recoil wobbler, *rcw*, a new neurological mutation in the proximal region of mouse Chromosome 10

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Mutation (allele) symbol: *rcw*

Mutation (allele) name: recoil wobbler

Gene symbol: $Grm1^{rcw}$

Strain of origin: 129S1/SvImJ

Current strain name: C3FeLe.Cg *a*-$Grm1^{rcw}$/GrsrJ

Stock #005494 (jaxstrain.jax.org)

Phenotype categories: neurological/behavioral:motor capabilities/coordination/movement

Origin and Description

The *rcw* mutation arose spontaneously in the 129S1/SvImJ (Stock # 002448) colony of The Jackson Laboratory in 1998. A female phenotypic deviant was outcrossed to an agouti C3HeB/FeJ (Stock #000658) male and the mutation was recovered in the second generation, which indicates recessive inheritance. Visibly, *rcw*/*rcw* mice are classified by 2.5- 3 weeks of age. Their constant wobbly gait frequently throws them off balance, but only briefly, as they show immediate and normal righting response. When walking, they often brace for balance with their fore and hindfeet extended forward or sideways. Their heads lurch mildly and the mutants often sit on their haunches when motion falters. Their forefeet show normal grip strength when the mutant mice are pulled gently backwards over a cage cover. When picked up by the tail, they may clasp their hindfeet. Even with this clenching, however, they show sufficient hindlimb strength to prevent falling when placed on an edge. Mutants live throughout adulthood and both genders breed; hence, the colony can be maintained by homozygous mutant x heterozygote. After seven generations of such brother x sister matings, we began a congenic to C3FeLe.B6-*a* (Stock # 000198) using the classic backcross-intercross mating scheme. This non-agouti C3 strain was chosen for transplantation of mutant ovaries into agouti hosts C3FeB6 -$A/A^{w\text{-}J}$ F1 (Stock # 001203) or C3SnSmn.CB17-$Prkdc^{scid}$/J (Stock # 001131) should the mutant phenotype lose viability as advanced inbreeding lessens hybrid vigor. To date, at the fourth backcross generation, the mutant phenotype has remained stable and ovarian transplantation is not necessary. The mutant phenotype is fully penetrant; heterozygous siblings produced 11 mutant mice from a total of 37 (29%). DNA was cryopreserved before the congenic matings were initiated and is available as 129S1;C3Fe-*rcw* (Stock # 004418). Embyros will be cryopreserved after the congenic strain has reached N5F2 as >85% of the genome will be homozygous C3FeLe.B6-*a* alleles.

Genetic Analysis

An intercross with CAST/Ei using 56 F2 mutant progeny (112 meioses), standard PCR protocols, and MIT microsatellite markers located the new mutation to mouse Chromosome 10. Analysis of our data with the Map Manager program gave the following order and recombination distances in centimorgans +/- standard error: [*D10Mit49*, *D10Mit75*]-(1.79 +/- 1.25)-[*rcw*, *D10Mit77*, *D10Mit28*]-(0.91 +/-0.90)-[*D10Mit80*, *D10Mit154*]. The mapping data have been submitted to MGI. Both CDS (2003) and MGSC (2003) agree with our mapping order. MGSC, as displayed by Ensembl (2003), places *rcw* within a 5.3 Mb interval of chromosomal bands 10A1-A2 between our flanking markers *D10Mit49* at map position 6.3 Mb and *D10Mit80* at map position 11.4 Mb.

Several genes within the flanked interval are plausible candidates for the *rcw* mutation. B-tomosyn, fragment (ENSMUSG00000019790, OMIM 604586, 2003) has a putative human homolog, *STXBP5*, (syntaxin binding protein 5, ENSG00000164506). Rat tomosyn colocalizes with syntaxin-1 in the cerebellum and is implicated in neurotransmitter release (Fujita, Y. et al 1998). Disruptions of the gene named epilepsy, progressive myoclonic epilepsy, type 2 gene alpha cause neurodegeneration and myoclonus epilepsy in humans (*EPM2A*, OMIM 607566, 2003) and mice (*Epm2a,* MGD 2003). Lafora inclusion bodies are present in various tissues of some, but not all, forms of this neuropathy. Mutations in glutamate receptor, metabotrophic 1 cause cerebellar ataxia in both humans (*GRM1*, OMIM 604473, 2003) and mice (*Grm1*, MGD 2003). Phenotypically, *rcw*/*rcw* mice resemble *Lc*/+ (lurcher) mutants. Coincidentally, lurcher is a mutation on mouse Chromosome 6 of glutamate receptor ionotropic, delta 2 (*Grid2*, MGD 2003). Mice with mutations in *Epm2a* and *Grm1* were not locally available for complementation tests.

Pathology

Our routine histology[1] screen found no major anomalies. Midline sagittal cerebellar sections from mutants and sibling controls found no differences in folial count and patterning. The granule cell region of the cerebella of *rcw*/*rcw* mice is normal. Slight gaps in the Purkinje cell layer of aged mutants is also seen in controls. Limb musculature and myelination are normal. No Lafora bodies were detected in sections stained with PAS of cerebellum, spinal cord, heart, leg muscle, and liver from adult *rcw*/*rcw* mice.

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References

CDS, Celera Discovery System.

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Manley KF (1993) A MacIntosh program for storage and analysis of experimental mapping data.

Mamm Genome 4, 303-3313.

MGD 2003, Mouse Genome Database, Mouse Genome Informatics Project, The Jackson Laboratory, Bar Harbor, ME.

MGSC 16.30.1, Mouse Genome Sequencing Consortium 2003.

OMIM 2003 Online Mendelian Inheritance in Man.

Protocols

[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.