Trembler 2 Jackson, a spontaneous point mutation in Pmp22

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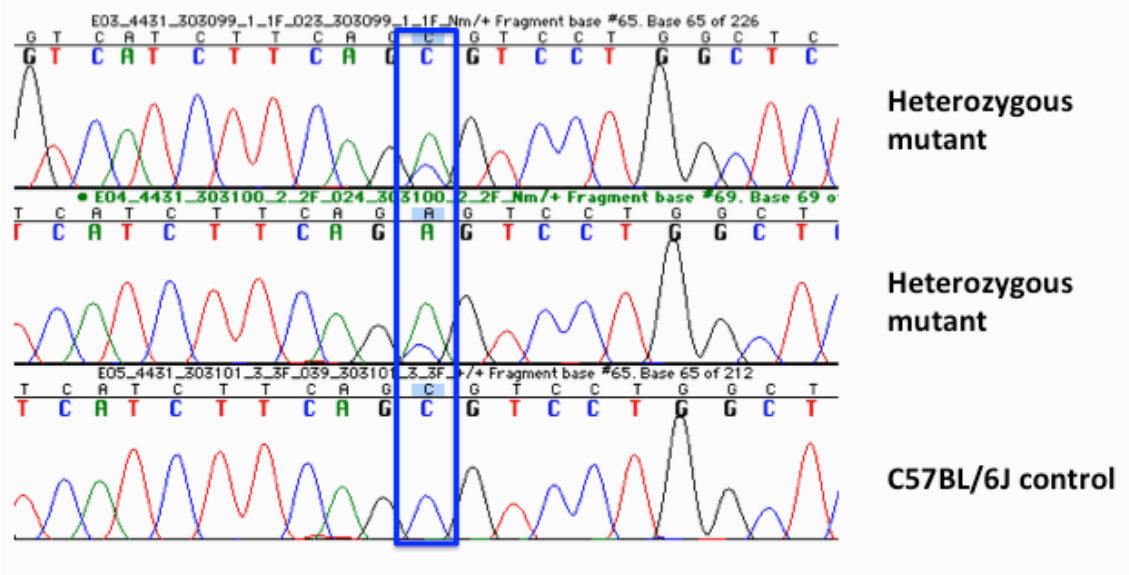
Mutation (allele) symbol: *Pmp22*$^{Tr\text{-}2J}$
Mutation (allele) name: trembler 2 Jackson
Gene symbol: *Pmp22*
Strain of origin: C57BL/6J
Current strain name: C57BL/6J-*Pmp22*$^{Tr\text{-}2J}$/GrsrJ
Stock #021550 (jaxmice.jax.org)
Phenotype categories: neurological

Abstract:
Mutations in *PMP22* cause varied peripheral demyelinating neuropathies in humans. We have characterized a dominant mouse mutation in *Pmp22*, designated trembler 2 Jackson. This spontaneous point mutation yields a serine to arginine missense mutation in the second transmembrane domain. Heterozygotes develop a rapid tremor and ataxia with peripheral demyelination, but this mutation causes a less severe phenotype than the original trembler mutation. This mutant provides a new model for correlating PMP22 mutations with phenotypic outcome.

Origin and description:
In 2012 a novel mutation that causes tremors, abnormal motor coordination, and abnormal gait was identified in a C57BL/6J strain in which a spontaneous mutation in *Kif1a* was segregating. Genotyping confirmed that the *Kif1a* mutation was absent from the new mutant subline. Affected animals carrying the new mutation can be identified by approximately three weeks of age by a rapid tremor, which persists throughout life, spasticity in the muscles of the lower back and limbs, and reduced ability to organize hind limb movements, producing an unsteady, wobbly gait. The mode of inheritance of this new mutation was established by mating a mutant female to a C57BL/6J male. Both normal and affected progeny were recovered, proving that this mutation is inherited in a dominant manner. Heterozygous mice have a normal life span. Male heterozygotes have a high rate of failed breeding: only two of six male heterozygotes placed with C57BL/6J females have produced offspring. Therefore, the colony is maintained by mating a heterozygous female with a C57BL/6J male.

Genetic analysis:
Whole exome sequencing was used to identify candidate coding mutations. A single nucleotide variant (SNV) was found in the candidate gene peripheral myelin protein 22 (*Pmp22*). Primers were generated that produce a 237 base pair product spanning the predicted mutation: *Pmp22* forward CCATTCCCAGGCTTGTCTAA and *Pmp22* reverse CTTGGGGAACAAGTCCTTCA . Sanger sequence analysis of genomic DNA from one

normal and two heterozygous mice confirmed the presence of a single nucleotide transversion from C to A on Chromosome 11 at position 62,964,670 (MGSCv37), which is in exon 4 of *Pmp22*. This missense mutation results in the replacement of serine with arginine at residue 76 of the 160 amino acid protein (S76R).



A single C to A transition at position 62,964,670 (MGSCv37) boxed in blue yields a serine to arginine substitution at residue 76 of the 160 amino acid protein. The presence of both alleles in heterozygous mutant samples is shown in the comparison of sequence chromatograms above.

This is the second mutation in the Pmp22 gene to arise at The Jackson Laboratory and it has therefore been designated trembler 2 Jackson, *Pmp22*$^{Tr\text{-}2J}$.

An independent variant at this same genomic position (rs26940153) has been identified in the inbred strain PWK/PhJ, but there a C to T transition results in a synonymous codon change of AGC to AGT leaving the serine at residue 76 unchanged. The trembler 3 Harwell mutation (*Pmp22*$^{Tr\text{-}3H}$) is a point mutation causing a serine to threonine substitution at amino acid 72, just 4 residues from the location of the trembler 2 Jackson mutation. The phenotype of *Pmp22*$^{Tr\text{-}3H}$ heterozygotes is also mild, including a tremor with onset at 3 weeks of age, even though less conservative amino acid substitutions at the same residue in humans have resulted in Dejerine-Sossa syndrome1, a severe phenotypic expression of *PMP22* mutations.

Pathology

A routine pathological screen[1] of two heterozygous males and two wild-type controls all at 5 weeks of age found severe myelin deficiency in the peripheral nerves of only the heterozygotes. Additional assessment of one sixteen-week-old female heterozygote revealed peripheral nerves that were abnormally large and cellular and containined very little myelin. Luxol fast blue staining confirmed the myelin deficiency in the peripheral nerves. The eyes of three mutants and one control animal were examined by

ophthalmoscopy and found to be normal. Hearing was assessed by auditory-evoked brainstem response testing[2]: at 44 days of age one mutant and one control mouse showed normal hearing; at 36 days of age two mutants had normal hearing and one mutant was completely deaf; and at 32 days of age one mutant and two control mice showed normal hearing but two mutants were hearing impaired. Therefore, hearing impairment sometimes results from this mutation but penetrance is incomplete, at least in the heterozygote. All feet were assessed on ten awake, adult, heterozygous mutants and none showed any shape anomaly resembling *pes cavovarus*.

Discussion:

This new spontaneous allele causes a less severe phenotype than several other mutant alleles of *Pmp22*, including the original trembler mutation, in that no juvenile mortality, paralysis, or seizures have been observed. The phenotype does include diminished male fertility, hearing loss with incomplete penetrance, peripheral nerve demyelination, rapid tremor with onset by 3 weeks of age, wobbly gait, and spasticity in the muscles of the lower back and limbs.

PMP22 is a hydrophobic glycoprotein that includes four membrane-spanning domains and is found in peripheral myelin. The original trembler ($Pmp22^{Tr}$) allele is also a point mutation, resulting in the substitution of aspartic acid (charged) for glycine at protein position 150, predicted to reside in the fourth transmembrane domain. Trembler Jackson ($Pmp22^{Tr\text{-}J}$) substitutes proline for leucine (hydrophobic) at protein position 16, in the first transmembrane domain. These mutations, which both introduce non-conservative amino acid changes within transmembrane domains of the PMP22 protein, have been predicted to result in the inability of the protein to integrate properly into the cellular membrane. Abnormal aggregation of mutant PMP22 protein has been observed in both mutant alleles, suggesting that these proteins are not properly integrated or not correctly targeted for cellular trafficking and degradation. The $Pmp22^{Tr\text{-}2J}$ mutation reported here is predicted to be a tolerated amino acid change within the second transmembrane domain. This may result in a semi-functional protein, able to be entirely or partially integrated into the cellular membrane, thus leading to a milder phenotype. Further studies are needed to test the function of the $Pmp22^{Tr\text{-}2J}$ mutant protein.

With its milder phenotype relative to several other mouse *Pmp22* mutants, the trembler 2 Jackson mutation provides a mouse model for comparative assessments of a mild point mutation in a single transmembrane domain relative to more severe mutations in PMP22. This may have predictive value relating to the development of the phenotypic array of human diseases caused by PMP22 mutations, including Charcot-Marie-Tooth Disease (types 1A and 1E), Roussy-Levy syndrome, Dejerine-Sottas syndrome, and hereditary neuropathy with liability to pressure palsies. While the phenotype is not detected in very young mice and there is no indication of *pes cavovarus*, trembler 2 Jackson mutants have the gait ataxia and demyelinating peripheral neuropathy found in Dejerine-Sottas syndrome, Charcot-Marie-Tooth disease, and Roussy-Levy syndrome, as well as the static tremor that also develops in some of these patients. Type 1E Charcot-Marie-Tooth disease includes hearing deficits in some, but not all, cases, as is true of trembler 2 Jackson mutants. Thus, the trembler 2 Jackson mutation may be useful in assessing

contributing factors causing hearing loss in this disease.

Acknowledgements:
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References:

Isaacs AM et al, Identification of a new Pmp22 mouse mutant and trafficking analysis of a Pmp22 allelic series suggesting that protein aggregates may be protective in Pmp22-associated peripheral neuropathy. Mol Cell Neurosci. 2002 Sep;21(1):114-25.

[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.