The first *Tmem79* mutant mouse

Authors: Son Yong Karst, Melissa L. Berry, Heather E. Fairfield, Laura G. Reinholdt, David E. Bergstrom, and Leah Rae Donahue

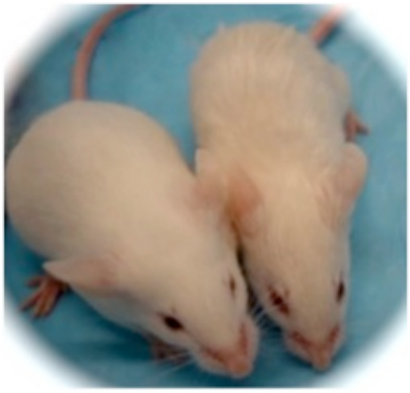
Source of Support: This research was supported by NIH/NCRR grant RR01183 to the Mouse Mutant Resource (Leah Rae Donahue, PI) and Cancer Center Core Grant CA34196.

Mutation (allele) symbol: *Tmem79*^{m1J}
Mutation (allele) name: mutation 1 Jackson
Gene symbol: *Tmem79*
Strain of origin: 129;FVB-Tg(PTH-cre)4167Slib/J
Current strain name: 129;FVB-*Tmem79*^{m1J}/GrsrJ
Stock #014103 (jaxmice.jax.org)
Phenotype categories: Skin and Hair

Abstract
A recessive mouse mutation that arose spontaneously causes abnormal hair growth and irritation around the eyes. Through SNP genotyping, traditional mapping to a position on Chromosome 3, and sequence analysis using the Illumina HiSeq high-throughput sequencing platform and Sanger method, the causative mutation has been identified as a point mutation in the gene transmembrane protein 79 (*Tmem79*). This is the first phenotype associated with a mutant allele of *Tmem79*. Therefore, this mutant strain is a novel tool for understanding the function of TMEM79 in hair development.

Origin and Description
This spontaneous recessive mutation was discovered by Tim Leach in the 129;FVB-Tg(PTH-cre)4167Slib/J strain at The Jackson Laboratory in 2007. These mutants present with an off-white coat color and sparse hair. Additionally, in young mice the coat appears slightly shiny and some adults display mild irritation around their eyes. The hair phenotype is obvious by 7 to 8 days post partum. These mutants are otherwise healthy, fertile and live a normal lifespan. The transgene was bred out of the mutant subline and the mutant phenotype remained the same.

Tmem79^{m1J} homozygotes on the left and right with sibling control between them

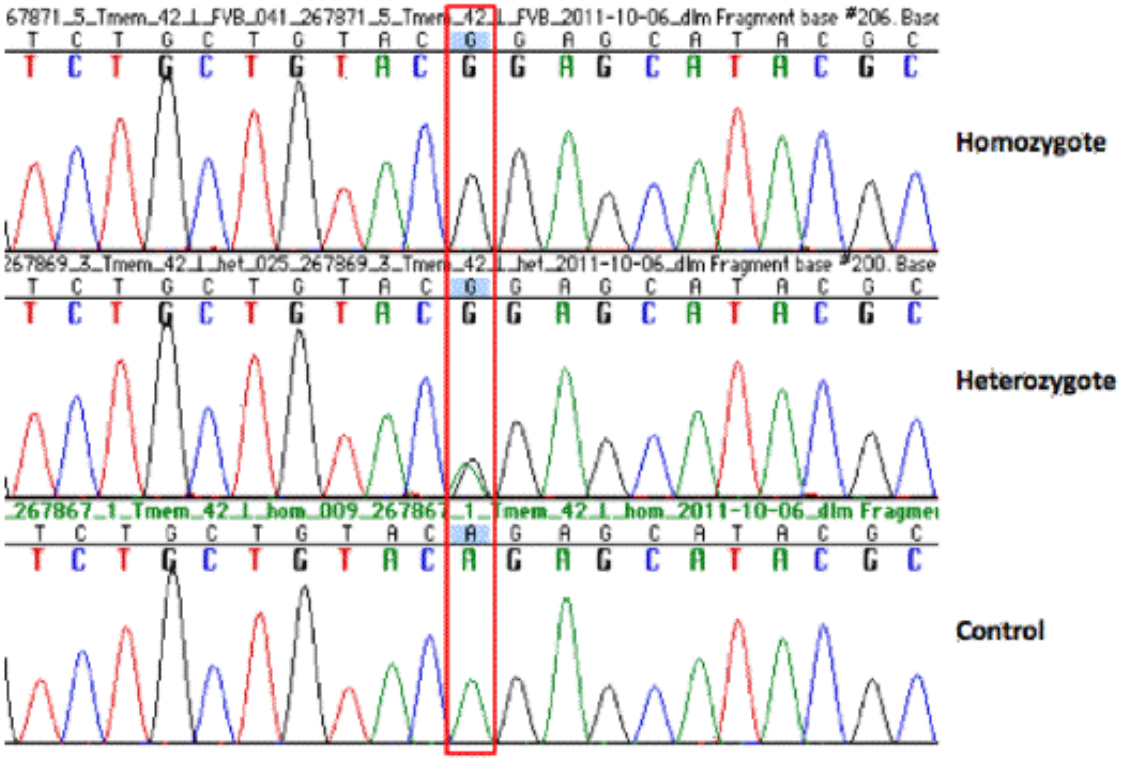


Tmem79^{m1J} homozygote on the right and sibling control on left

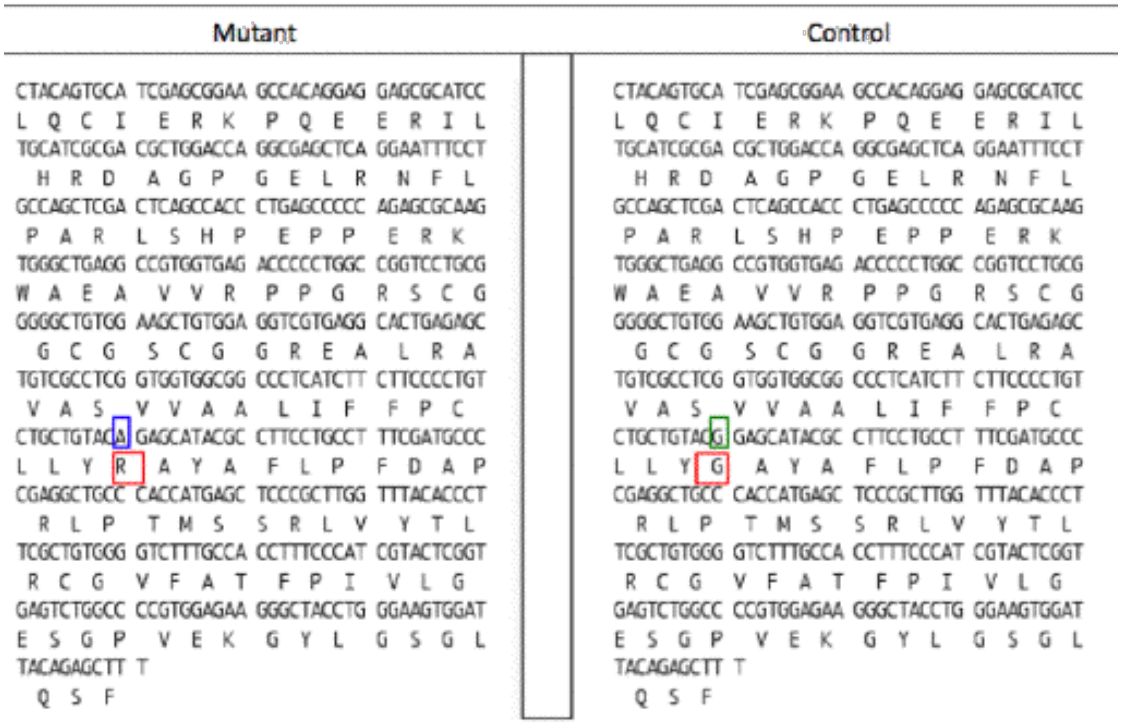
Genetic Analysis

Mutant animals were outcrossed to A/J mice to establish heritability. No affected mice were found in the F1 generation. Intercrossing of unaffected F1 animals resulted in affected F2 animals, indicating a recessive mode of inheritance. Affected F2 mice were generated for linkage analysis and fine mapping. Using standard SNP protocols, linkage analysis for this mutation was completed in the Fine Mapping Laboratory at The Jackson Laboratory. This mutation mapped to Chromosome 3, between NCBI 37 position 86182591 bp and NCBI 37 position 107370499 bp.

Whole exome sequencing was then performed to identify candidate coding 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 3 in transmembrane protein 79 (*Tmem79*). Primers were generated that produce a 489 base pair product spanning the predicted mutation: *Tmem79* forward (TTCGTGCCCATTGATCTACA) and *Tmem79* reverse (AGAGAGTGGCAGGTTCAGGA). Sequence analysis of two mutant genomic DNA samples compared to genomic DNA from four unaffected animals confirmed a single nucleotide transition from G to A at position 88136922 in *Tmem79*. This is a missense mutation and causes an amino acid change from glycine to arginine at protein position 214. This residue is in the first of five putative transmembrane domains.



Comparison of DNA sequence chromatograms of the *Tmem79*^{m1J} homozygote, heterozygote and control sequence. The red boxed region corresponds to the green and blue boxed regions shown in the sequence image.



A portion of the protein coding region of *Tmem79*. The control DNA sequence and its amino acid translation are shown on the right, and the *Tmem79*^{m1J} mutation 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 214 from glycine to arginine. This change is indicated by a red box in the control and the mutant sequence.

Pathology

A routine pathological screen of a mutant and a control at 15 weeks of age and electroretinography of a mutant at 17 weeks of age showed the retinal degeneration phenotype attributable to *Pde6b*^{rd1}, which is a strain characteristic of FVB, not a characteristic attributable to this mutation. Hearing assessments by auditory brainstem response testing of one homozygote at age 13 weeks showed normal hearing. Assessment of the four hair types revealed an anomaly in the zigzag hairs. Normal zigzag hairs are generally straight for approximately one third their length. They then angle approximately 15 degrees followed by another straight length for approximately one third of their length. They then angle again by 15 degrees in the opposite direction followed by another straight length. However, *Tmem79*^{m1J} homozygotes display one or two additional kinks in the zigzag hairs and are not straight at the ends. No aberrations were apparent in the other three hair types.

Discussion

This is the first reported phenotype of a *Tmem79* mutant allele. *Tmem79* has been predicted to encode a phosphoprotein with a transmembrane helix that has 5 transmembrane domains. The single G to A transition discovered by exome sequencing is expected to result in a change from the non-polar hydrophobic amino acid glycine to the

positively charged amino acid arginine in the first of 5 transmembrane domains of a transmembrane helix. The cellular and organism-wide function of this gene is currently unknown.

Acknowledgements
We thank Tim Leach for discovery of the mutant, Roderick Bronson and Coleen Kane for pathological screening, Chantal Longo-Guess for hearing assessment, and Ron Hurd for eye examinations.