Two new spontaneous mutations in the *Lmx1a* gene, dreher 10 Jackson and dreher 11 Jackson.

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Mutation (allele) symbols: *dr-10J, dr-11J*

Mutation (allele) name: dreher 10 Jackson; dreher 11 Jackson

Gene symbol: *Lmx1a*

Strains of origin: 129P3/J (10J); NOD.CB17-*Prkdc*^{scid}/J (11J)

Strain names: 129P3/J- *Lmx1a*$^{dr\text{-}10J}$/J (stock #5619); STOCK *Lmx1a*$^{dr\text{-}11J}$/J (stock #7786)

Phenotype categories: circling/hearing loss/ head toss

Origin and Description

The dreher 10 Jackson mutation arose spontaneously in the 129P3/J mouse strain at the Jackson Laboratory, and dreher 11 Jackson arose in the NOD.CB17-*Prkdc*^{scid}/J inbred colony spontaneously. To eliminate the *Prkdc*^{scid} mutation, *dr-11J* mutant mice were crossed to C57BL/6J mice three times, creating the new strain name STOCK *Lmx1a*$^{dr\text{-}11J}$/J. Mice homozygous for the *dr-10J and dr-11J* mutations are identical in their phenotype. Similar to the previously described *Lmx1a*^{dr} mice, mutants display head tossing and circling behavior indicative of inner ear vestibular dysfunction and possible hearing loss. Mutants tend to have slightly smaller bodies and shorter tails than littermates, occasional tail kinks and a normal lifespan. Mutant females breed but some mutant males never breed.

A homozygous mutant *dr-10J* mouse (top) compared with a normal littermate (bottom). Note the smaller size and shortened tail of the mutant.

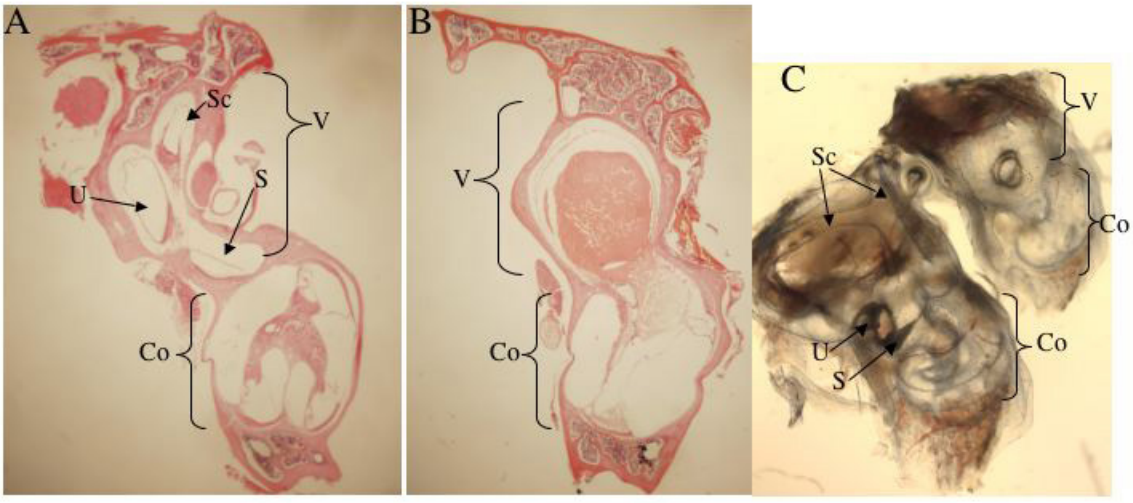
Genetic Analysis

Based on the similarity of the phenotype of these new mutations with that of the original *Lmx1a*^{dr} mice, direct allele tests were performed. A heterozygous *dr-10J* mouse was mated to a homozygous B6C3Fe-a/a-*Lmx1a*^{dr} mouse producing 5 litters of mice with 17 affected out of 31 progeny. A heterozygous *dr-11J* female was mated to a heterozygous *dr* mouse producing two litters with 2 affected mice out of 19 progeny. Both complementation tests proved allelism.

Pathology

A standard pathological screen[1] of one homozygous *Lmx1a*$^{dr\text{-}10J}$ mutant at 8 and 30 weeks of age showed abnormalities of the brain. The brains from homozygous (*dr-10J/ dr-10J)* mutants have an overall reduced size and the cerebellar foliation is reduced as compared to littermate controls.

Histological sections of the inner ear showed that the membranous labyrinth is under developed. Severe inner ear abnormalities include a reduction in overall size, undeveloped semicircular canals, no distinguishable utricle or saccule, and a shortened cochlea lacking compartmentalization.



Inner ears of control and dreher-10J mice.

(A,B) H&E stained cross-sections (4μm) of inner ears from a +/dr^{10J} (A) and a dr^{10J}/dr^{10J} (B) adult.

(C) Whole mount preparation of inner ears from a +/dr^{10J} control mouse (left) and a dr^{10J}/dr^{10J} mutant (right) viewed by light microscopy.

Brackets identify the cochlea (Co) and vestibular apparatus (V). Arrows indicate the utricle (U), saccule (S) and semi-circular canals (Sc). These compartments are malformed and underdeveloped in the mutant dr^{10J}/dr^{10J} ear (B,C-right).

Hearing as assessed by ABR of two mice homozygous for the *dr10J* mutation and two littermate controls determined that the mutants were deaf and control littermates retained normal hearing. The dreher 11J mutation was not assessed for hearing because of the confounding hearing loss from the NOD background strain.

Additional histological Methods:

Cross sections of inner ears were obtained in the following manner. Mice were anesthetized and perfused through the left ventricle of the heart with phosphate-buffered saline (PBS) followed by Bouin's fixative. Cross sections were obtained by dissecting the inner ears out of the skull, immersing them in Bouin's fixative for 24 hours, decalcifying in Cal-Ex solution for 6 hours and embedding in paraffin. Sections were cut (4 μM thick), mounted on glass slides and counterstained with hematoxylin and eosin (H&E). Sagittal sections of the brain were prepared in a similar manner. After perfusion, the brain was dissected, fixed in Bouin's for 7 days and embedded in paraffin. Sections were cut 6 μm thick, mounted on glass slides and counterstained with H & E. All slides were examined on an Olympus BX40 light microscope and digital images were captured with the Olympus DP70 Camera (Olympus Optical co., LTD, Tokyo, Japan).
To prepare whole mounts, inner ears were dissected from the temporal bone and flushed with neutral-buffered formalin through a hole made at the cochlear apex. Inner ears were then immersed in neutral-buffered formalin, dehydrated in ethanol, and cleared in methyl salicylate overnight.

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