A new spontaneous mutation in myosin VIIA

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Source of Support: This research was supported by NIH grants OD010972 to the Mouse Mutant Resource (Leah Rae Donahue, PI) and Cancer Core Grant CA34196.

Mutation (allele) symbol: *Myo7*$^{ash1\text{-}13J}$

Mutation (allele) name: shaker 1, 13 Jackson

Gene symbol: *Myo7a*

Strain of origin: C.Cg-*Gata1*^{tm6Sho}/J

Current strain name: C.Cg-*Myo7a*$^{sh1\text{-}13J}$/GrsrJ

Stock #: 022311

Phenotype categories: behavior

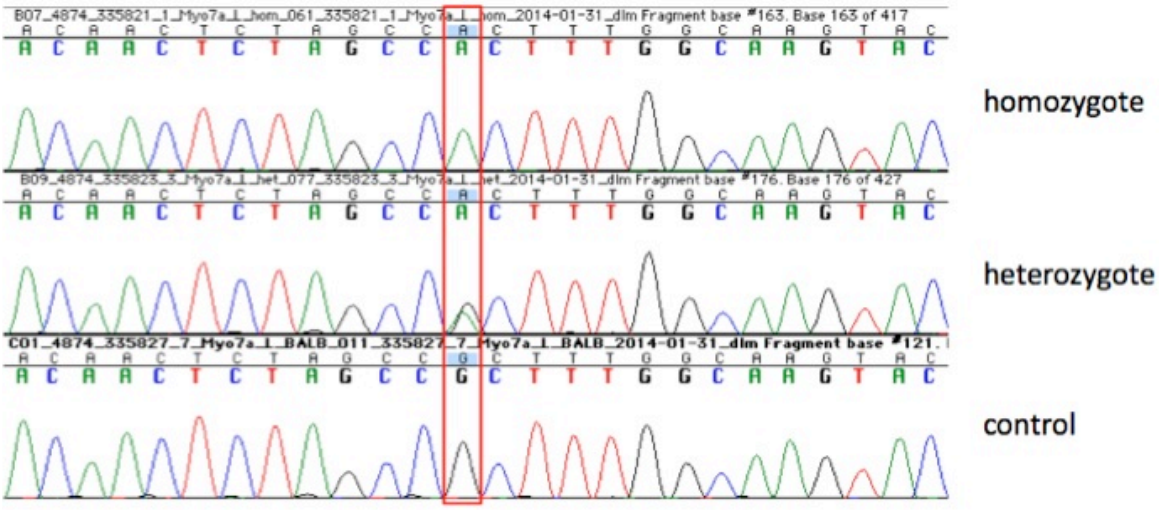
Abstract:
A novel spontaneous mutation that causes head tossing has been characterized and identified as a point mutation in *Myo7a* by its comparable phenotype, map position on Chromosome 7, and sequence analysis of the causative mutation using the Illumina HiSeq (high-throughput sequencing platform) and Sanger method.

Origin and Description:
A new recessive mutation that causes rapid, near continuous head tossing with some head shaking and head bobbing was identified in the C.Cg-*Gata1*^{tm6Sho}/J colony at The Jackson Laboratory. Homozygotes live a normal life span, and can reproduce. In addition to the head tossing, the gait is lower to the ground with the feet held wider apart than normal, likely for balance. The tail is often held aloft, even straight up in the air, as if for balance, and there is an increased tendency to move backward. Circling behavior has not been noted.

Genetic Analysis:
This new mutation was shown to have recessive inheritance by mating a mutant to an unrelated 129S1/SvImJ mouse. The unaffected F1 hybrid offspring were backcrossed to a mutant mouse, and affected N2 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 7, between position 63,768,461 bp and position 106,153,798 bp (MGSCv37). This map position includes myosin VIIA (*Myo7a*) and the phenotype is like that of shaker 1 mutants of *Myo7a*.

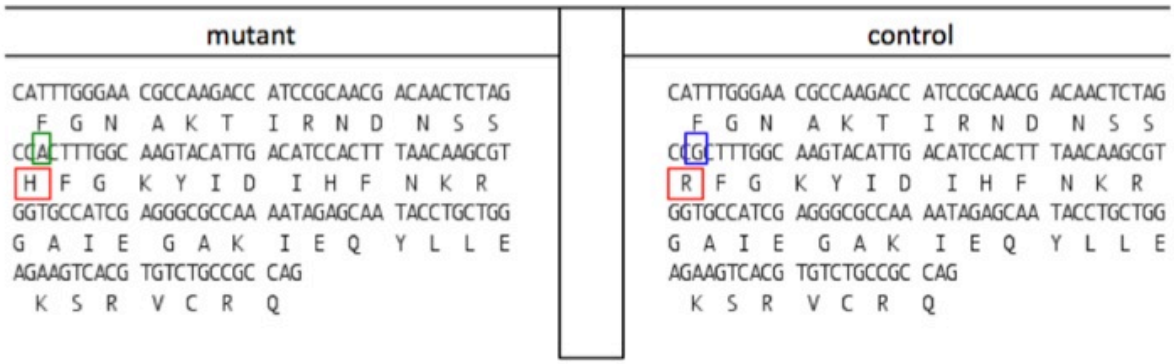
Whole exome sequencing followed by single nucleotide variant (SNV) and INDEL (insertion/deletion) analysis including Burrows-Wheeler alignment (BWA) and

SAMtools were 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 transition from G to A at position 98,097,488 on Chromosome 7 (GRCm38/mm10) in myosin VIIA (*Myo7a*) was detected in these data. Primers were generated that produce a 500 base pair product spanning the predicted mutation: Myo7a Left (GGAGCTGGCAAGACAGAGAG) and Myo7a Right (GAAGTGGGCACCAGGATTG). Sequence analysis of genomic DNA from two mutants compared to genomic DNA from three unaffected animals confirmed the presence of this SNV.



Comparison of DNA sequence chromatograms from *Myo7a*$^{sh1\text{-}13J}$ homozygote, heterozygote and BALB/cByJ control. The red boxed region corresponds to the green and blue boxed regions shown in the sequence figure.

This is a missense mutation predicted to change amino acid 212 from arginine to histidine in a protein that is predicted to be 2215 amino acids long. This substitution of one positively charged amino acid for another occurs in the myosin head motor domain, as do several other phenotypic point mutations in mouse *Myo7a*. This is the thirteenth mutation in *Myo7a* to arise at The Jackson Laboratory so this new allele has been designated shaker 1, 13 Jackson, *Myo7a*$^{sh1\text{-}13J}$. The impact on expression, protein stability and function remain to be characterized. The X-linked *Gata1*^{tm6Sho} mutation, which was homozygous in the mice initially assessed, causes complete ablation of the eosinophil lineage, but homozygotes have no outward behavioral or neurological phenotype so this mutation is not thought to alter the outward phenotype of the *Myo7a*$^{sh1\text{-}13J}$ mutation. In support of this, the *Myo7a*$^{sh1\text{-}13J}$ mutation has been bred by backcross-intercross of *Myo7a*$^{sh1\text{-}13J}$ homozygous females to BALB/cByJ males for three generations with no alteration of phenotype.



A portion of the protein coding region of *Myo7a*. The control DNA sequence and its amino acid translation are shown on the right, and the *Myo7a*$^{sh1\text{-}13J}$ 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 212 from arginine to histidine, enclosed by red boxes in the control and the mutant sequence.

Acknowledgements:
The authors thank Jennifer Aiguier for discovery of this mutant.