A spontaneous Zfp191 mouse mutation that alters 18 carboxyl terminal amino acids

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Mutation (allele) symbol: *Zfp191*$^{hmcns-2J}$

Mutation (allele) name: hypomyelinated central nervous system 2 Jackson

Gene symbol: *Zfp191*

Strain of origin: BALB/cByJ congenic

Current strain name: CByJ(Cg)- *Zfp191*$^{hmcns-2J}$/GrsrJ

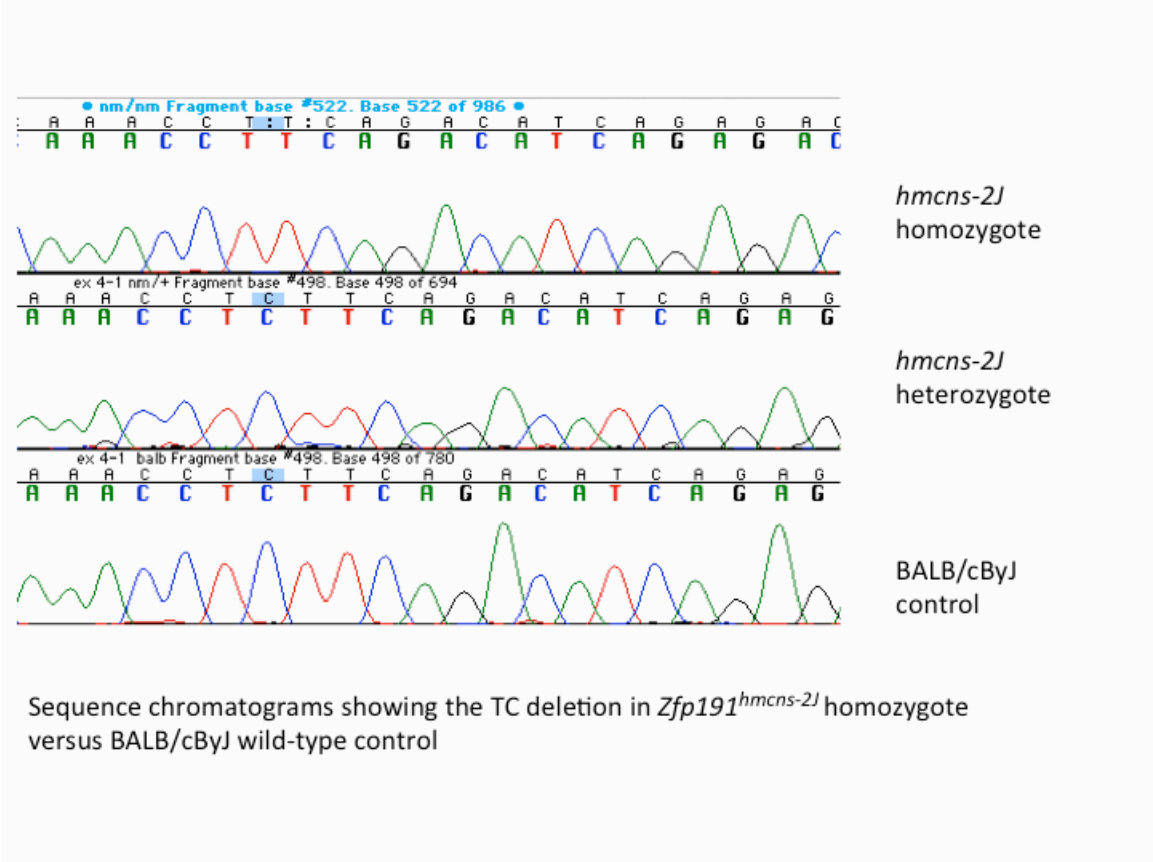
Stock #022138 (jaxmice.jax.org)

Phenotype categories: neurological

Origin and Description
In 2012 a spontaneous mutation was identified in a BALB/cByJ congenic strain that was already carrying a dominant head-tossing phenotype. The new mutation can be identified as early as fourteen days of age by a whole body tremor that is most pronounced when the mouse is moving. This new mutation was selectively bred away from the head-toss mutation. In addition to the tremor, both males and females have tonic seizures. As the affected mice age, the severity of the tremor becomes more pronounced, and they do not survive past four to five weeks of age.

Genetic Analysis
Because these mutant mice do not survive long enough to breed, the mode of inheritance of the new mutation was established by intercrossing phenotypically normal siblings of mutants. These unaffected mice produced offspring that expressed the mutation, proving that this is a recessive mutation. To determine the chromosomal location of the mutation, mice proven heterozygous by progeny testing were mated to 129S1/SvImJ mice. The F1 progeny were sibling intercrossed, DNA was isolated from the affected F2 offspring, and a linkage assignment was determined using standard SNP mapping procedures. The mutation mapped to Chromosome 18. Because of the map location and phenotypic similarities with the mutant *Zfp191*$^{hmcns-2J}$ (hypomyelinated central nervous system), Sanger sequence analysis of *Zfp191* was performed. Using the primer pair *Zfp191* forward CCTTTCCCTTCAAGTTCTTTCC and *Zfp191* reverse

CCCTTACAGAACACTCCAATG, which produce a 955 base pair product from wild-type sequence, a deletion of two base pairs, C at position 24,014,204 and T at position 24,014,205 (GRCm38), was identified from 2 homozygous mutants.



Sequence chromatograms showing the TC deletion in *Zfp191*$^{hmcns-2J}$ homozygote versus BALB/cByJ wild-type control

This 2 base pair deletion, which falls within exon 4 of the *Zfp191* gene, causes a frame shift that results in the substitution of 20 new amino acids in place of the last 18 amino acids of this 368 amino acid protein.

Wild-type *Zfp191* Sequence:
AGC TCA AAC CTC TTC AGA CAT CAG AGA CGA CAC AAT GCA GAA AAA CTT TTG AAT GTT GTG AAG GTT TAA
S S N L F R H Q R R H N A E K L L N V V K V .

Zfp191$^{hmcns-2J}$ Sequence:
AGC TCA AAC CTT CAG ACA TCA GAG ACG ACA CAA TGC AGA AAA ACT TTT GAA TGT TGT GAA GGT TTA AGA AAT TAG
S S N L **Q T S E T T Q C R K T F E C C E G L R N .**

Wild-type and *hmcns-2J* mutant transcript and translated protein sequences showing highlighted in red the TC nucleotides that are deleted from the mutant, and the altered reading frame in the mutant, underlined in blue, and resulting substitution of 20 distinct amino acids, in bold type, in place of the 18 terminal amino acids of the wild-type sequence.

This is predicted to alter the last of four zinc finger domains.

Pathology

A routine pathological screen[1] of three mutants at four weeks of age showed very little myelin in the central nervous system but normal myelination in the peripheral nervous system. The eyes of four mutants and two controls at three weeks of age were examined

by ophthalmoscopy and found to be normal. Hearing, assessed by auditory brainstem response testing[2] of seven mutants and four control mice at four weeks of age revealed severe hearing loss in four mutants, elevated thresholds at higher frequencies in one mutant, and normal thresholds in two mutants and all four controls. Hearing assessment has not been reported for other published *Zfp191* mutants.

Discussion

This two base pair deletion, predicted to replace the 18 carboxyl terminal amino acids of ZFP191 with 20 distinct amino acids, yields a phenotype nearly as severe as the *Zfp191*$^{tm1.2Pop}$ null mutation or the *Zfp191*^{hmcns} mutation, which causes a premature stop codon before the last 155 amino acids of this 368 residue protein. Each of those mutations causes tonic seizures and death by approximately 35 days of age due to hypomyelination, similar to the phenotype of *Zfp191*$^{hmcns-2J}$ homozygotes, which also have shortened lifespan. The *Zfp191* ^{tm1Jifu} null mutant, on the other hand, causes death by embryonic day 7.5 due to impaired cell proliferation.

[1]**Standard Histology Protocol used in the Mouse Mutant Resource:** For fixation of tissues, mice were deeply anesthetized with tribromoethanol (avertin) until they no longer displayed a withdrawal reflex in the hind limbs and then perfused intracardially with Bouin's fixative following a flush of the vasculature with saline solution. After soaking in Bouin's for one week to demineralize bones, tissues were dissected. Six segments of spine with axial muscles and spinal cord in situ, representing cervical, thoracic and lumbar spinal segments, were dissected. The brain was removed and sliced into 6 cross sectional pieces at the levels of olfactory lobes, frontal cortex, striatum, thalamus, midbrain, rostral and caudal medulla with cerebellum. Midsagittal slices of hind leg through the knees were prepared. Slices of basal skull through the pituitary and inner ears were taken. Both eyes, salivary glands and submandibular lymph node, trachea plus thyroid and sometimes parathyroid were removed and cassetted. A longitudinal slice of skin from the back was removed. The thymus, slices of lung, and a longitudinal slice of heart were cassetted. Similarly slices of liver through gall bladder, kidney with adrenal attached, pancreas and spleen were prepared. The stomach was sliced longitudinally to include both squamous and glandular portions. Loops of small intestine from 3 levels and slices of large intestine and cecum were removed, as were slices of urinary bladder. The whole uterus, with ovaries attached, was taken. In males testes were sliced longitudinally. The accessory male organs including seminal vesicles, coagulating gland and prostate were removed en block. Altogether in most cases all tissue fit into a total of 10 cassettes. The cassettes were processed in an automatic tissue processor to dehydrate tissues which were then embedded in paraffin. Six micron sections were cut and stained with hematoxylin and eosin (H&E). Sections of brain and spinal cord in vertebral bones also were stained with luxol fast blue (LFB) for myelin and cresylecht violet (CV) for cellular detail.

[2]**ABR thresholds** in mice are determined using a semi-automated computer system (Intelligent Hearing Systems, Miami, Florida). Subdermal needle electrodes are inserted at the vertex and ventrolaterally to both ears of anesthetized mice. Specific auditory stimuli from 10-100 dB SPL are delivered binaurally through plastic tubes from high frequency transducers. ABR thresholds are obtained, in an acoustic chamber, for clicks and for 8, 16, and 32 kHz pure-tone pips.

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