Cacna1a$^{tg\text{-}7J}$; a new neurological mutation on Chromosome 8

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Mutation (allele) name: tottering 7 Jackson

Mutation (allele) symbol: *Cacna1a*$^{tg\text{-}7J}$

Mutation (allele) name: tottering 7 Jackson

Gene symbol: *Cacna1a*

Strain of origin:B6.129P2-*Nos3*^{tm1Unc}

Phenotype categories: neurological

Origin and Description

The new dominant *Cacna1a*$^{tg\text{-}7J}$ mutation was discovered by Melanie Atherton in a production colony of B6.129P2-*Nos3*^{tm1Unc} mice at The Jackson Laboratory. Mice homozygous for the *tg-7J* mutation can be recognized after weaning age by their shaky, wobbly gait that resembles a subtle hotfoot walk. The hindquarters are most affected. Mutant male mice do breed.

Genetic Analysis

Inheritance of the *Cacna1a*$^{tg\text{-}7J}$ mutation was determined to be dominant when affected progeny were produced from a mating of a C57Bl/6J female mouse to a *Cacna1a*$^{tg\text{-}7J}$ male mouse. Using our standard mapping protocols a heterozygous *Cacna1a*$^{tg\text{-}7J}$ female mouse was mated to a C57Bl/6J male. The progeny from this cross were then backcrossed to C57BL/6J and produced 58 affected progeny that were used for linkage analysis. The *Cacna1a*$^{tg\text{-}7J}$ mutation maps between *D8Mit77* (NCBI 37 position 84.7 Mb) and *D8Mit250* (NCBI 37 position 93.4 Mb) and is non-recombinant with *D8Mit78* (NCBI 37 position 86.9 Mb). *Cacna1a*^{tg} is mapped to NCBI 37 position 86.9 Mb. Based on phenotype and map position and because two other dominant alleles of *Cacna1a*^{tg} were found in exon 25, that exon was sequenced and a change was found in two affected mice, while a control mouse matched the genome project sequence. The change is a C to T mutation at position 3920 in the coding sequence (in exon 25 in the genome), it changes Threonine 1307 to Isoleucine.

Pathology

A pathological screen[1] of a male mutant at 5 weeks of age showed apoptotic sperm in the epididymus and another mutant at 12 weeks of age had a tiny testis but spermatogenesis was still occurring which is unusual for a small testis. Hearing as assessed by auditory-

evoked brainstem response testing[2] (ABR) of a male mutant, a female mutant, and two heterozygotes, all at 6 weeks of age, was normal. The eyes of mutants and control littermates were examined with an ophthalmoscope and vision was determined to be normal. An electroretinogram was performed on a 5 week old female mutant and was normal.

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[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] Auditory-Evoked Brainstem Response (ABR) Thresholds

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. ABR thresholds of all mice and strains tested are entered in spreadsheet files for storage, easy access, and for the production of periodic progress reports. Click-evoked ABR waveforms, obtained at threshold (T) and at T+10, T+20 and T+30 dB or each mouse, are also stored for future reference. Mice of the CBA/CaJ strain are tested periodically as references for normal hearing, and for monitoring the reliability of the equipment and testing procedures.