Overall Diluted Appearance, A New Diluted Color Mutation on Chromosome 14

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Mutation (allele) symbol: *oda*

Mutation (allele) name: overall diluted appearance

Strain of origin: STOCK Tg(CAG-EGFP)D4Nagy/J

Current strain name: B6.Cg-*oda*/GrsrJ

Stock #008548 (jaxmice.jax.org)

Phenotype categories: pigmentation

Origin and Description

A new autosomal recessive color mutation, named overall diluted appearance (*oda*), was found in STOCK Tg(CAG-EGFP)D4Nagy/J by Cheryl MacLean at The Jackson Laboratory. Mice homozygous for *oda* are characterized easily by two weeks of age by their overall diluted steel grey coat color on a nonagouti background. This mutation also dilutes the color of the ears, feet and tail. Both sexes of homozygotes are viable and live a normal lifespan. Intercrossing heterozygotes produces the expected Mendelian ratio of homozygotes. This colony is maintained by breeding a C57BL/6J mouse to a homozygote of either gender and then intercrossing the obligate heterozygous offspring. The transgene has been bred out of this mutant subline, but the phenotype caused by this mutation has remained the same.

Genetic Analysis

The *oda* locus was mapped using a cross between a mutant with the overall diluted appearance and the inbred strain C57BL/6J. All F1 progeny were normal and the F2 generation showed normal Mendelian inheritance for a recessive mutation. Using the standard mapping protocols of The Mouse Mutant Resource[1], DNA was obtained from F2 affected animals and used to map this mutation. The *oda* locus mapped to Chromosome 14 near marker *D14Mit10* with 2 recombinants out of 42 meioses.

This map position is some distance from dopachrome tautomerase, a gene for which mutations cause a coat color dilution similar to *oda*, but *Dct* remains a candidate gene. No allele test was performed with a *Dct* mutant because none was readily available, but allele tests were performed with mutants of Hermansky-Pudlak syndrome 3 homolog (*Hps3*) and Rab geranylgeranyl transferase alpha subunit (*Rabggta*). A complementation test cross between *oda* and $Hps3^{coa}$ (cocoa) on a nonagouti background proved them non-allelic. The offspring in a complementation cross between a $Rabggta^{gm}$ (gunmetal) homozygote and an *oda* homozgote produced offspring that were not true black in color

in that they appeared slightly dull compared with C57BL/6J, but were not significantly diluted in color like that of either gunmetal or overall dilute.

Whole exome sequencing[2] of *oda* did not reveal any candidate mutations on Chromosome 14. Failure to identify any candidates in protein coding sequence, splice sites, or UTRs may indicate that the mutation resides in a non-coding or regulatory region. Additionally, the causative mutation may be in the coding region, but remains undetectable using current informatic methods.

Pathology

A routine pathological screen on individual animals showed no histological lesions in one female or one male homozygote examined at five months of age. The eyes of one male and two female homozygotes examined showed that all had normal eyes and confirmed that transgene driving expression of green fluorescent protein had been bred out of this mutant subline. Hearing tests on one female and one male heterozygote and one female and one male homozygote at three months of age showed that all had normal hearing.

Discussion

Overall diluted appearance is a new recessive coat color mutation that shows a dilution of the coat color in affected mice and is mapped to Chromosome 14. Both sexes are viable and fertile. While *Hps3* has been ruled out as the mutated gene, *Dct* and *Rabggta* remain possible candidates. Sequencing of the introns and promoter for these genes may reveal the molecular lesion underlying this new mutation.

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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

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

Manly KF, Cudmore RH Jr, and Meer JM (2001) Map Manager QTX, cross-platform software for genetic mapping. Mamm Genome12: 930-932.

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Truett GE, Heeger P, Mynatt RL, Truett AA, Walker JA, and Warman ML(2000) Preparation of PCR-quality mouse genomic DNA with hot sodium hydroxide and Tris (HotSHOT). Biotechniques 29:52-54

[2]Exome Sequencing Protocol

The exome sequencing data referred to in this website were analyzed using tools and workflows provided by Genome Quest1 including processes for mapping (HS3), SNP calling and annotation of variants. Our analysis focused on novel variants, which were not positioned in repetitive sequence, had expected allele ratios (>0.95 for homozygous variants and >0.2 for heterozygous variants), and displayed sufficient locus coverage (at least 5X for homozygous variants and 10X for heterozygous variants) for effective mutation discovery. High priority was given to protein coding or splice variants within mapped regions, as well as unique variants that were not found in other exome data sets or in the Sanger Mouse Genomes Database2. Following these analyses, re-sequencing of additional mutant and unaffected samples was performed to validate and determine the most likely causative mutation.