Wobbly locomotion; a new neurological mutation causing an abnormal gait.

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Mutation (allele) symbol: *wblo*

Mutation (allele) name: wobbly locomotion

Gene symbol: *wblo*

Strain of origin: B6;129-*Gla*^{tm1Kul}/J

Current strain name: B6;129-*Gla*^{tm1Kul} *wblo*/GrsrJ

Stock #012625 (jaxmice.jax.org)

Phenotype categories: Neurological

Origin and Description

Mice carrying the new spontaneous recessive wobbly locomotion mutation (*wblo*) were found by Sean Sullivan in a production colony of B6;129-*Gla*^{tm1Kul}/J mice at The Jackson Laboratory. Mice homozygous for the wobbly locomotion mutation display impaired movement coordination that results in their walking with a wobbly gait. The wobbly locomotion phenotype can be first observed when the affected mice reach about 5 weeks of age. As adult mutant mice age, the wobbly gait phenotype becomes less severe. Homozygous *wblo* females and males are fertile and live a normal life span.

Genetic Analysis

A female mouse homozygous for the wobbly locomotion mutation was mated to a male CAST/EiJ mouse. The unaffected F1 hybrid mice produced from this cross were then intercrossed and used to generate 48 affected F2 mice that were utilized for linkage analysis.

Using the standard mapping procedures of The Mouse Mutant Resource[1], with 96 meiosis tested, the recessive wobbly locomotion mutation was mapped to Chromosome 6 and is located between *D6Mit38* (NCBI 37 position 100.0 Mb) and *D6Mit55* (NCBI 37 position 114.2 Mb) and is non-recombinant with *D6Mit65* (NCBI 37 position 101.3 Mb), *D6Mit64* (NCBI 37 position 103.7 Mb), *D6Mit104* (NCBI 37 position 110.9 Mb), *D6Mit107* (NCBI 37 position 112.4 Mb), and *D6Mit23* (NCBI 37 position 112.5 Mb).

A plausible candidate gene involving the nervous system lies close to our flanked segment. Chemically induced mutant de Leon (*dleon*) mice exhibit motor neuron axons

that project aberrantly shortly after exiting the lumbar spinal cord (Lewcock JW et al.). Mice with *dleon* mutations were not available for complementation tests. The physical location of the *dleon locus* is unknown but its genetic position is on Chromosome 6 at or near 51.0 cM. Our distal flanking marker (*D6Mit55*) is listed at 47.9 cM.

Pathology

A routine pathological examination[2] of one mutant at nine months of age showed dystrophic axons in the spinal cord and in the substantia gelatinosa. Central chromatolysis was observed in the central root ganglia.

Hearing as assessed by auditory brainstem response testing of two mutants at four months of age was normal. At three months of age the eyes of one mutant mouse were tested by electroretinograph (ERG) and were clinically normal.

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References

Mouse Genome Informatics (2010) ref # J: 132726 Lewcock JW et al., The ubiquitin ligase Phr1 regulates axon outgrowth through modulation of microtubule dynamics. Neuron 2007 Nov 21; 56(4): 604-20.

Protocols

[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.