The bouncy alleles are mutations in the *Kcnn2* gene

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Mutation (allele) symbol: *Kcnn2*$^{bc\text{-}7J}$

Mutation (allele) name: bouncy 7 Jackson

Gene symbol: *Kcnn2*

Strain of origin: B6.129P2-*Apoe*^{tm1Unc}/J

Current strain name: B6.Cg-*Kcnn2*$^{bc\text{-}7J}$/GrsrJ

Stock #016099 (jaxmice.jax.org)

Abstract:
In 1963 Priscilla Lane identified a recessive neuromuscular mutant at The Jackson Laboratory that she called bouncy (*bc*). Since that time eight additional bouncy alleles have been identified. Through mapping, high-throughput sequencing, and allele testing we have now proven that the bouncy 7 Jackson mutation and therefore the series of noncomplementing bouncy alleles are mutations in the gene potassium intermediate/small conductance calcium-activated channel subfamily N member 2 (*Kcnn2*).

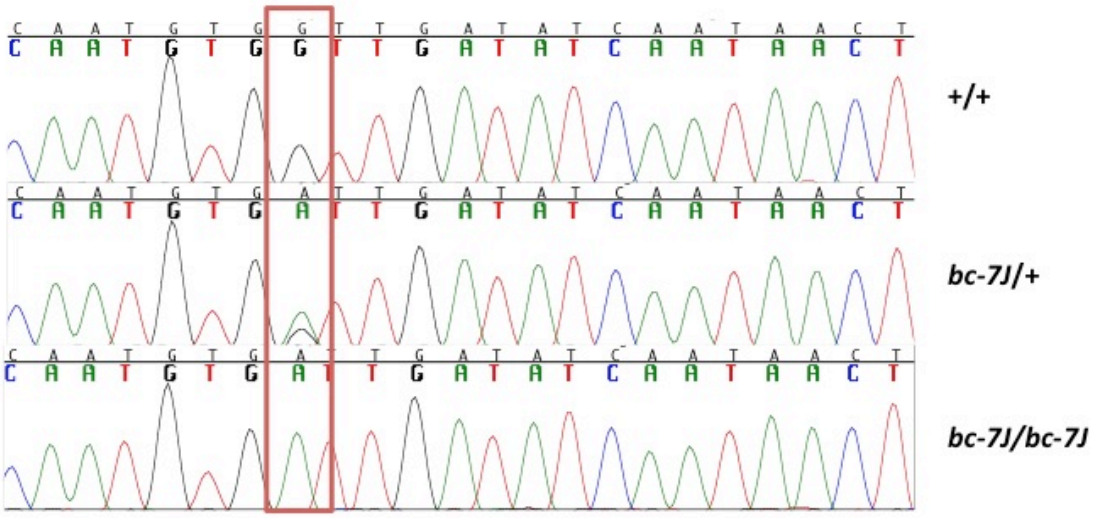
Phenotype categories:
A recessive neurological mutation that causes a moderate tremor and impaired gait was identified in the B6.129P2-*Apoe*^{tm1Unc}/J strain at The Jackson Laboratory. Mutants are noticeable as early as 2-3 weeks of age and are smaller than littermates. Despite the tremor these mice seem to have a normal life span. Light microscopic assessment of the sperm determined that the male mutants have viable sperm, yet they do not breed. To determine the mode of inheritance a mutant female was outcrossed to C57BL/6J and no mutants were identified in the offspring, but when these wild-type offspring were intercrossed they produced mutants, proving this a recessive mutation. The colony is maintained by mating homozygous females with heterozygous males. Genotyping confirmed that the *Apoe*^{tm1Unc} allele was bred out of this mutant subline.

Pathology:
A routine pathological screen of one female mutant at the age of 10 weeks showed atrophy of the thymus and a male mutant at 22 weeks showed partial testicular atrophy. A male mutant and control at 18 weeks had mild hydrocephalus, which is common in C57BL/6J mice and is not a significant phenotype of this mutation. The eyes of two

mutants and two controls at 11 weeks of age were examined by ophthalmoscopy and found to be normal. Hearing, assessed by auditory-evoked brainstem response testing of two mutant and two control mice, showed normal hearing at 4 weeks of age.

Genetic Analysis:
To determine the chromosomal location of the mutation, homozygous females were mated to CAST/Ei males, the F1 progeny were intercrossed and DNA was isolated from affected F2 offspring. Using standard SNP mapping procedures, this mutation was mapped to a Chromosome 18 segment located between *D18Mit236* (39.5Mb) and *D18Mit57* (48.8 Mb). Because of the phenotypic similarity, heterozygous females were bred with males heterozygous for the bouncy 3 Jackson mutation and three out of twelve offspring had the mutant phenotype proving this new mutation to be a new allele of bouncy, bouncy 7 Jackson. Because of the map position and the phenotypic similarity with *Kcnn2*$^{tm1.1Jpad}$ homozygotes, a complementation test was also done by breeding B6.129(Cg)-*Kcnn2*$^{tm1.1Jpad}$/J heterozygous females with males heterozygous for bouncy 3 Jackson. Two out of the seven offspring had this phenotype of tremor and impaired gait, proving the bouncy series of mutations to be alleles of *Kcnn2*. This was confirmed by breeding bouncy 7 Jackson heterozygotes with *Kcnn2*$^{tm1.1Jpad}$ heterozygotes and finding four of the fifteen affected progeny to have the mutant phenotype.

A custom DNA-Seq array was designed to target the region of the genome containing the *Kcnn2* gene (Chr18: 45,393,000-45,882,000, MGSCv37). Data analysis for bouncy 7 Jackson revealed a single nucleotide variant of G to A on Chromosome 18, at position 45,814,900 (see sequence below). This variant was also detected via whole exome sequencing, which was executed simultaneously. This nonsense mutation in the fourth exon of *Kcnn2* is predicted to result in the amino acid change of TGG (W) to TGA (stop) at protein position 345 (*Kcnn2*W345*). This mutation was validated in DNA samples from 3 wild-type, 3 heterozygous, and 3 homozygous bouncy 7 Jackson animals from the colony using PCR amplification with Kcnn2_bc7J_1L (GCACATGGAATACTAAGACTGTCAA) and Kcnn2_bc7J_1R (TGGAAAGGGGAAAACACTCA) and Sanger sequencing.



Comparison of sequence chromatograms from wild-type and heterozygous control samples, and homozygous *bc-7J/bc-7J* samples. Double chromatogram in *bc-7J/+* sample and single chromatogram in *bc-7J/bc-7J* sample indicates a SNV at position 45,814,900 on chromosome 18 (MGSCv37).

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