Wooly: A New Hair Mutation on Mouse Chromosome 11

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Mutation (allele) symbol: *wly*

Mutation (allele) name: wooly

Gene symbol: *wly*

Strain of origin: NOD/LtJ

Current strain name: NOD/LtJ-*wly*/J

Stock #004774 (jaxmice.jax.org)

Phenotype categories: skin and hair

Abstract
A new autosomal recessive hair mutation named wooly has been found in the Mouse Mutant Resource (MMR) at The Jackson Laboratory. It is characterized by having a wooly coat as compared to its normal coated littermates and is recognizable by four weeks of age. This mutation maps to Chromosome 11 between *D11Mit313* (28 cM) and *D11Mit261* (34.15 cM), which is near, but not allelic with, the mouse hair mutation waved-2 (*wa-2*).

Origin and Description
This mutation was found in 2001 in the strain NOD/LtJ at F92 by Andrea Kelley in the mouse room AX-8 at The Jackson Laboratory and brought to the MMR deviant search program. Homozygous mutant mice are recognized by their wooly coats. Mutant animals have straight whiskers. Both sexes are viable and fertile.

Genetic Analysis
A homozygous wooly male mutant was mated to a female C57BL/6J and produced all normal F1 progeny. Using our standard MMR procedures[1], these F1s were intercrossed and produced 54 F2 affected animals which were used to determine the chromosomal location of the wooly mutation. Affected mice from the linkage cross phenotypically resembled the wooly mutation on its original background. Linkage on Chr 11 was first detected with *D11Mit186* and *D11Mit51* (both markers near waved-2). The linkage cross DNA samples were then typed for six additional Chr 11 markers. The recombination estimates with standard errors and the best gene order are:
centromere -- *D11Mit226* --16.2 +/- 7.6 -- [*D11Mit186*, *D11Mit51*] -- 8.2+/- 3.7 -- *D11Mit20* -- 4.0 +/-2.0 --*D11Mit140* -- 1.9 +/- 1.4 -- *D11Mit313* -- 1.0 +/- 1.0 --wly --1.9 +/- 1.4 -- *D11Mit261* --3.3 +/- 2.4 -- *D11Mit5*. Gene order and recombination frequencies were calculated with the Map Manger computer program (Manley 1993). The complete Chr 11 linkage data for 54 F2 *wly/wly* mice has been deposited in the Mouse Genome

Database, accession #J:85256. Based on the Ensembl assembly for Chr 11, the chromosomal position for *wly* is between 56892125 bp (*D11Mit313*) and 62805134 bp (*D11Mit261*).

When linkage was originally detected on proximal Chr 11, a direct test for allelism was set up with waved-2 (*wa-2*). Two homozygous wooly females were crossed to a male waved-2 homozygote and produced no affected progeny out of 16 born. A second mating of two homozygous wooly females to a male heterozygote waved–2 produced no affected progeny out of 12 born. Based on the allelism test results and the additional microsatellite marker typing listed above, wooly is not an allele of waved-2.

Pathology
In three mutant mice at 5 months of age, giant hair follicles still in anagen were observed. There was no subdermal fat. Microscopic examination of auchene, guard, and zigzag hair types were all normal. A histological examination of the skin showed it was normal. Auditory-evoked brainstem response (Zheng et al. 1999) testing was done on two homozygotes and two heterozygotes at ten weeks of age. All mice had the same level of severe hearing loss that was due to a genetic background affect. Mice of the NOD/LtJ strain have severe hearing loss by 2 months of age. The eyes of two mutant and two control animals at 7 weeks of age were examined with an ophthalmoscope and all were normal.

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References
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[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 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.

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