A mouse mutation in the *Large* gene with muscular dystrophy and retinoschisis

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Mutation (allele) symbol: $Large^{myd\text{-}3J}$

Mutation (allele) name: myodystrophy 3 Jackson

Strain of origin: NOD.Cg-$Prkdc^{scid}$ $Il2rg^{tm1Wjl}$/SzJ

Current strain name: STOCK $Large^{myd\text{-}3J}$/GrsrJ

Stock #008581 (jaxmice.jax.org)

Phenotype categories: craniofacial, neuromuscular, eye

Origin and Description

Myodystrophy 3 Jackson ($Large^{myd\text{-}3J}$) is an autosomal recessive mutation that arose in the NOD.Cg-$Prkdc^{scid}$ $Il2rg^{tm1Wjl}$/SzJ (Stock #005557) colony at The Jackson Laboratory in 2006. Homozygous mutants have a muscle degeneration phenotype usually not visible until two to three months of age. When the phenotype does express, the mice are unable to splay their back legs outward when held up by the tail as unaffected mice can do. The condition worsens with age as mice sway more as they walk until their back legs are dragging. Some mice have exhibited symptoms as early as wean age but most are older. Mice with the muscle phenotype also have retinoschisis, or a splitting of the layers of the retina.

C57BL/6J alleles were introduced into the colony when ovaries from a homozygous *myd-3J* mutant were transplanted into a C3SnSmn.CB17-$Prkdc^{scid}$/J female, that was then was mated to a C57BL/6J male. Since the introduction of the C57BL/6J alleles, the colony has been intercrossed consecutively for five generations as of this writing. Also, the $Prkdc^{scid}$ and $Il2rg^{tm1Wjl}$ alleles have been bred out of this $Large^{myd\text{-}3J}$ stock.

Female homozygotes are fertile but males are not. Heterozygotes from both sexes are fertile. The colony is maintained by heterozygote x heterozygote mating, or by mating a female homozygote and a male heterozygote.

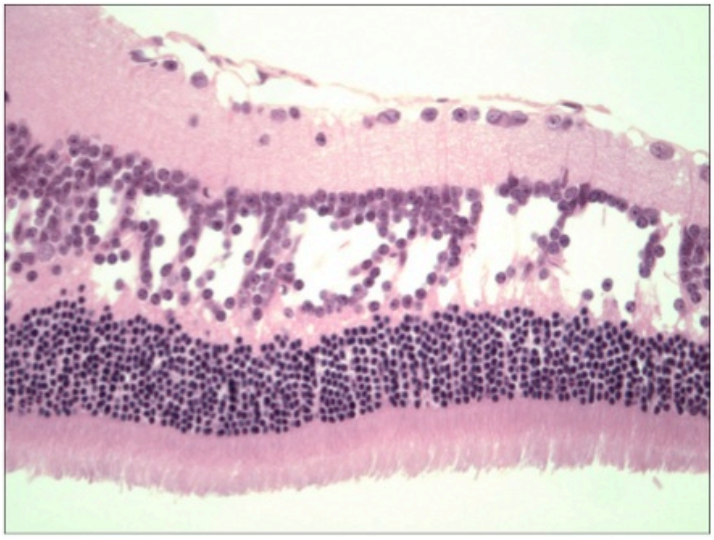
Genetic Analysis

The same C57BL/6J background that was incorporated into the colony was also used to map the mutation. A homozygous female on the NOD.Cg-$Prkdc^{scid}$ $Il2rg^{tm1Wjl}$/SzJ background was crossed to a C57BL/6J male. Obligate heterozygotes were mated and mutants appeared in the F2 generation. Using our standard mapping protocol, spleen and tail tips were collected and stored at -80 C. The mutation maps between *D8Mit65* and *D8Mit249*. These markers are at 44.4 Mb and 83.8 Mb respectfully with the *Large* gene located at 75.7 Mb. (Ensembl.org)

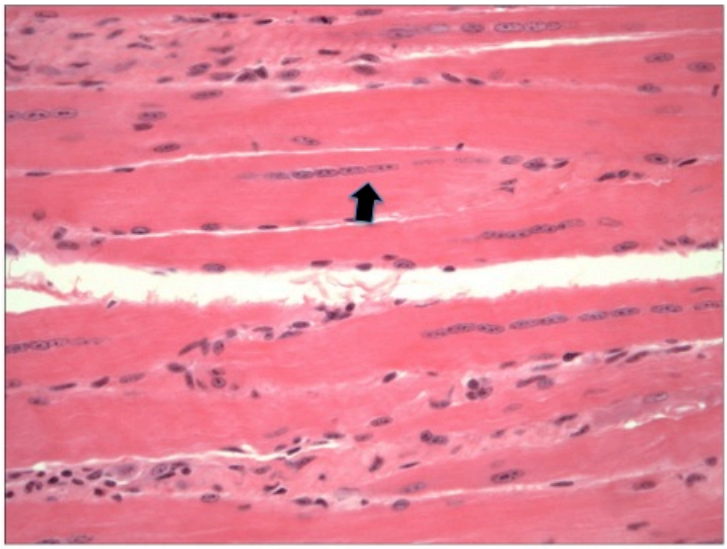
Due to the similarities of our *myd-3J* phenotype and the published *Large* alleles, a direct test for allelism was done with B6.CAST(Cg)-*Large*^{vls}/Pjn. A female *myd-3J/myd-3J* was mated with a male *+/vls* and nine out of 32 F1 offspring had the muscle degeneration phenotype proving allelism. The coding region for the *Large* gene in the *myd-3J* strain was sequenced but the lesion was not found.

Pathology

Using our standard pathology screen[1], three mutants age four, five and eight months all had retinoschisis and muscle degeneration. The linkage of these two phenotypes was further determined by visually scoring mice for the muscle mutation and then looking at the retinas as part of the clinical eye exam. Mice with both muscle degeneration and retinoschisis were confirmed in seven out of eight mice examined, with the one unconfirmed for retinoschisis noted as having a bad retina. Of fourteen mice analyzed that did not have a muscle mutation, thirteen did not have retinoschisis; the condition of one mouse could not be determined due to a cloudy lens.



The clefts in the inner nuclear layer are diagnostic of retinoschisis



Typical of muscular dystrophy there is degeneration of muscle fibers and regeneration, evidenced by rows of centrally located muscle fiber nuclei. (see arrow)

Hearing was assessed by auditory-evoked brainstem response. An examination of four mutants and six unaffected (ranging from two to nine months old) determined that one out of four mutants could hear, as could four out of six unaffected mice. Three mutants were completely deaf. This, coupled with the high-frequency hearing loss of the two unaffected mice, suggests that the *myd-3J* mutation may affect hearing with incomplete penetrance.

Discussion

There are one transgenic and two spontaneous strains of mice with the *Large* gene disrupted. The *myd-3J* allele would be the third reported spontaneous mutation of this gene. The other two spontaneous alleles - *myd* and *vls*- are reported to have abnormal cardiac morphology and dilated cardiomyopathy. No heart defects are reported in the *enr-Tg(MpbReg)36Pop*; likewise the *myd-3J* exhibits no heart defects based on the pathology of two mutants, one at 33 and another at 37 weeks of age. Also, while the two other spontaneous *Large* alleles have an abnormal retinal phenotype, the $Large^{myd\text{-}3J}$ mouse mutation is the first reported retinoschisis model that is not X-linked. There is no eye phenotype reported for the *Large* transgene model. These observations make the $Large^{myd\text{-}3J}$ mutation unique and a potentially useful model for eye research.

Acknowledgements

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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.