A Remutation at the *Grem1* locus named limb deformity 3 Jackson (*Grem1*$^{ld\text{-}3J}$)

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Mutation (allele) symbol: *Grem1*$^{ld\text{-}3J}$

Mutation (allele) name: gremlin1 limb deformity 3 Jackson

Gene symbol: *Grem1*

Strain of Origin: NOD.CB17-*Pkdc*^{scid}/J

Current strain name: NOD.Cg-*Prkdc*^{scid}/J-*Fmn1*$^{ld\text{-}3J}$/GrsrJ (formerly called *Grem1*$^{ld\text{-}3J}$)

Stock #: 004688 (jaxmice.jax.org)

Phenotype categories (key words): skeletal, limbs

Origin and Description

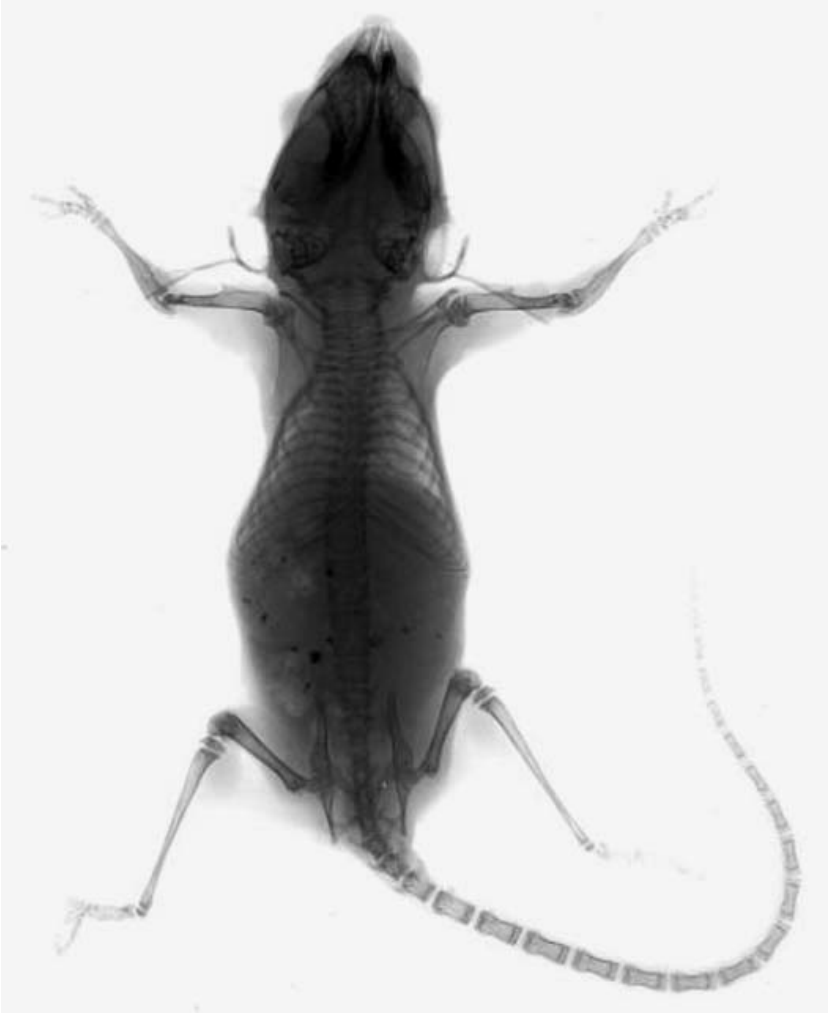
The *Grem1*$^{ld\text{-}3J}$ mutation was found in a production colony at The Jackson Laboratory in 2000 by Alissa Buzzell. This recessive mutation causes disorganization defects affecting all four feet from wrists/ankles distally. The front feet have disorganized metacarpals and 3 digits; rear feet have similar disorganized carpels but some have only one digit. Mice appear to have no radius and no fibula, but x-rays show they are fused into one flat bone. Females may breed but males do not. One mutant male had only one kidney. This remutation maps to Chr 2 and is non-recombinant with *D2Mit131* and *D2Mit163* for the 68 meioses tested.

Pathology

Our standard pathology screen[1] showed that the joint spaces in the knee are dilated in these mutants. Lymph nodes are depleted but this may be due to the *scid* in the background strain. The eyes of four 5-month-old mice (two mutants and two controls) were examined with an ophthalmoscope. All four had normal vision. Auditory-evoked brainstem response threshold testing (Zheng et al. 1999) of hearing showed deafness in all animals tested. This is likely due to a background effect because the strain NOD/Lt has very early age related hearing loss.

Skeletal characterization. Mice homozygous for the *Grem1*$^{ld\text{-}3J}$ mutation have appendicular skeletal abnormalities characteristic of the original limb deformity mutation discovered at Oak Ridge National Laboratory. Syndactyly and oligodactyly appear on all four feet, and the radius and ulna are fused to one flat, somewhat triangular bone. Long bone abnormalities are restricted to elements distal to the elbow or knee, whereby the humerus and femur are normal, as are bones of the pelvic and pectoral girdle. The morphology of spinal vertebrae is unaffected. This x-ray of a 3-week-old homozygous

Grem1$^{ld\text{-}3J}$ male is representative of this skeletal dysmorphology.



Genetic Analyses

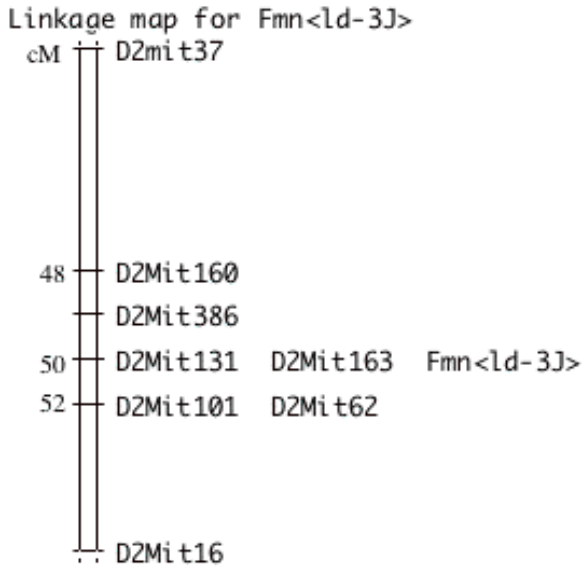
Grem1$^{ld\text{-}3J}$ is inherited as a recessive mutation as shown by traditional linkage cross analysis. The data showed no visible mutants in the F1 generation (0/15) and segregation in the F2 (84 *nm*/707 total progeny) was 11.8% mutants, much lower than the expected 25%. For linkage analysis, an intercross was utilized to produce mutant mice. CAST/Ei females were mated to NOD.CB17-*Pkdc*^{scid} *Grem1*$^{ld\text{-}3J}$ homozygous males. F1 hybrids from this initial cross were then intercrossed to produce the F2 progeny. The F2 progeny were scored visually for phenotype and spleens and tail tips from 34 *Grem1*$^{ld\text{-}3J}$ homozygous animals were collected and stored at -70C for subsequent DNA typing to map the mutation.

DNA isolation. DNA was extracted from the frozen tail tips of 34 mutant (homozygous) F2 mice produced in the linkage cross by a standard Hot Sodium and Tris (Hot SHOT) procedure (Truett, et al., 2000).

Polymerase chain reaction. PCR primer pairs (MapPairs, Research Genetics, Huntsville

Ala.) of microsatellite markers *D2Mit37, D2Mit160, D2Mit386, D2Mit131, D2Mit163, D2Mit101, D2Mit62 and D2Mit16* were used to localize the mutation on Chr 2. 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).

Mutation segregation ruled out Chromosome X linkage. A genome sweep of F2 progeny from the CAST intercross was begun by typing DNA samples from 34 $Grem1^{ld-3J}/Grem1^{ld-3J}$ F2 animals for segregating MIT microsatellite markers starting with chromosomal areas where mutations with similar phenotypes are located. Linkage of Fmn^{ld-3J} was first detected on Chr 2 with *D2Mit62*. DNA samples were then typed for seven additional Chr 2 markers. The recombination estimates and best gene order are centromere-[*D2Mit37*]-7.3+/-4.2-[*D2Mit160, D2Mit386*]-1.5+/-1.5-[*D2Mit131, D2Mit163*, *Grem1ld-3J]*-1.5+/-1.5-[*D2Mit101, D2Mit62*]-4.8+/-3.4-[*D2Mit16*].



Linkage analysis. Gene order and recombination frequencies were calculated with the Map Manager computer program (Manley), a MacIntosh program for storage and analysis of experimental mapping data. The complete Chr 2 linkage data for 34 F2 $Grem1^{ld-3J}/Grem1^{ld-3J}$ mice have been deposited in the Mouse Genome Database, accession number J:82800.

Allelism Tests. A test for allelism with *ld-J* was positive, with 6 mutants of 72 born.

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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.