A Mouse Mutation in *Lrp4*.

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Mutation Symbol: *Lrp4*$^{mdig\text{-}2J}$

Mutation Name: malformed digits 2 Jackson

Strain of Origin: SB/LeJ

Current Strain Name: SB;C3Sn-*Lrp4*$^{mdig\text{-}2J}$/GrsrJ

Stock #010968 (jaxmice.jax.org)

Phenotype Category: craniofacial, teeth, skeleton, tail

Origin and Description

The *mdig-2J* mutation was discovered in January 2008 in the SB/LeJ colony (Stock #000269). Overall breeding performance of the *mdig-2J* subline was poor, coupled with the fact that mutants rarely bred. C3H/HeSnJ (Stock #000661) was introduced and the mixed background not only continued to rescue the phenotype but also allowed mutants from both sexes to breed therefore improving the breeding performance in the colony. All affected mice have missing toes on every foot. Mutants also have a coarse tail. Additionally, *Lrp4*$^{mdig\text{-}2J}$ mutants may have one or two extra upper incisors but the tooth phenotype is incompletely penetrant.

The satin (*Foxq1*^{sa}) and beige (*Lyst*^{bg}) mutations are still in the background and are segregating. The colony is maintained by heterozygote by homozygote matings and the reciprocal.

Genetic Analysis

mdig-2J is a recessive spontaneous mutation determined when a *mdig-2J/+* on the SB/LeJ background was mated to a C3H/HeSnJ. There were no mutants in the F1 generation (0/18) and F2 matings produced ten mutants out of 33 F2 pups.

To determine a genetic map position, a heterozygote from the SB/LeJ background was mated with an FVB/NJ (Stock #001800) and spleen and tail from affected F2 offspring were collected and stored at -80 C. DNA was extracted using standard phenol extraction. Polymerase Chain Reaction was done with MIT or Research Genetics primer pairs. Fifteen mutants were scored and the mutation maps to Chromosome two with zero percent recombination at *D2Mit66* at 84.6 Mb. The distal flank *D2Mit395* at 119.3 Mb was confirmed with one recombinant from 14 mice, or 3.5% recombination. Before a proximal flank was determined, the candidate gene *Lrp4* at 91.3 Mb described our missing digit phenotype.

An allele test was done mating a heterozygous DBA/1LacJ-*Lrp4*^{mdig}/GrsrJ (stock #004423) to a homozygous SB;C3-*Lrp4*^{mdig}/GrsrJ. Three out of seven F1 pups exhibited

the missing digit phenotype validating that this is a new mutation in the *Lrp4* gene.

Pathology

According to our standard pathology screen, a five-week-old mutant had no lesions except for the missing toes. The coarser tail is also clearly affected upon gross inspection but the pathology revealed no lesions.

The eyes of three mutants and three littermate controls at ten-weeks-of age were checked with our standardized eye exam. They all had retinal degeneration consistent with the *Pde6b*^{rd1} mutation for which both SB/LeJ and C3H/HeSnJ inbred strains are homozygous. Since retinal degeneration can mask other eye mutations, an attempt to determine an eye phenotype unique to the *Lrp4*$^{mdig\text{-}2J}$ mutation was done by mating a homozygous SB;C3-*Lrp4*$^{mdig\text{-}2J}$/GrsrJ to a C3A.BLiA-*Pde6b*$^{+}$/J mouse (Stock #001912). A mutation in the *Pde6b* gene causes retinal degeneration or *rd* and this *Pde6b*+ strain is valuable for this assessment in that it does not have retinal degeneration that is typical in the other C3H lines.

This mating produced five F1 mice all heterozygous for both *Lrp4*$^{mdig\text{-}2J}$ and *Pde6b*^{rd1} so they had normal eyes at two months of age. Of sixteen F2 mice born, only one mouse was homozygous for *Lrp4*$^{mdig\text{-}2J}$. This mutant was also homozygous for *Pde6b*^{rd1} as were five unaffected siblings. The remaining ten F2 mice that did not display the *Lrp4*$^{mdig\text{-}2J}$ phenotype had normal eyes demonstrating that *Pde6b*^{rd1} is segregating as expected. The search to uncover an eye phenotype unique to the *Lrp4*$^{mdig\text{-}2J}$ is still inconclusive.

Hearing was assessed by Auditory-Evoked Brainstem Response. Four heterozygotes and three homozygotes at three-months-of age all had good hearing.

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