A novel point mutation in *Tpp1* provides a new model for late-infantile neuronal ceroid lipofuscinosis

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

Source of Support: This research was supported by grants RR001183 and 0D010972-35 to the Mouse Mutant Resource (Leah Rae Donahue, PI) and by the Cancer Center Core Grant CA34196 awarded to The Jackson Laboratory

Mutation (allele) symbol: $Tpp1^{m1J}$

Mutation (allele) name: mutation 1 Jackson

Gene symbol: *Tpp1*

Strain of origin: C57BL/6J

Current strain name: STOCK $Tpp1^{m1J}$ /GrsrJ

Stock #012876 (jaxmice.jax.org)

Phenotype categories: neurological

Abstract
A new, recessive mutation that causes progressive motor defects and premature death has been characterized and identified as a point mutation in a splice donor site of the tripeptidyl peptidase I gene (*Tpp1*). This mutant provides a model for late-infantile neuronal ceroid lipofuscinosis with some advantages over existing models.

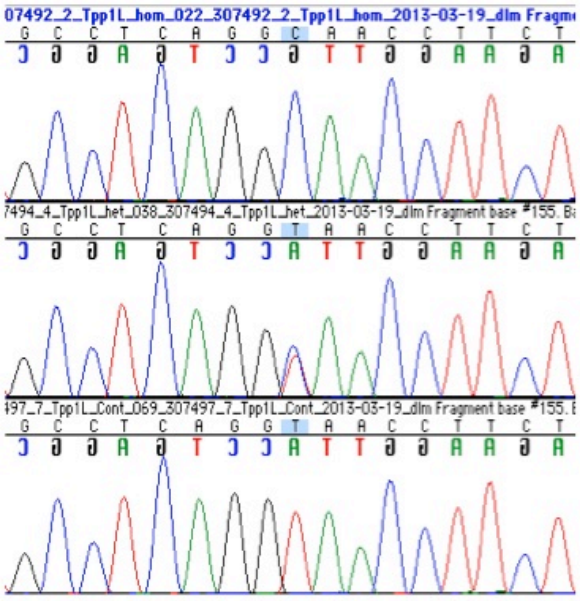
Origin and Description
A mutant was identified in the progeny of an ENU mutagenized C57BL/6J male that was part of a region-specific mutagenesis screen performed in the laboratory of Dr. Simon John. The region-specific ENU screen identified mutants in the rump white (*Rw*) deletion region of Chromosome 5 by breeding the mutagenized C57BL/6J male to C3Fe.Cg-*Rw* heterozygous females, breeding the F1 offspring heterozygous for *Rw* and any ENU-induced mutations to a C3Fe.Cg-*Hm* +/+ *Rw* mate, then selecting the offspring that were found to be heterozygous for *Rw* and any ENU-induced mutations. These were bred back to their F1 father to produce a G3 population in which some pups were homozygous for ENU-induced mutations and some were still heterozygous[1]. This new mutant was identified in this G3 population and was transferred to The Mouse Mutant Resource for characterization. The mutant subline was sibling inbred to homozygosity and at F18 was outcrossed once to C57BL/6J and then sibling intercrossed again to homozygosity. This STOCK background, which is homozygous for nonagouti, consists predominantly of C57BL/6J and C3HeB/FeJ with possible trace contributions from C3H/HeH, 101/H and other undefined backgrounds.

This new mutant is noticeable because of a tremor and hunched posture by 3.5 to 4 months of age. The tremor initially appears intermittent and more pronounced when walking, but the tremor becomes constant as the symptoms worsen with time. The symptoms rapidly progress, the mutants display diminishing locomotion, and all die by 6 months of age. The colony is maintained by sibling inbreeding homozygotes, but because of this shortened lifespan mutant pairs only produce one or two litters. When heterozygotes are needed a separate outcross to C57BL/6J is done.

Genetic Analysis
Mutant animals were outcrossed to FVB/NJ mice to establish heritability. No affected mice were found in the F1 generation. Intercrossing unaffected F1 animals produced 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 7, between MGSCv37 position 58,658,647 bp and position 123,960,956 bp.

Whole exome sequencing was 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. The exome data were analyzed using tools and workflows provided by Galaxy including processes for mapping (BWA), SNP calling (VCF Tools), and annotation of variants. Our analysis focused on novel variants, which were not positioned in repetitive sequence, had expected allele ratios (>0.95 for homozygous variants and >0.2 for heterozygous variants), and displayed sufficient locus coverage (at least 5X for homozygous variants and 10X for heterozygous variants) for effective mutation discovery. High priority was given to protein coding or splice variants within mapped regions, as well as unique variants that were not found in other exome data sets or in the Sanger Mouse Genomes Database. Following these analyses, re-sequencing of additional mutant and unaffected samples was performed to validate and determine the most likely causative mutation.

A single nucleotide polymorphism was found at position 112,897,395 (MGSCv37) on Chromosome 7 in tripeptidyl peptidase 1 (*Tpp1*). Primers were generated that produced a 408 base pair product spanning the predicted mutation: *Tpp1* Left (GCACAGGAGCCCTTCTTACA) and *Tpp1* Right (TGGGAAAGCCATAGTAAGTATTCAGA). Sanger sequence analysis of two homozygous mutants' genomic DNA compared to genomic DNA from five unaffected animals confirmed this single nucleotide transition from T to C, which overlaps a *Tpp1* splice donor site. This new mutation has been designated $Tpp1^{m1J}$ for mutation 1 Jackson.



Chromatograms comparing the sequence from C57BL/6J, *Tpp1*^{m1J} heterozygote, and homozygote with the T to C transition in *Tpp1* highlighted in blue

Pathology

We assessed 7 homozygotes at various ages with our standard pathological screen[a]. At 6 weeks of age one female and one male homozygote were found to have only a few inflamed and degenerating muscle fibers. At 17 weeks of age one female displayed eosinophilic and luxol fast blue positive inclusions in the cytoplasm of motor neurons in spinal cord and other large neurons in the brain. At 19 weeks of age one male and one female homozygote were found to have inclusions in neurons, with myelin figures in the white matter, and inclusions also in muscle and in bone marrow macrophages. Small muscle fibers and rowing of nuclei in the muscle fibers were also observed in these two homozygotes. Subsequent assessment of one female homozygote and one male homozygote at 10 weeks of age revealed that the myopathy found at 17 and 19 weeks of age had begun to present in these 10-week-old homozygotes.

Retinal degeneration is a standard phenotype in children with *TPP1* mutations causing late-infantile neuronal ceroid lipofuscinosis. However, ophthalmoscopy of 3 female homozygotes and 2 male homozygotes all at 17 weeks of age failed to detect any defects. Subsequent retinal and fundus images by optical coherence tomography of one homozygote at 18 weeks of age showed normal retina but spots on the fundus. This may be the beginning of ocular defects and further assessment remains to be done on older homozygotes.

Discussion

This ENU-induced point mutation is a transition in a splice donor site just downstream of exon 8 of *Tpp1*, a gene that encodes an essential lysosomal exopeptidase. Human *TPP1* mutations cause late-infantile (type 2) neuronal ceroid lipofuscinosis, a recessive, neurodegenerative lysosomal storage disease that involves seizures, ataxia, myoclonus, cerebral atrophy, retinal degeneration, and premature death. Clinical onset is usually between 2 and 4 years of age and death results generally between 10 to 15 years of age. $Tpp1^{m1J}$ provides a new model of this human disease with pre-wean onset and reasonably rapid progression.

An engineered hypomorphic Arg446His mouse allele, $Tpp1^{tm1.1Plob}$, on a congenic C57BL/6J background causes a comparatively very mild phenotype, with tremor onset after 1 year of age, and median survival of 603 days. $Tpp1^{tm1.1Plob}$ was generated via cre-mediated excision from the $Tpp1^{tm1Plob}$ allele, which retains the neomycin selection cassette in intron 1 and disrupts splicing results in diminished enzyme activity. The $Tpp1^{tm1Plob}$ allele causes a stronger and earlier disease phenotype on a predominantly C57BL/6J background, with constant tremor by 7 weeks of age, ataxia with shortened stride and splaying of the hind-limbs by 4 months of age, and a median survival of 132 days. On a coisogenic 129S1/Sv background the $Tpp1^{tm1Plob}$ allele has a slightly less severe phenotype, with median survival of 155 days[2]. By comparison, the $Tpp1^{m1J}$ point mutation on this mixed genetic background of predominantly C3HeB/FeJ and C57BL/6J provides a model for late-infantile neuronal ceroid lipofuscinosis with similar onset and mortality as $Tpp1^{tm1Plob}$, but the $Tpp1^{m1J}$ point mutation falls in a non-coding region and lacks any potential caveats associated with neomycin phosphotransferase.

Acknowledgements

The authors thank Simon John and his laboratory for identification of this mutant, Chantal Longo-Guess for hearing assessment, and Norman Hawes, Ron Hurd, and Bernard FitzMaurice for eye examinations.

References

1) John Schimenti and Maja Bucan, Functional Genomics in the Mouse: Phenotype-Based Mutagenesis Screens, Genome Research, 1998, Jul; 8(7) p698-710.

2) Sleat DE; El-Banna M; Sohar I; Kim KH; Dobrenis K; Walkley SU; Lobel P, Residual levels of tripeptidyl-peptidase I activity dramatically ameliorate disease in late-infantile neuronal ceroid lipofuscinosis. Mol Genet Metab, 2008, 94 (2) 222-33

Footnotes:

a) 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.