Darkened dorsal; a new coat color mutation on Chromosome 2

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Mutation (allele) symbol: *Dkd*

Mutation (allele) name: darkened dorsal

Gene symbol: *Dkd*

Strain of origin: LPT/Le

Current strain name: LPT;C3-*Dkd*/GrsrJ

Stock #006058 (jaxmice.jax.org)

Phenotype categories: skin and hair

Origin and Description

A new spontaneous semidominant coat color mutation has been identified and named darkened dorsal (*Dkd*). Mice carrying this mutation were discovered by Rebecca Rhodes in a colony of LPT/Le at the Jackson Laboratory. Mice homozygous for this mutation are identified by a darkened dorsal stripe that can be seen when the first coat of hair comes in. The LPT;C3-*Dkd*/GrsrJ colony is maintained by homozygous matings.

A mouse homozygous for the darkened dorsal mutation is shown on the left with a littermate control on the right. Both are three weeks of age. See the allele detail page of MGI for more photos.

Genetic Analysis

Using our standard mapping protocols[1] the *Dkd* mutation was mapped to Chromosome 2. It maps between *D2Mit260* (NCBI 36 position 149.0 Mb) and *D2Mit148* (NCBI 36 position 178.5 MB) and is non-recombinant with *D2Mit286* (NCBI 36 position 154.3Mb) and *D2Mit196* (NCBI 36 position 160.2 Mb).

Pathology

A pathological screen of two homozygous mutant mice was performed at 20 weeks of age and no gross abnormalities were observed.

Hearing as assessed by auditory brainstem response testing (ABR) of four homozygous mutants at four weeks of age was normal.

The eyes of four homozygous mutants at four weeks of age were examined with an ophthalmoscope and were determined to be normal.

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[1]Standard Mapping Protocol used in The Mouse Mutant Resource

Linkage crosses

To map new mouse mutations we use CAST/Ei, an inbred strain of *Mus musculus castaneus*, as our standard linkage testing strain. In some cases, because of breeding difficulties or reduced phenotypic penetrance, we use other strains. An intercross of F1 hybrids is usually used to analyze linkage of recessive mutations and a backcross to analyze linkage of dominant mutations. Our goal is to produce enough informative mice from each mapping cross to test the recombinational products from at least 100 meioses.

DNA isolation

DNA is extracted from the frozen tail tips of mutant (homozygous) F2 mice or backcross progeny by a standard hot sodium hydroxide and Tris (Hot SHOT) procedure (Truett, et al., 2000) or from spleens using standard phenol extraction methods.

Polymerase chain reaction

PCR primer pairs (MapPairs, from Research Genetics, Huntsville, Ala., or from Integrated DNA Technologies, Coralville, Ia.) are used to type MIT microsatellite markers positioned throughout the genome. PCR reactions contain 20 ng genomic DNA in 10 ul containing 50 mM KCL, 10 mM Tris-HCL (pH 9.0 at 250C), and 0.01% Triton-X-100, 2.25 mM MgCl2, 100 nM of each primer (forward and reverse), 100 uM of each of four deoxyribonucleoside triphosphates, and 0.5 Units of Taq DNA polymerase (Amplitaq from Applied Biosystems #N808-0145). Amplification consists of one cycle of denaturation at 940C for 3 minutes followed by 30 cycles, each consisting of 940C for 15 sec. denaturation, 550C for 2 minutes of annealing, and 720C for 2 minutes of extension. After the 30 cycles, the final product is extended for 7 minutes at 720C. The PCR products are run on 2.5% Metaphor agarose gels or 6% polyacrylamide (non-denaturing) gels. The gels are then stained with ethidium bromide, destained with distilled water, visualized on a UV light table, and photographed.

Pooled DNA Method (Taylor et al 1994).

In the case of an intercross, a pool of DNA prepared from 25-30 mutant F2 mice is compared with DNA from F1 hybrids. In the case of a backcross, a DNA pool from 25-30 mutant N2 mice is compared with a pool from 25-30 unaffected N2 mice. These DNA samples are typed by PCR for MIT markers located throughout the genome. For linked markers, the mutant strain allele will predominate in the DNA pool from mutant mice compared with controls. When a particular marker indicates linkage by analysis of the pooled sample, individual DNA samples are typed with that marker and additional markers in the same region to confirm linkage. Once a linkage is confirmed, additional DNAs from individual mice are typed to obtain a finer map position.

Linkage analysis

Gene order and recombination frequencies are calculated with the Map Manager computer program (Manley1993, 2001), a MacIntosh program for storage and analysis of genotyping data.

References

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