive mutation that causes dorsal stripes and necrotic dental pulp has been characterized and identified as a point mutation of the *Map3k11* gene by its similar phenotype, map position on Chromosome 19, and sequence analysis of the causative mutation using the Illumina HiSeq (high-throughput sequencing platform) and Sanger method.

Origin and Description
This new recessive mutation arose spontaneously in the STOCK-*Bdnf*^{tm3Jae}/J mouse strain at The Jackson Laboratory and was identified by Serrena Lovley. Mice homozygous for this new mutation exhibit bright red dorsal lines from head to tail and from left to right across the crown of the head, at the base of the neck in front of the shoulders, and on the lower part of the trunk. These dorsal lines are easily recognized at 3 to 4 days of age. Mutants that exhibited dorsal lines early in life showed no evidence of the dorsal line at 3 weeks of age.

This dorsal defect phenotype also co-segregates with abnormal tooth growth. Mutants appear frail, are smaller than their littermates, and are occasionally found dead before or just after wean age. Those mutants that do survive, eventually develop normal teeth and grow to normal size. Mutants are fertile, and can live a normal life span. Because of poor teeth and frailty, mutants are usually weaned at age 4-6 weeks of age.



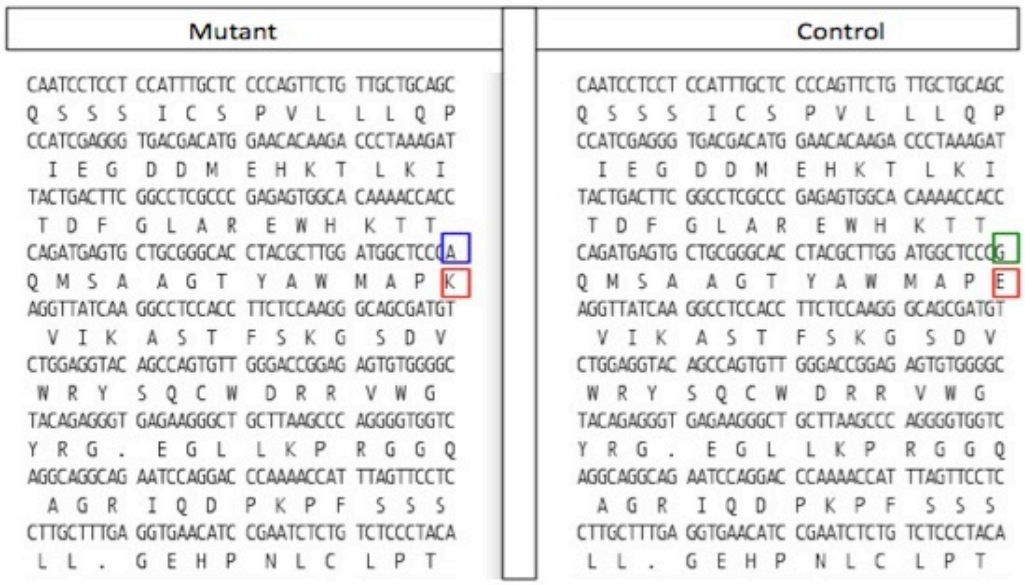
Two homozygotes, on right, and one control, on left, at 6 days of age



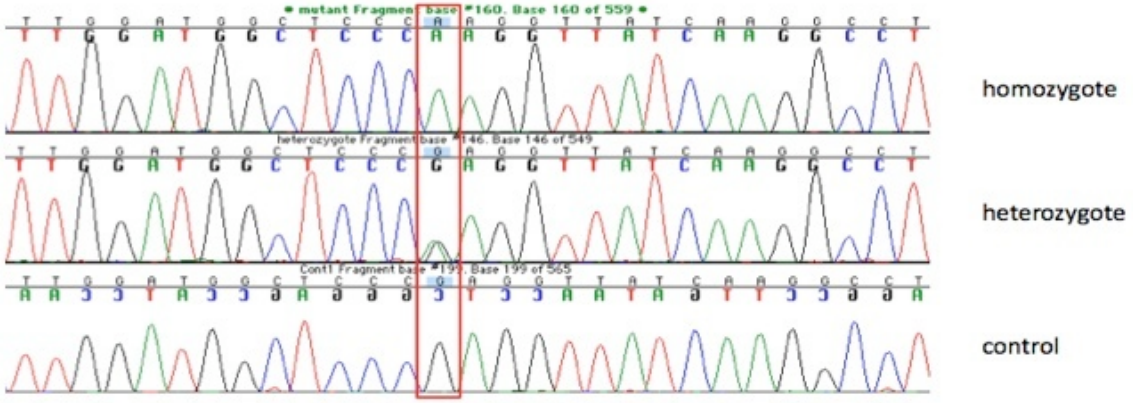
Two homozygotes (center) at 11 days of age, displaying dorsal stripes, flanked by wildtype controls

Genetic Analysis
A homozygote was mated with CAST/EiJ producing only non-affected F1 progeny, proving that this mutation is recessive. Intercrossed F1 progeny generated affected F2 animals for linkage analysis. Using our standard mapping procedure this mutation was mapped to Chromosome 19, between *D19Mit59* (NCBI 37 position 53.2 Mb) and *D19Mit126* (NCBI 37 position 107.8 Mb). No recombination was found with *D19Mit77* (NCBI 37 position 65.5 Mb) and *D19Mit44* (NCBI 37 position 77.1 Mb). Sixty-six (66) meioses were tested.

This mapping data demonstrated linkage to Chromosome 19 and whole exome sequencing was used to identify candidate mutations in the mapped region. Briefly, genomic DNA was enriched for coding sequence by hybridization-based capture with probes representing 54 Mb of annotated coding sequence. The enriched DNA was then sequenced using the Illumina HiSeq high throughput sequencing platform. A single nucleotide polymorphism was found on Chromosome 19 in mitogen-activated protein kinase kinase kinase 11 (*Map3k11*). To validate the putative mutation, primers *Map3k11* forward (CAGGATGACTGTGAGATGCG) and *Map3k11* reverse (TGTGAGCATTTGTGTCTGGG), predicted to produce 625 base pair products flanking the variant, were synthesized. Sequence analysis of genomic DNA from two mutants when compared to genomic DNA from four unaffected animals identified a single nucleotide transition from G to A at 5695295 in *Map3k11*. This is a missense mutation and causes the codon change E293K.



A. A portion of the protein coding region of *Map3K11*. The control DNA sequence and its amino acid translation are shown on the right, and the *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 is predicted to change amino acid 293 from glutamate to lysine. This change is indicated by a red box in the control and the mutant sequence.



B. Comparison of DNA sequence chromatograms of the *m1J* homozygote, heterozygote and control sequence. The red boxed region corresponds to the green and blue boxed regions shown in A.

Pathology

A routine pathological screen of one mutant at age 4 weeks showed poor red pulp in the spleen, rare dysplastic follicles in the skin and small adipocytes. One mutant at age 5 weeks showed no lesions; skin sections looked normal. Two mutants at age 4 weeks and one mutant at age 5 weeks showed necrotic tooth pulp. Two mutants at age 8.5 weeks demonstrated necrosis of the tooth pulp of the incisor teeth, perhaps due to limited blood supply. The mutants also displayed infarcts of the pulp of roots of incisor teeth. The pulps appear necrotic, presumably due to loss of blood supply. Two mutants at age 27 weeks showed no evidence of the dorsal stripe, one mutant showed no lesions and the other mutant had oral papillomata. One mutant at age 30 weeks of age showed the distal portions of incisor teeth becoming necrotic. The tooth roots, however appeared normal. Teeth from older animals did not appear necrotic and looked normal. Thus, both the dorsal skin and dental phenotypes are transient in nature.

Hearing, assessed by auditory brainstem response testing (ABR) of one mutant and one control mouse, showed hearing loss at 5 weeks age. One mutant and one control mouse showed good hearing at age of two months. One mutant and one control mouse showed hearing loss at age of two months. Results indicating both normal and diminished hearing were found in both *Map3k11*^{m1J} mutant and control mice at different test sessions. These tests were done prior to removal of the *Bdnf*^{tm3Jae} allele and may have been influence by *Bdnf* genotype.

Acknowledgements

The authors thank Serrena Lovley for discovery of the mutant, Roderick T. Bronson and Coleen Kane for pathological screening, Heping Yu for ABR testing.