Spinner 2J, a new spontaneous mutation in the *Tmie* gene

Kelly L. Kane and Kenneth R. Johnson

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Mutation (allele) symbol: *Tmie*$^{sr\text{-}2J}$

Mutation (allele) name: Spinner 2 Jackson

Gene symbol: *Tmie*

Strain of origin: B6MMTVCre/Cre18

Current strain name: B6(Cg)-*Tmie*$^{sr\text{-}2J}$/J

Stock #008834 (jaxmice.jax.org)

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

Origin and Description:
The recessively inherited, spontaneous mutation named spinner 2 Jackson (*sr-2J*) was identified in a B6MMTVCre/Cre18 research colony of mice. Mutant mice display head tilting and circling behaviors, which are commonly indicative of vestibular dysfunction and often accompanied by hearing loss. Mutant mice were repeatedly backcrossed to C57BL/6J mice and then intercrossed, creating the new inbred strain designated B6(Cg)-*Tmie*$^{sr\text{-}2J}$/J (Stock #008834). The colony is currently maintained by brother/sister mating of a homozygous male and heterozygous female.

Genetic Analysis
An intercross was performed with CAST/EiJ mice, and 28 mutant F2 animals were analyzed. Using our standard mapping practice, the mutation was mapped to the region of Chromosome 9 where the *Tmie* gene is located. Previous mutations identified in the *Tmie* gene are known to cause circling behavior and hearing loss. To determine if the mutant mice carry a mutation in the *Tmie* gene, primers were designed flanking each of the gene's 6 exons. Each primer set was used to PCR amplify and sequence the exons. A G to A transition was identified in the critical G nucleotide of the 3' splice acceptor site adjacent to exon 5 (Fig. 1). To verify this mutation, 5 mutants and 4 heterozygous littermate controls were analyzed. All mutants carry the A nucleotide and all heterozygotes carry both the G and A nucleotides at this position (Fig. 1). Ablation of the exon 5 splice acceptor site in mutant DNA prevents formation of the wildtype *Tmie* transcript (Fig. 2).

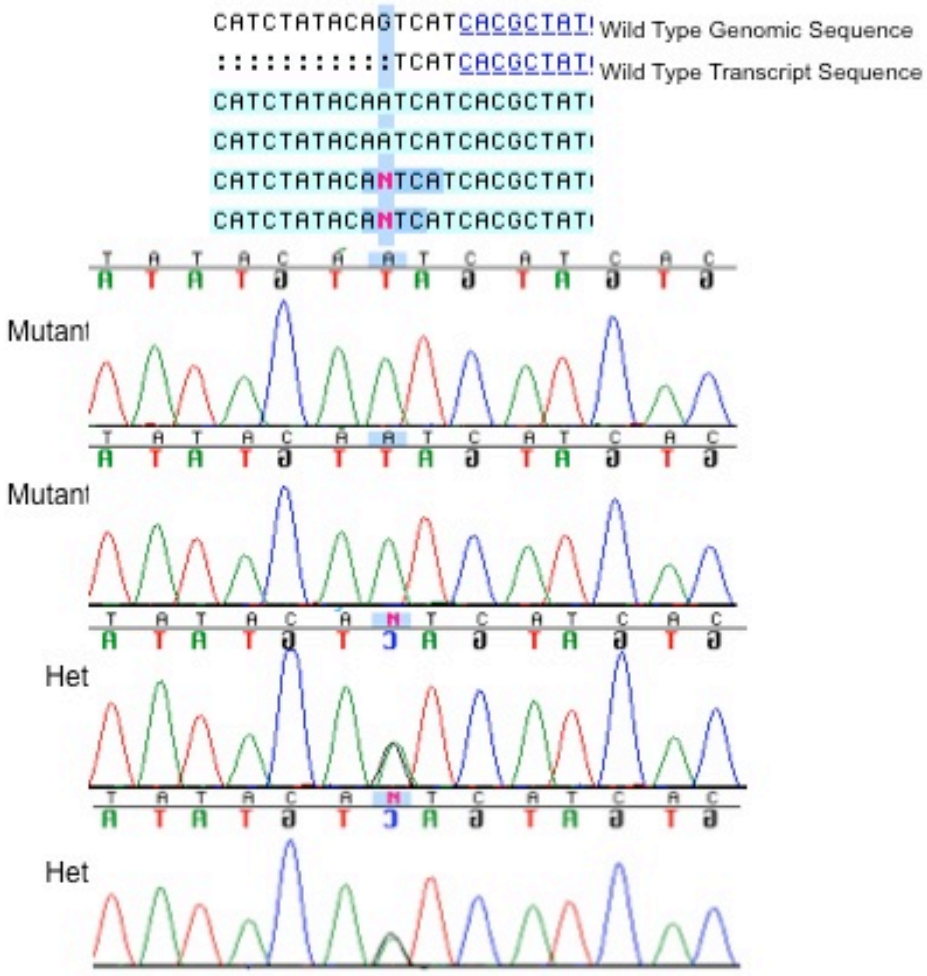

Fig. 1. Sequencing data with corresponding chromatograms for genomic DNA from two mutant mice and two heterozygous littermates. The PCR amplified region containing exon 5 was sequenced using the reverse primer. Highlighted is the critical 3' splice acceptor G nucleotide.

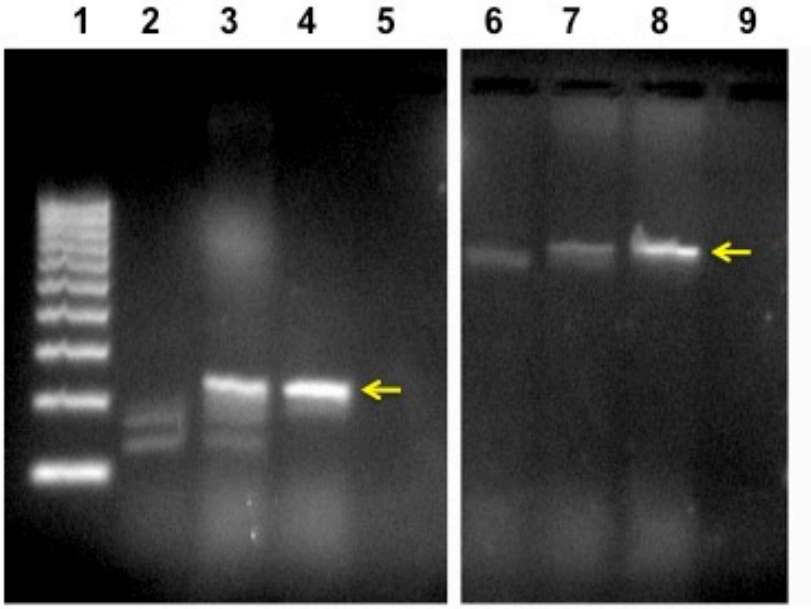

Fig. 2. PCR products from brain cDNA of mutant (lanes 2 and 6), heterozygous (lanes 3 and 7) and wild type mice (lanes 4 and 8) amplified using a forward primer within exon 4 and reverse primers within exon 5 (lanes 2-5) and within exon 6 (lanes 6-9). Lane 1 is a 100 bp DNA ladder while lanes 5 and 9 are PCR blanks (no template DNA). PCR products corresponding to the expected sizes for a normal *Tmie* transcript (yellow arrows) are seen in the controls and heterozygotes but are absent in mutant mice. Smaller PCR products can be seen in the mutants and heterozygotes.

Pathology

A routine pathological screen of a 36-week-old mutant female and a heterozygous male littermate control revealed no abnormalities other than mild hydrocephalus in the mutant. This mild hydrocephalus could be due to the C57BL/6J background and is likely not caused by the mutation. The auditory brainstem response (ABR) was used to assess the hearing of 4 mutant mice at 5 weeks of age. All were deaf, showing no response to the highest stimulus presented (100dB).

Acknowledgements

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