A New Mutation, *mdig*, in the *Fnbp4* - *Alx4* - *Ext2* Region of Mouse Chromosome 2

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Mutation (allele) symbol: *mdig*

Mutation (allele) name: malformed digits

Gene symbol: *mdig*

Strain of origin: DBA/1LacJ

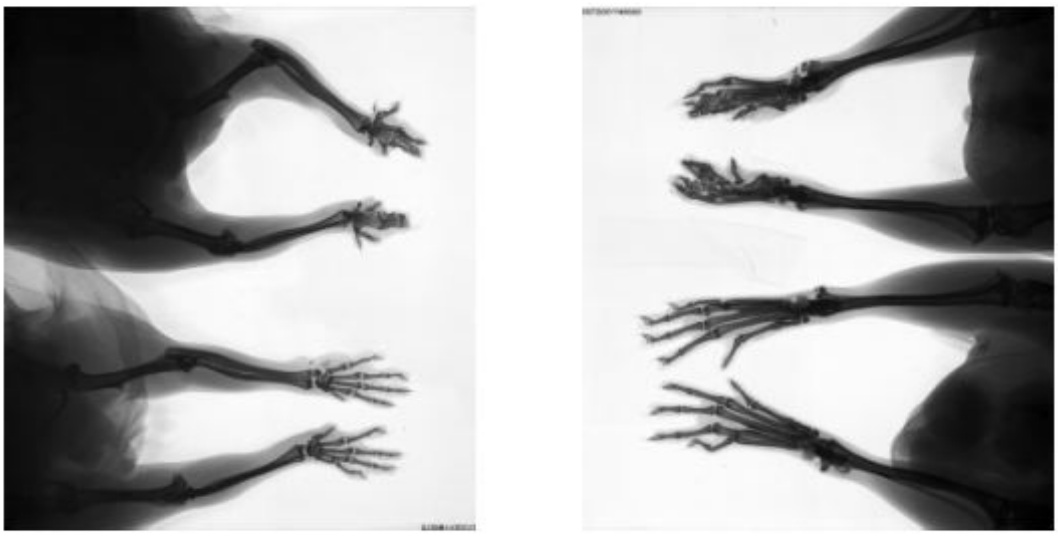
Current strain name: DBA/1LacJ-*Lrp4*^{mdig}/GrsrJ

Stock #004423 (jaxmice.jax.org)

Phenotype categories: skeleton, digits

Origin and Description

The recessive mutation arose spontaneously in the DBA/1LacJ (Stock #001140) production colony of The Jackson Laboratory in 1999. Both genders of homozygotes live and breed through adulthood; hence, the colony is maintained by mating homozygous x heterozygous siblings (either sex of each genotype). Penetrance of the mutant phenotype is ~100% as 51 mutants were classified from a total of 104 progeny (49%) from homozygous x heterozygous matings. X-rays and alizarin staining show that on this background homozygous mutants exhibit variable brachydactyly and syndactyly of all four feet; forefeet may also show incomplete polydactyly with partial toe buds or long toenail-like protusions. Body and limb bones are similar in shape and length to sibling controls.



The top image of the left X-ray is an adult *mdig*/*mdig* showing digit anomalies of the forefeet. The top image of the right X-ray shows digit anomalies of the hindfeet of the same mutant mouse. Similarly, the bottom images are of a normal *mdig*/+ control.

Genetic Analysis
An intercross with CAST/Ei using 44 mutant F2 progeny, standard PCR protocols, and MIT microsatellite markers located the new mutation to mouse Chromosome 2.

D2Mit37Hoxd	DDDDDDDHDD	HDHDDDDDHD	DDDDDDDHDD	DDD	
D2Mit331	DDDDDDDDDD	DDDDDDDDDD	DDDDDDDHDD	DDDDDDDDDD	DDDC
D2Mit272	DDDDDDDDDD	DDDDDDDDDD	DDDDDDDDDD	DDDDDDDDDD	DDDC
D2Mit96	DDDDDDDDDD	DDDDDDDDDD	DDDDDDDDDD	DDDDDDDDDD	DDDD
D2Mit15	DDDDDDDDDD	DDDDDDDDDD	DDDDDDDDDD	DDDDDDDDDD	DDDD
mdig	*d d d d d d d d d d*	*d d d d d d d d d d*	*d d d d d d d d d d*	*d d d d d d d d d d*	*d d d d*
D2Mit386	DDDDDDDDDD	HDDDDDDDDH	DDDDDDDDDD	DDDDDDDDDD	DDDD
D2Mit221	DDDDDDDDDD	HDDDDDDDDH	DDDDDDDDDD	DDDDDDDDDD	DDDD
D2Mit300	DDHDDDDDDD	HDDDDDDDDH	DDDDDDDDDD	DDDDDDDDDD	DDDD
D2Mit99	DDHDDDDDDD	HDDDDDDDDH	DDDDDHDDDD	DDDDDDDDDD	DDDD

Typing of 44 *mdig*/*mdig* F2 progeny (88 meioses) from the CAST/Ei x DBA/1LacJ intercross. Codominant Mit typings are homozygous DBA/1LacJ (D), homozygous CAST/Ei (C) and heterozygous (H); all are shown in upper case. The recessive inheritance of the *mdig* mutation is shown in italicized lower case (*d* represents DBA/1LacJ-*mdig*/*mdig*).

Analysis of our data with the Map Manager program predicted the following order in centimorgans: *D2Mit37*(*Hoxd*)-(6.2 +/- 2.9)-*D2Mit331*-(1.1 +/- 1.1)-*D2Mit272*-(2.3 +/- 1.6)-*D2Mit96*, *D2Mit15*, *mdig*-(2.3 +/- 1.6)-*D2Mit386*, *D2Mit221*-(1.1 +/- 1.1)-*D2Mit300*-(1.1 +/- 1.1)-*D2Mit99*. The mapping data is available as MGD accession number J:83757. Mouse genome sequence data from Celera Discovery System (CDS) and Mouse Genome Sequencing Consortium (MGSC) agree with our mapping order. MGSC as displayed by Ensembl places candidate genes *Fnbp4*, *Alx4* and *Ext2* in chromosome band 2E1 within the 3.5 Mb region defined by our flanking markers *D2Mit272* at 91.8 Mb to *D2Mit386* at 95.3 Mb. These three genes are good candidates for *mdig* because their disruption could possibly cause the type of digit malformation seen in *mdig* mutant mice.

The *mdig* mutation was tested for allelism with three other mutations on Chr 2 that affect limb development. The test for allelism with B6C3Fe-a/a-$Alx4^{lst\text{-}J}$ (Stock #000221) was inconclusive. Each $Alx4^{lst\text{-}J}$/+ breeder showed an extra toe on one or both hind feet, but we saw only 7 affected mice in a total of 138 progeny from $Alx4^{lst\text{-}J}$/+ x *mdig*/*mdig* and reciprocal matings. The penetrance of *Alx4* alleles is known to be strain-dependent and influenced by modifying genes (MGD 2003). The test for allelism with LDJ/Le-$Fmn^{ld\text{-}J}$/$Atrn^{mg}$ (Stock # 000289) was negative as we saw no affected mice in a total of 30 progeny from *ld-J*/+ x *mdig*/*mdig* matings. The test for allelism with B6C3Fe *a*/*a*-$Hoxd13^{spdh}$/J (Stock #002875) was also negative as we saw no affected mice in a total of 33 progeny from $Hoxd13^{spdh}$/$Hoxd13^{spdh}$ x *mdig*/*mdig* matings.

Pathology

Our routine screen[1] of other tissues showed no anomalies.

Acknowledgements

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References

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

MGSC12.3.1, Mouse Genome Sequencing Consortium (ensembl.org/Mus_musculus)

Manley KF (1993) A MacIntosh program for storage and analysis of experimental mapping data. Mamm Genome 4, 303-3313.

[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.