

## A new spontaneous mutation named X-linked stripe.

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

Mutation (allele) symbol: *Xls*

Mutation (allele) name: X-linked stripe

Gene symbol: *Xls*

Strain of origin: (C57BL/6J x CBA/Ca-*Pdss2*<sup>kd</sup>) F1

Current strain name: CBACaGnLe.Cg-*Xls*/GrsrJ

Stock #005274 (jaxmice.jax.org)

Phenotype categories: Color

### Abstract

A new spontaneous dominant X-linked mutation causing a striped coat has been characterized in the Mouse Mutant Resource (MMR) at the Jackson Laboratory and named X-linked stripe (*Xls*). The new mutant mice have a phenotype and chromosomal location similar to that of the previously described Tabby (*Eda*<sup>Ta</sup>) mutation, but a direct test for allelism was not possible.



A female mouse from the CBACaGnLe.Cg-*Xls*/J colony showing the striped coat

### Origin and Description

The new X-linked stripe mutation was identified in the offspring resulting from an in vitro fertilization of C57BL/6J eggs fertilized with sperm from CBA/Ca-*Pdss2*<sup>kd</sup> homozygous males in 2001. After one outcross to C3H/HeSnJ this mutation was subsequently maintained by continuous backcross of a heterozygous female to a

CBA/CaGnLeJ male and reached generation N15 in 2007. Heterozygous female mice affected by the *Xls* mutation have an agouti coat color with stripes and a white patch is always observed on the left hind flank. Hemizygous mutant males do not survive past birth.

### Genetic Analysis

X-linked stripe is inherited as a dominant mutation on the X Chromosome, as shown by traditional breeding, in which a heterozygous mutant female mouse was outcrossed to an unrelated C3H/HeSnJ male mouse. Mutant females were observed in the F1 mice produced by this cross proving that the mutation has dominant inheritance. A female affected with the *Xls* mutation was backcrossed to a CBA/CaGnLe/J male. The progeny from this cross produced normal appearing  $+/+$  females and  $+/Y$  males and affected  $+/Nm$  females. No affected males ( $Nm/Y$ ) were ever born.

Using standard MMR mapping procedures<sup>1</sup> a linkage cross was set up by mating 2 female mice heterozygous for *Xls* with 1 male CAST/Ei mouse. Five affected F1 females from this cross were then backcrossed to  $+/Y$  males from the CBACaGnLe.Cg-*Xls*/J colony. These matings produced 32 affected female mice of which 21 were used for linkage analysis. *Xls* was confirmed to be on the X Chromosome by linkage with MIT markers *DXMit113* (NCBI 36 position 90.0 Mb), which showed no recombination with *Xls*, and *DXMit41* (NCBI 36 position 97.3 Mb), which showed 9.5% recombination. Although phenotype and map position are similar to those of the Tabby (*Eda<sup>Ta</sup>*) mutation (NCBI 36 position 96.1Mb), a direct test for allelism was not possible. Two other X-linked genes with similar phenotypes, striated (NCBI 36 position 69.1Mb) and patchy fur (NCBI 36 position 73.3Mb), were ruled out as candidate genes based on map position.

### Pathology

A routine pathological screen of two mice carrying the *Xls* mutation at 19 weeks of age revealed no gross abnormalities.

Hearing, as assessed by auditory brainstem response (ABR) testing on two mutant mice and two littermate controls at 3 months of age, was normal.

The eyes of two mice carrying the *Xls* mutation were examined with an ophthalmoscope and no abnormalities were observed.

### Acknowledgements

The authors wish to thank Chantal Longo-Guess and Heping Yu for hearing assessment, Norm Hawes for the eye examinations, and Rod Bronson for the pathological review.

### Footnotes

#### 1) Standard Mapping Protocol used in the MMR

##### 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 MgCl<sub>2</sub>, 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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