Luxate-like 2 (*Lxl2*), a remutation to Luxate (*Lx*)

Patricia F. Ward-Bailey, Belinda S. Harris, Leah Rae Donahue, Roderick T. Bronson, Kenneth R. Johnson, and Muriel Davisson

Source of Support:This research was supported by NIH/NCRR grant RR01183 to the Mouse Mutant Resource (M.T. Davisson, PI) and Cancer Center Core Grant CA34196.

Mutation Symbol: *Lxl2*

Mutation Name: Luxate-like 2

Strain of origin: AKR/J

Current strain name: B6;AKR-*Lxl2*/GrsrJ

Stock #004502 (jaxmice.jax.org)

Phenotype category: limbs

Abstract
Luxate like 2 (*Lxl2*) is a remutation to luxate (*Lx*). *Lxl2* arose in a production colony of AKR/J mice at The Jackson Laboratory in 1998. This dominant mutation causes the animal to present with all four limbs at odd angles to the body. There are extra toes on all four limbs and the rear legs are oriented backward. Severe arthritis of the knee was observed in one homozygous female, but no other histological lesions were seen. We used a backcross to map the *Lxl2* mutation to Chr 5. The most likely gene order places the mutation between *D5Mit1* and *D5mit13* in 53 tested meioses. Luxate-like 2 could not be directly tested for allelism by complementation analysis with luxate because both are dominant; however, its identical phenotype and genetic map position indicate allelism.

Origin and Description
The *Lxl2* remutation was found at The Jackson laboratory in 1998 by Jennifer Haycoch in a pooled mating in a colony of AKR/J mice (JR # 648) in a production colony in Room MP14.

The phenotype when heterozygote shows extra toes on all four feet and the rear legs twisted backward. It is believed that homozygous *Lxl2* mutants die in utero because a homozygous mutant has never been born. When hetrozygotes are mated together, and the resulting *Lxl2*/? animals are progeny tested, only *Lxl2*/+ are found and no homozygotes are produced.

This remutation is being transferred to a C57BL/6J background for maintenance because the breeding was poor on the AKR/J background. Currently it is at N2 on C57BL/6J. DNA was saved from the original mutant background.

Mutants are easily identified at birth by their twisted rear legs and extra digits on all four limbs. The *Lxl2* mutation is maintained by mating a female heterozygote to a +/+ male. Males heterozygous for the *Lxl2* mutation do not always breed.

Phenotypic Characterization

X-ray image of whole mouse clearly shows twisted limbs and extra digits characteristic of *Lxl2*/+ mutants.

Histological techniques. Tissues for histopathological examination were prepared from both *Lxl2*/+ and control animals. Tissues were removed from animals deeply anesthetized with tribromoethanol (Avertin) and fixed by intracardiac perfusion of Bouin's solution

following a flush of saline. After demineralization in Bouin's, cross sections of spine with spinal cord *in situ*, and midsagittal sections of hind leg were prepared. Cross sections of brain and most somatic organs were prepared. Organs sampled were eyes, pituitary, salivary glands, thyroids, lung, heart, thymus, liver, spleen, pancreas, stomach, small intestine, colon, cecum, kidneys, adrenals and reproductive organs. Sections of all tissues were stained with hematoxylin and eosin (H&E). Brain and spinal cord sections were also stained with luxol-fast blue-cresylecht violet (LFB-CV) No lesions were observed in any organ. Specimens from 2 *Lxl2* and 2 +/? mice were cleared and stained with alizarin red S and alcian blue to demonstrate bone and cartilage (Green, M.C., 1952). Apart from the twisted rear legs and extra digits on all four limbs, no abnormalities were observed.

ABR threshold tests (Zheng 1999) of four 7-week-old and two 40-week-old mutants indicated normal hearing.

Genetic Analyses

Lxl2 inheritance as a dominant mutation was shown by outcrosses to both C57BL/6J and CAST/Ei. In both outcrosses, affected animals were seen in the F1 generation. For linkage analysis, a backcross was utilized to produce mutant mice. The offspring of a cross between a female AKR/J mutant and a male C57BL/6J were crossed back to C57BL/6J +/+ animals. The backcross progeny were scored visually for phenotype and spleens and tails tips from 53 mutant animals were collected and stored at -70C for subsequent DNA typing to map the mutation. DNA was extracted from the frozen tail tips of 53 mutant produced in the linkage cross by a standard hot sodium and Tris (HotSHOT) procedure (Truett,et al., 2000). PCR primer pairs (MapPairs, Research Genetics, Huntsville Ala.) of microsatellite markers *D5Mit47, D5Mit70, D5Mit1, D5Mit349, D5Mit386, D5Mit387, D5Mit13, D5Mit255*, and *D5Mit205* were used to localize the mutation on Chr 5. PCR analyses were carried out in 10 ul total volume reactions containing 20 ng genomic DNA, by previously described methods (Ward-Bailey et al. 1996). Markers on Chr 5 were chosen first for linkage analysis because this mutation causesa phenotype that looks like Luxate (*Lx*). *Lxl2* mapped to the same position on Chr 5 as *Lx*. The gene order and recombination estimates with standard errors, calculated with the Map Manger computer program (Manley 1993), are centromere-[*D5Mit47, D5Mit70*]-3.85+/- 2.67-[*D5Mit1*]-7.69+/-3.70-[*D5Mit349, D5Mit386, D5Mit387*, ***Lxl2***]-3.03+/- 2.98-[*D5Mit13*]-6.45+/- 4.41-[*D5Mit255*].

Acknowledgments

We thank the following for their excellent technical expertise: Coleen Marden, Jane Maynard, Heping Yu, Qing Yin Zheng, Norm Hawes

References Cited

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

Mouse Genome Database (MGD) Mouse Genome Informatics Project, The Jackson Laboratory, Bar, Harbor, Maine. World Wide Web (www.informatics.jax.org)

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

Ward-Bailey PF, Johnson KR, Handel MA, Harris BH, Davisson MT. (1996) A new mouse mutation causing male sterility and histoincompatibility. Mamm. Genome 7, 793- 797.

Zheng QY, Johnson KR, Erway LC (1999) Assessment of hearing in 80 inbred strains of mice by ABR threshold analyses. Hear Res 130, 94-107.