No eyelid is a spontaneous missense mutation in *Frem2*

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Mutation (allele) symbol: *Frem2*^{ne}

Mutation (allele) name: no eyelid

Gene symbol: *Frem2*

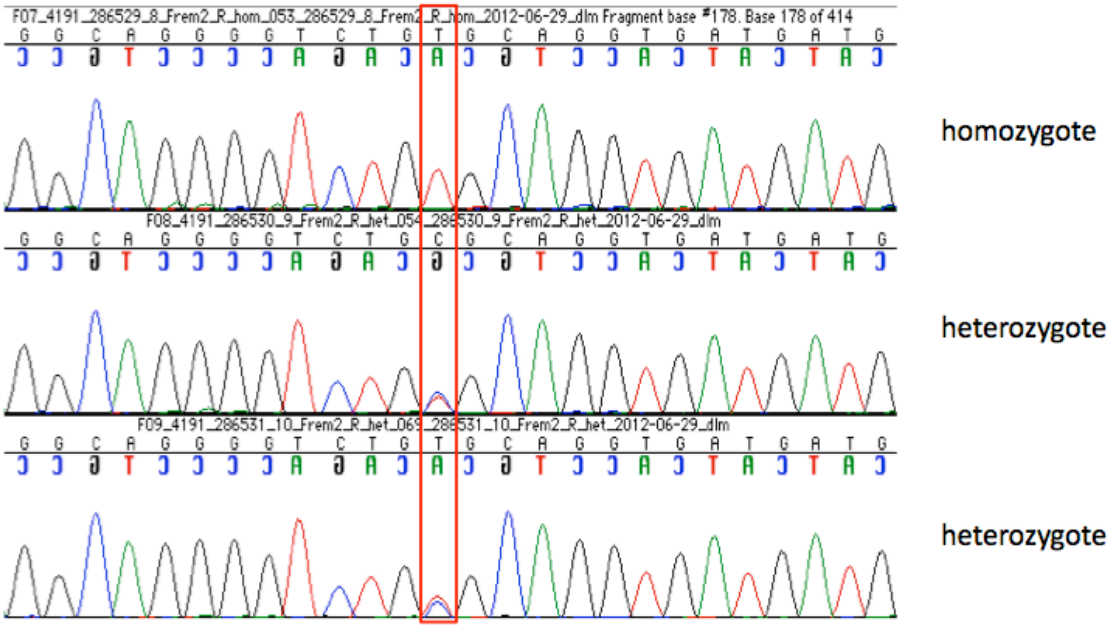
Strain of origin: STOCK Tg(CAG-Bgeo/GFP)21Lbe/J X CAST/EiJ

Current strain name: STOCK *Frem2*^{ne}/GrsrJ

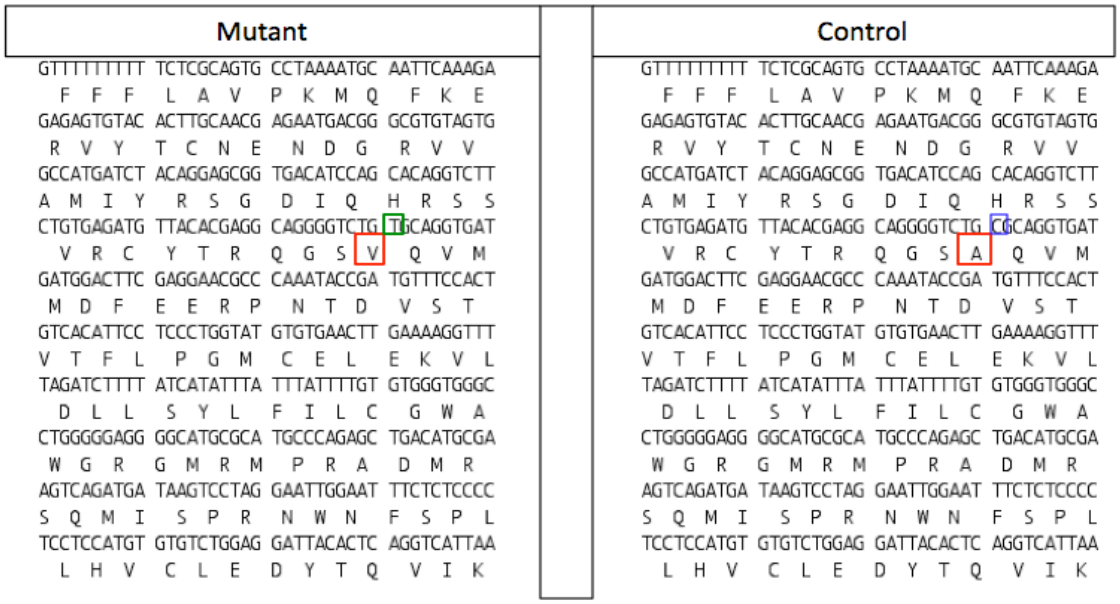
Stock #006857 (jaxmice.jax.org)

Phenotype categories: eye, skeleton, coat color, kidney

The no eyelid mutation arose spontaneously at The Jackson Laboratory, and was characterized by Michelle Curtain. Mapping data and phenotype strongly indicated that no eyelid might be a mutation in Fras1 related extracellular matrix protein 2 (*Frem2*) and we have confirmed this hypothesis. Whole exome sequencing was 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 *Frem2*. Primers were generated that produce a 627 base pair product spanning the predicted mutation: *Frem2* Left (TTGACAGTGGTCTCTCGAGGT) and *Frem2* Right (TTGTCAGTGCACATTTGGTG). Sequence analysis of two mutant genomic DNA samples compared to genomic DNA from three unaffected animals confirmed a single nucleotide transition from C to T at position 53351597 (NCBI build 37, mm9) in *Frem2*. This is a missense mutation and causes an amino acid change from alanine to valine at protein position 2160.



Comparison of DNA sequence chromatograms from *Frem2*^{ne} homozygote, heterozygote control. The red boxed region corresponds to the green and blue boxed regions shown in the sequence figure.



A portion of the protein coding region of *Frem2.* The control DNA sequence and its amino acid translation are shown on the right, and the *Frem2*^{ne} mutant DNA and its translation on the left. A single nucleotide transition is enclosed by a green box in the mutant sequence and a blue box in the control sequence. The mutation that is predicted to change amino acid 2160 from ananine to valine, enclosed by red boxes in the control and the mutant sequence.

FREM2 is a 3160 amino acid single-pass type 1 membrane protein that is important for extracellular matrix interactions. Residue 2160 is in the fourth of five extracellular Calx-beta domains and is located in a stretch of amino acids that are evolutionarily highly conserved (VRCYTRQGS**A**QVM). *Frem2*^{ne} provides an excellent mouse model for Fraser syndrome, a developmental disorder cuased by mutations in either *FRAS1* or *FREM2* that result in defective cell-cell interactions. That a single amino acid change in a Calx-beta domain of *FREM2* can result in such a profound phenotype has already been shown in a human Fraser syndrome patient by Jadeja et al., who identified an E1972K mutation believed to impact the calcium binding pocket at the interface of the second and third Calx-beta domains. While *Frem2*^{ne} is a less severe molecular mutation in *Frem2* than that of other mouse mutations that model Fraser syndrome, the incompletely penetrant mutant phenotypes of malformed digits, syndactyly, absence of eyelids, cryoptophthalmos, microopthalmia, malformed ear pinnae, and one case of renal agenesis all parallel the central phenotypes of human Fraser syndrome.

The *Frem2*$^{my\text{-}F11}$ mouse mutation introduces a stop codon truncating the predicted protein after 1880 residues and thus deleting four of the five Calx-beta motifs (Timmer et al). Timmer's group reported that the *Frem2*$^{my\text{-}F11}$ allele causes a less severe phenotype when the genetic background includes contributions from *M. m. castaneus*. The genetic background on which *Frem2*^{ne} has been assessed includes contributions from CAST/EiJ, and the STOCK *Frem2*^{ne}/J line was selected for increased viability after outcrossing to CAST/EiJ. The CAST/Ei modifier or modifiers of Frem2 remain to be characterized.

References:
Jadeja et al., 2005 May;37(5):520-5. Timmer et al., 2005 Aug;102(33):11746-50.