Spontaneous fracture 2 Jackson, a second mutation of the gulonolactone oxidase gene (*Gulo*).

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Mutation (allele) symbol: $Gulo^{sfx\text{-}2J}$

Mutation (allele) name: spontaneous fracture 2 Jackson

Gene symbol: Gulo

Strain of origin: RB156BNR/Ei-*rul*/J

Current strain name: RB156Bnr/Ei *rul*-$Gulo^{sfx\text{-}2J}$/GrsrJ

Stock #005354 (jaxmice.jax.org)

Phenotype categories: skeletal

Abstract

We have identified a remutation to spontaneous fracture ($Gulo^{sfx}$) by a direct test for allelism. The phenotype of this remutation is identifiable at 4-5 weeks of age when the mutants appear smaller than their control littermates and begin to hobble about their cage. Between 5 and 8 weeks of age the mutants develop rear limb paralysis and many die by 8 weeks of age. The phenotypic characteristics of this mutation are similar to the original mutation spontaneous fracture ($Gulo^{sfx}$) except for the eye phenotype described below that is inherent in the background strain on which the $Gulo^{sfx\text{-}2J}$ mutation arose.

Origin and Description

This recessive mutation arose in 2002 in the strain RB156BNR/Ei-*rul*/J (ruffled) which is maintained in the Mouse Mutant Resource at the Jackson Laboratory. The ruffled mutation in the background strain is still segregating in the RB156BNR/Ei *rul*-$Gulo^{sfx\text{-}2J}$ strain and when homozygous causes the $Gulo^{sfx\text{-}2J}$ mice to have a ruffled looking coat.

Genetic Analysis

A direct test for allelism was set up by mating a female heterozygote carrying this new mutation to a male $Gulo^{sfx}$ heterozygote. This cross produced three affected mutants out of 10 born, proving that the new mutation is allelic with $Gulo^{sfx}$.

Pathology

X-Ray analyses of seven mutants between 4 and 8 weeks of age show thin cortical bone, absence of trabeculae, and complete fractures of the femur with mineralized callus in all

seven mice. The forelimbs and spine were less affected than the rear legs, but all bone displayed thin cortices and very little, if any trabeculae. Bone mineral density was measured by PIXImus in 3 mutants and very low amounts of mineral were detected in the overall mutant skeleton compared to age matched littermates.

Three mutants were perfused for our standard pathological screen[1] and found to have severe osteolysis with fibrous osteodystrophy, and thin cortical bone, particularly in long bones. These 3 mutants all had complete fractures in the femur or at the knee near the epiphysis and beneath the growth plate. Fracture sites have extensive callus formation (See slide).

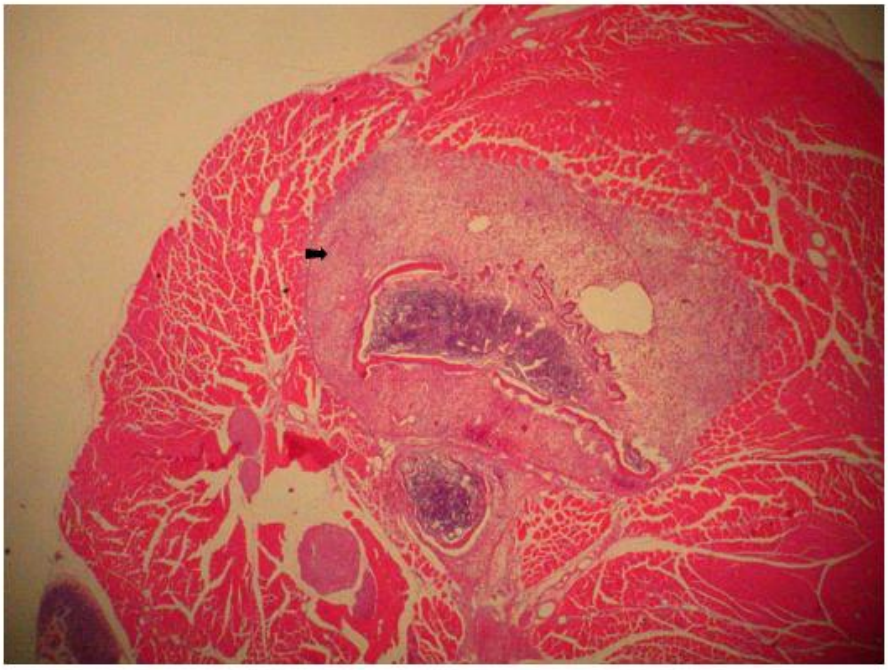

Figure 1: Fracture site with extensive callus formation. (see arrow)

The severity of the fractures in *Gulo*$^{sxf\text{-}2J}$ appears to be worse than in the original spontaneous fracture mutant. It is possible that a modifying gene is present in the RB156BNR/Ei background that exacerbates the fracture phenotype. Mutants also had cataracts and rosettes and wavy outer nuclear layer of retinas. The eyes of a female mutant were checked using an ophthalmoscope and it was found to have cataracts on both eyes (as the strain background has characteristically) It has not yet been determined if the rosettes and wavy outer nuclear layer of the retinas is also characteristic of the background strain.

Hearing as assessed by auditory-evoked brainstem response testing of three mutants and three controls tested at one month of age was normal.

Discussion

A remutation to spontaneous fracture has been confirmed by a direct test for allelism. The phenotype of this remutation is more severe than the original *Gulo*^{sfx}

mutation, suggesting a modifying gene may be present in the RB156BNR/Ei-*rul*/J background strain.

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References

MGD 2005, Mouse Genome Database, Mouse Genome Informatics Project, The Jackson Laboratory, Bar Harbor, ME.

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.