Dark-like: A New Recessive Color Mutation that is Located on Mouse Chromosome 7

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Source of Support: This research was supported by grants NIH/NCRR RR01183 to the Mouse Mutant Resource (M.T. Davisson, PI) and Cancer Center Core Grant CA34196.

Mutation (allele) symbol: *dal*

Mutation (allele) name: dark-like

Gene symbol: *dal*

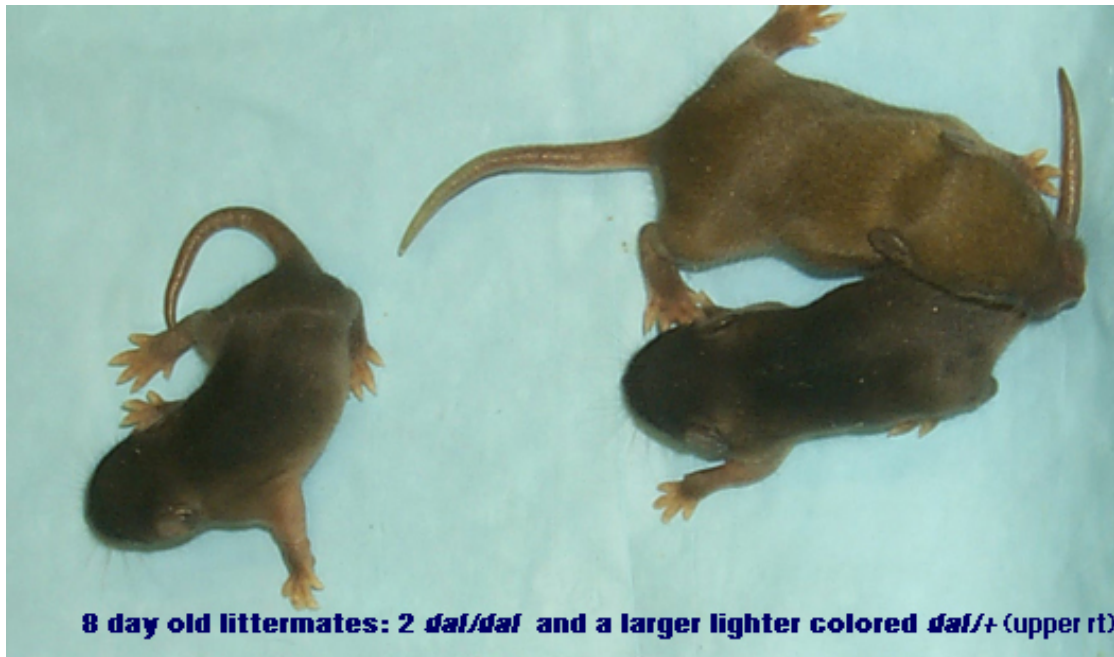
Strain of origin: CBA/J

Current strain name: CBA/J-*dal*/GrsrJ

Stock #003398 (jaxmice.jax.org)

Phenotype categories: color, skeletal, metabolic, reproductive

Abstract
A new recessive mouse mutation causing a darkened coat color, smaller size, gonad abnormalities, and dark staining urine has occurred on Mouse Chromosome 7 and has been named dark-like. A previously described mutation named dark maps to the same chromosomal region. Dark-like may be a remutation to dark (*da*) however a test for allelism was not possible because dark is thought to be extinct.



8 day old littermates: 2 *dal/dal* and a larger lighter colored *dal/+* (upper rt)

Origin and Description
The dark-like mutation arose in the research colony of Dr. Eva Eicher at The Jackson Laboratory. Mice homozygous for the *dal* mutation are easily recognized at 14 days of

age by their darkened coats which are darker than littermate controls but more subtle than mahogany (*mg*) and by their smaller size (see photo above). The darker coat color becomes more subtle with age. Some mutant mice appear, both phenotypically (small size) and pathologically (dense bones), to have skeletal abnormalities however X-rays appear normal and do not indicate the dense bones that are observed in histological sections. Both males and females breed normally until 5 months of age. By 7 months of age both males and females have reproductive abnormalities (see pathology below).

Genetic Analysis

Using standard mapping protocols of The Mouse Mutant Resource a mapping backcross (BC) was set up between dark-like and B.D-Ca^{16J}. DNA from 56 BC progeny were used to map the mutation. Because of the similarity of the phenotype to dark, mapping was started on Chromosome 7 and linkage was first indicated with *D7Mit82.* All individual DNAs from the linkage cross were then typed for 4 additional markers to flank the mutation. The recombination estimates and best gene order are centromere-[*D7Mit56*, *D7Mit294*]- 4.7 +/- 1.1- *dal*- 4.1 +/- 1.0 - *D7Mit247* - 3.1 +/- 0.9 -[*D7Mit82-D7Mit120*]. Gene order and recombination frequencies were calculated with the Map Manager computer program (Manley), a MacIntosh program for storage and analysis of experimental mapping data.

The penetrance of the dark-like phenotype appears to be incomplete. Twenty-three backcross mice were classified as mutant by coat color and all were confirmed to be homozygous for the mutant strain allele by flanking DNA markers. However, 33 backcross mice were classified as non-mutant by normal coat color, but flanking markers indicate that 6 of these mice are homozygous for the mutant strain allele. Therefore, penetrance of the mutant phenotype is estimated as 23/29 = 79%.

The 6 misclassified mice are homozygous mutants that did not show the expected coat color phenotype.

Pathology

Our standard pathology screen[1] revealed that in homozygous mutant mice at 6 weeks of age there were vacuolated cells at the cortical medullary junction of the adrenal gland, mild hydrocephalus, and dense bone. At 7 months of age, females had no follicles and many corpora lutea; and males had mild testicular degeneration with increased Leydig cells.

Serum assays for Albumin, BUN, creatine, bilirubin, and iron showed no significant differences between 7 heterozygotes and 10 homozygotes tested.

ABR (auditory brainstem response) testing revealed no significant hearing loss in 3 +/*dal* and 2 *dal*/*dal* mutants tested at 14 weeks of age.

Mutants do have retinal degeneration (*rd-1*) characteristic of most CBA strains.

Discussion

The phenotype description of dark (MGD) is very similar to dark-like. Dark homozygotes

are described as smaller than normal and the darkening of the coat becomes more subtle with age. The urine of mutants turning the shavings a darker yellow than normal was not mentioned in the original dark description. The chromosomal location for dark is on Chr 7 at 12 cM and we have placed dark-like between 8 and 16 cM with our flanking markers *D7Mit294* and *D7Mit247*. Dark-like may be a remutation to dark but a test for allelism was not done because of the unavailability of the dark mutation.

Acknowledgements
The authors wish to thank Coleen Marden, Heping Yu and Norm Hawes for excellent technical assistance.

References

Manley KF (1993) A MacIntosh program for storage and analysis of experimental mapping data. Mamm Genome 4,303-313

Mouse Genome Database (MGD) Mouse Genome Informatics Project, The Jackson Laboratory, Bar Harbor, Maine. World Wide Web (www.informatics.jax.org)

Zheng QY, Johnson KR, Erway LC(1999) Assessment of hearing in 80 inbred strains of mice by ABR threshold analyses. Hear Res 130, 94-107.

[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.