Chocolate-like, a spontaneous mutation causing a dark brown coat color in the mouse.

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Mutation (allele) name: chocolate-like

Mutation (allele) symbol: *chtl*

Gene symbol: *chtl*

Strain of origin:B6.129P2-*Nos2*^{tm1Lau}

Current strain name: B6(129P2)-*chtl Nos2*^{tm1Lau}/GrsrJ

Stock #004684 (jaxmice.jax.org)

Phenotype categories: coat color

Origin and Description

The chocolate-like mutation was found at The Jackson Laboratory in 1998 in the B6.129P2-*Nos2*^{tm1Lau} strain at generation N7F4 + F16. This mutant has an overall lighter coat, tail, and ears than a black a/a control. Both females and males breed and it is maintained as a homozygous stock. Stock # 002609-B6.129P2-*Nos2*^{tm1Lau} is the background control. This recessive mutation, which we have named chocolate-like (gene symbol, *chtl*), maps to Chr 7 between the markers *D7Mit211* and *D7Mit321* and is non recombinant with *D7Mit31*. Based on linkage map position and Ensembl location for the markers used, it was thought that this was a remutation to chocolate (*cht*). A test for allelism with *cht* however did not produce the expected chocolate colored mice, but instead produced a dull grey phenotype.

Pathology

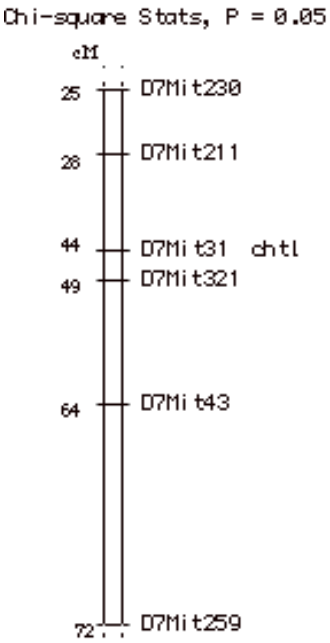
A routine pathological screen of one female and one male *chtl/chtl* mice at 11 weeks of age showed no lesions. Hearing was assessed by ABR threshold analysis (Zheng et al. 1999) of 2 *chtl/chtl* mice tested at 4 months of age. The ABR results showed that the homozygous mutants had normal hearing. The eyes of two homozygous mutants were examined with an ophthalmoscope and were determined to be normal.

Genetic analysis

chtl is inherited as a recessive mutation as shown by segregation in the traditional linkage cross analysis described below. The progeny produced showed no visible mutants in the F1 generation (0/26) and segregation in the F2 (23 *cht*l/398 total progeny) produced 5.78% mutants, less than the expected 25%. For linkage analysis, an intercross was utilized to produce mutant mice. A CAST/Ei female was mated to a B6.129P2-*Nos2*^{tm1Lau} homozygous mutant male. F1 hybrids from this initial cross were then

intercrossed to produce the F2 progeny. The F2 progeny were scored visually for phenotype and spleens and tail tips from 21 homozygous mutant animals were collected and stored at -70C for subsequent DNA typing to map the mutation. DNA was extracted from the frozen tail tips of 21 mutant (homozygous) F2 mice produced in the linkage cross by a standard Hot Sodium and Tris (HotSHOT) procedure (Truett,et al., 2000). Polymerase chain reaction. PCR primer pairs (MapPairs, Research Genetics, Huntsville Ala.) of microsatellite markers *D7Mit230, D7Mit211, D7Mit313, D7Mit321, D7Mit43*, and *D7Mit259* were used to localize the mutation on Chr 7. PCR analyses were carried out in 10 ul total volume reactions containing 20 ng genomic DNA, by previously described methods (Ward-Bailey et al. 1996).

Mutation segregation ruled out Chromosome X linkage. A genome sweep of F2 progeny from the CAST intercross was begun by typing DNA samples from 21 homozygous mutant animals for segregating MIT microsatellite markers, starting with Chromosomes 7and 14, chosen because mutations with similar phenotypes (*cht* and *slaty)* are located there. Linkage of *chtl* was first detected on Chr 7 with *D7Mit43*. DNA samples were then typed for five additional Chr 7 markers. The recombination estimates and best gene order are centromere-[*D7Mit230*]-5.06 +/-3.45-[*D7Mit211*]-7.65+/-4.16-[*D7Mit31, chtl*]-2.40+/-2.35-[*D7Mit321*]-9.76+/-4.53-[*D7Mit43*]-17.43+/-5.75-[*D7Mit259*].



Linkage analysis. 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 complete Chr 7 linkage data for 21 F2 *chtl/chtl* mice have been deposited in the Mouse Genome Database.

Allelism tests

chtl mice mated to DBA/2J mice was negative (all pups produced were *a/a*).
A test for allelism with homozygous *cht* mouse mated to a homozygous mutant mouse produced 32/32 progeny with a dull grey phenotype, unlike *cht* or *a/a*. These results

suggested *chtl* is a new allele of the *cht* gene that produces a different phenotype from the original *cht* allele. When two matings of the grey color F1s from the allele test progeny were mated together, however, they produced segregating litters for a total of 10 *cht* looking mice and 3 normal black *a/a* mice. The appearance of the black mice means either the two mutations are pseudoalleles or modifier genes can affect the phenotype expression.

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References

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