Gracile axonal dystrophy 2 Jackson; a neuromuscular mutation in the *Uchl1* gene.

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Mutation (allele) symbol: *gad-2J*

Mutation (allele) name: gracile axonal dystrophy 2 Jackson

Gene symbol: *Uchl1*

Strain of origin: WCB6F1/J-*Kitl*^{Sl}*Kitl*$^{Sl\text{-}d}$

Current strain name: B6.Cg-*Uchl1* $^{gad\text{-}2J}$/GrsrJ

Stock #: 13110 (jaxmice.jax.org)

Phenotype categories: Neuromuscular

Abstract

Mice affected by the new *Uchl1*$^{gad\text{-}2J}$ mutation have a neuromuscular phenotype causing an impaired locomotion and atrophy of hind limb muscles. PCR and sequence analysis determined that this is a mutation in the ubiquitin carboxy-terminal hydrolase L1 (*Uchl1*) gene.

Origin and Description

A new spontaneous recessive mutation has been discovered and characterized at The Jackson Laboratory. Mice displaying the *Uchl1*$^{gad\text{-}2J}$ mutation phenotype were found by Kristinam Croan in a production colony of WCB6F1/J-*Kitl*^{Sl}*Kitl*$^{Sl\text{-}d}$ mice.

Mice homozygous for the *gad-2J* mutation have a normal phenotype until about 6~7weeks of age when the neuromuscular phenotype starts to show. The hind legs of affected mice have weakness characterized by limb grasping, dragging or splaying of hind limbs while walking. Beginning at 12 weeks age, mutants decrease in body weight, and have difficulty moving, and the phenotype worsens progressively and becomes more severe with age. The mutant mice are eventually paralyzed, and die prematurely.

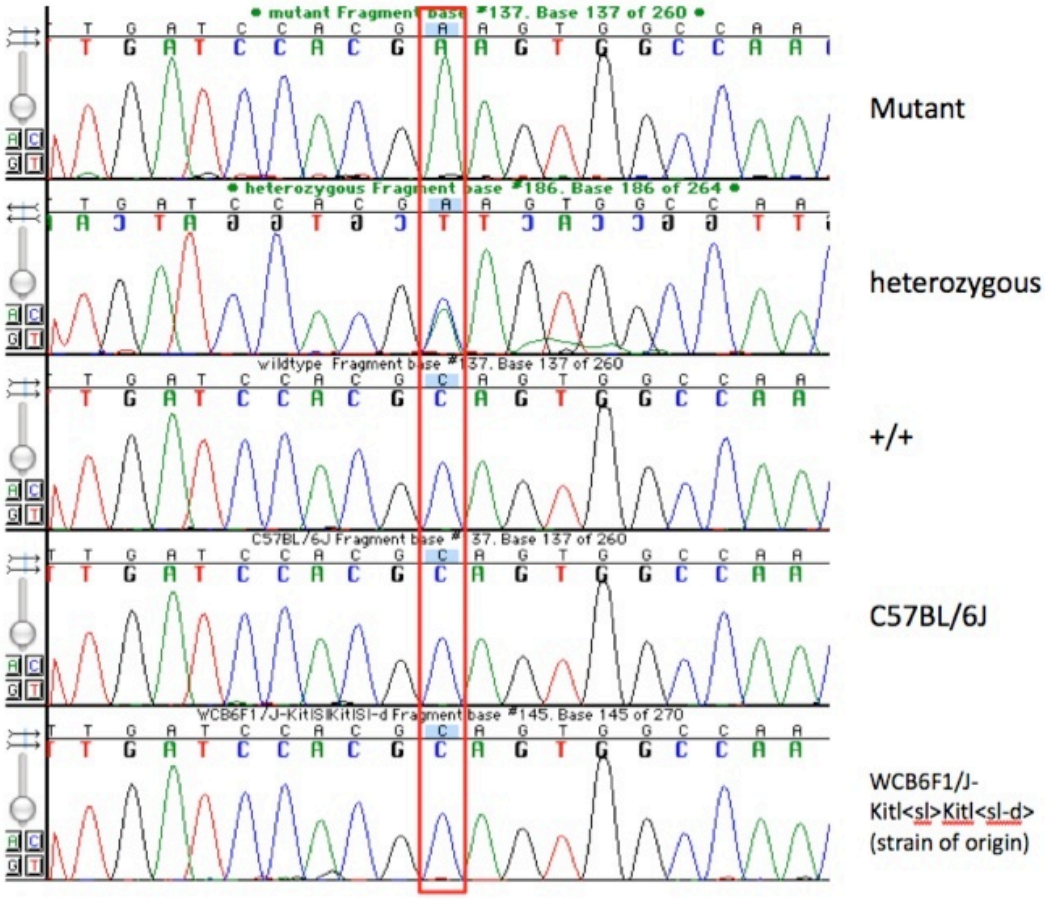
Homozygous mice are not able to breed. This colony is maintained by ovarian transplantation. Transplanted recipient females are crossed with C57BL/6J mice and then heterozygous mice produced are intercrossed.

Genetic Analysis

A mouse homozygous (ovarian transplanted) for the *Uch1*$^{gad\text{-}2J}$ was mated to CAST/EiJ mouse. This mating of mice produced unaffected F1 hybrid mice. The F1 hybrid mice

were then intercrossed and generated 42 affected F2 mice for linkage analysis. Using our standard MMR mapping protocol[1], this recessive *Uchl1*$^{gad\text{-}2J}$ mutation was mapped to Chromosome 5, between *D5Mit258* (NCBI 37 position 67.0Mb) and *D5Mit356* (NCBI 37 position 73.5Mb).

Based on phenotype and map location, *Uchl1* was considered a plausible candidate gene as it is involved in the neuromuscular system and lies close to our flanked segment. Sequence analysis revealed a mutation in exon 4. A PCR product from genomic DNA was used to sequence the *Uchl1* $^{gad\text{-}2J}$ mutation. Primers were generated that produce a 335 base pair product flanking exon 4 of the *Uchl1* wildtype allele; primer exon 4 Left (CTGGACCACCATCTGCTTAC) and primer exon 4 Right (CCCCACAAAGCTCTACTCTAACC). Sequence analysis of mutant DNA identified a single nucleotide changes from C to A was detected in exon 4.



Comparison of DNA sequence chromatograms of *Uchl1*$^{gad\text{-}2J}$ (enclosed in red box).

Pathology

A routine pathological examination[2] of one mutant at 21 weeks of age showed extensive neurogenic atrophy in hind legs and also degeneration of long tracks in the spinal cord. Hearing as assessed by auditory brainstem response testing (ABR) of one mutant at 8 week of age was normal.

The eyes of one mutant mouse at age 19weeks of age were tested by electroretinograph (ERG) and showed clinically normal eyes.

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References
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(Latest) J:159125 Nunes AF et al., "Neuropeptide Y expression and function during osteoblast differentiation--insights from transthyretin knockout mice." FEBS J 2010 Jan;277(1):263-75

[1]**Standard Mapping Protocol used in The Mouse Mutant Resource**

Linkage crosses
To map new mouse mutations we use CAST/Ei, an inbred strain of *Mus musculus castaneus*, as our standard linkage testing strain. In some cases, because of breeding difficulties or reduced phenotypic penetrance, we use other strains. An intercross of F1 hybrids is usually used to analyze linkage of recessive mutations and a backcross to analyze linkage of dominant mutations. Our goal is to produce enough informative mice from each mapping cross to test the recombinational products from at least 100 meioses.

DNA isolation
DNA is extracted from the frozen tail tips of mutant (homozygous) F2 mice or backcross progeny by a standard hot sodium hydroxide and Tris (Hot SHOT) procedure (Truett, et al., 2000) or from spleens using standard phenol extraction methods.

Polymerase chain reaction
PCR primer pairs (MapPairs, from Research Genetics, Huntsville, Ala., or from Integrated DNA Technologies, Coralville, Ia.) are used to type MIT microsatellite markers positioned throughout the genome. PCR reactions contain 20 ng genomic DNA in 10 ul containing 50 mM KCL, 10 mM Tris-HCL (pH 9.0 at 250C), and 0.01% Triton-X-100, 2.25 mM MgCl2, 100 nM of each primer (forward and reverse), 100 uM of each of four deoxyribonucleoside triphosphates, and 0.5 Units of Taq DNA polymerase (Amplitaq from Applied Biosystems #N808-0145). Amplification consists of one cycle of denaturation at 940C for 3 minutes followed by 30 cycles, each consisting of 940C for 15 sec. denaturation, 550C for 2 minutes of annealing, and 720C for 2 minutes of extension. After the 30 cycles, the final product is extended for 7 minutes at 720C. The PCR products are run on 2.5% Metaphor agarose gels or 6% polyacrylamide (non-denaturing) gels. The gels are then stained with ethidium bromide, destained with distilled water, visualized on a UV light table, and photographed.

Pooled DNA Method (Taylor et al 1994).
In the case of an intercross, a pool of DNA prepared from 25-30 mutant F2 mice is compared with DNA from F1 hybrids. In the case of a backcross, a DNA pool from 25-30 mutant N2 mice is compared with a pool from 25-30 unaffected N2 mice. These DNA samples are typed by PCR for MIT markers located throughout the genome. For linked markers, the mutant strain allele will predominate in the DNA pool from mutant mice compared with controls. When a particular marker indicates linkage by analysis of the pooled sample, individual DNA samples are typed with that marker and additional markers in the same region to confirm linkage. Once a linkage is confirmed, additional DNAs from individual mice are typed to obtain a finer map position.

Linkage analysis
Gene order and recombination frequencies are calculated with the Map Manager computer program (Manley1993, 2001), a MacIntosh program for storage and analysis of genotyping data.

References
Manley KF (1993) A MacIntosh program for storage and analysis of experimental mapping data. Mamm Genome 4: 303-313.

Manly KF, Cudmore RH Jr, and Meer JM (2001) Map Manager QTX, cross-platform software for genetic mapping. Mamm Genome12: 930-932.

Taylor BA, Navin A, and Phillips SJ (1994) PCR-amplification of simple sequence repeat variants from pooled DNA samples for rapidly mapping new mutations of the mouse. Genomics 21: 626-32.

Truett GE, Heeger P, Mynatt RL, Truett AA, Walker JA, and Warman ML(2000) Preparation of PCR-quality mouse genomic DNA with hot sodium hydroxide and Tris (HotSHOT). Biotechniques 29:52-54

[2]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.