Nude 2 Jackson: A new spontaneous mutation in *Foxn1*

Belinda S. Harris, Heather E. Fairfield, Melissa L. Berry, David E. Bergstrom, Roderick T. Bronson and Leah Rae Donahue

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Mutation (allele) symbol: *Foxn1*$^{nu\text{-}2J}$

Mutation (allele) name: nude 2 Jackson

Gene symbol: *Foxn1*

Strain of origin: B6.SJL-*Ptprca Pepc*^{b}/BoyJ

Current strain name: B6(SJL)-*Foxn1*$^{nu\text{-}2J}$/GrsrJ

Stock#: 016195 (jaxmice.jax.org)

Phenotype categories: athymic, skin, and hair

Abstract

We have identified a new recessive mutation that causes affected mice to exhibit the same abnormal skin phenotype as that caused by the original nude mutation. Mutants can be identified by approximately one week of age by their wrinkly skin. Homozygotes remain smaller in size and hairless throughout their lifespans. Vibrissae are very sparse or short and very curled. A direct test for allelism with *Foxn1*^{nu} mutants produced affected progeny, and SNP analysis and exome sequencing were used to confirm that this is a new spontaneous mutation in *Foxn1*.

Origin and Description

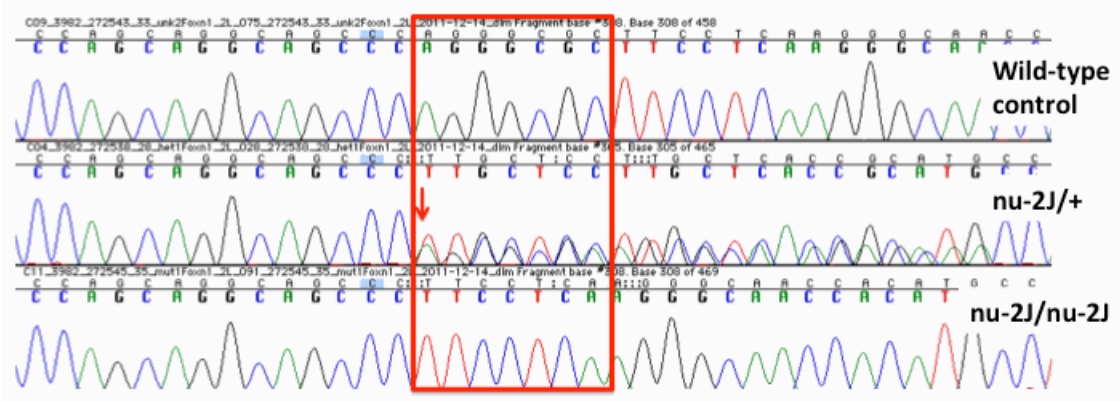
This new mutation was found by Phyllis Van Compernolle in a production colony of B6.SJL-*Ptprc*^{a} *Pepc*^{b}/BoyJ mice (stock #002014) at The Jackson Laboratory, and was initially identified by the wrinkled and hairless skin. Mutants are smaller in size than their littermates and have varying lifespans. Many do not live to adulthood and die before weaning age but some do live and may be fertile. They have either very sparse vibrissae, short and curled and sparse vibrissae, or almost no vibrissae visible at all. Male mutants may sire litters when mated to normal littermates or to an inbred mouse, while female mutants may be fertile but may bear no live pups or will have very few pups, thus making it necessary to foster their litters after birth. This mutation was proven recessive by outcrossing mutants to C57BL/6J. No mutants were found in the F1 offspring but mutants were found in the expected Mendelian ratio in the F2 population. Heterozygotes are fertile and live a normal lifespan. This strain has been maintained by backcross-intercross to C57BL/6J.

Genetic Analysis

SNP typing of F2 mice from an outcross-intercross involving C3H/HeSnJ mapped this new mutation to Chromosome 11 in the vicinity of Forkhead box protein N1

(*Foxn1*). Because the phenotype of this mutation is so similar to that of *Foxn1* mutants, a complementation test for allelism with $Foxn1^{nu}$ was performed. A female heterozygous for the new mutation was bred to a male homozygous for $Foxn1^{nu}$ and this mating produced 3 total pups of which 2 were born dead. Two females, one homozygous and one heterozygous for this new mutation, were bred to a homozygous $Foxn1^{nu}$ male. This produced four affected pups out of 13 total progeny with 8 born dead and one phenotypically normal, proving this new mutation to be an allele of *Foxn1*. Thus, this new allele has been designated nude 2 Jackson ($Foxn1^{nu\text{-}2J}$).

Exome sequencing[1] was used to identify candidate mutations in the mapped region. Analysis including the use of commercial alignment and SNP/INDEL calling tools indicated a single mismatch allele of C to G on Chromosome 11, at position 78,184,703 (NCBI build 37/mm9). This was predicted to cause a single amino acid change from arginine to proline (R114P) in *Foxn1*. Primers were generated that produce a 267 base pair product spanning the predicted mutation; *Foxn1* forward (ATCTCCAGACCCAGAGCAGA) and *Foxn1* reverse (CTCCTCAAAGGCTTCCAGTG). Sequence analysis (Sanger method) of additional genomic DNA samples from homozygotes, heterozygotes, and wild-type controls showed that the single mismatch detected by exome sequencing was instead a small 7-nucleotide deletion encompassing nucleotides 78,184,702-78,184,708 on Chromosome 11 in mutant DNA samples. This small deletion in exon 3 results in a frameshift mutation, leading to the introduction of 186 novel amino acids (beginning at protein position 114), and an early termination codon.



Comparison of sequence chromatograms from wild type and heterozygous controls and homozygous mutant sample. Double chromatogram in nu-2J/+ sample and single chromatogram in mutant sample indicates a 7 bp deletion encompassing nucleotides 78,184,702-78,184,708 on Chromosome 11 (NCBI37/mm9).

Pathology

A routine pathological screen[2] of one female and one male homozygote at eight weeks of age showed severe follicular dystrophy with clumping hair. Hair plucks, albeit with little hair able to be plucked, were taken from two mutants. Microscopic examination showed that the hair was tangled into clumps of broken hairs, indicative of fragile hair, but most of the hair types could be identified. Two homozygous females at 24 days of age were

dissected and found entirely athymic consistent with previous *Foxn1* mutants.

Auditory brainstem response (ABR) testing on two male homozygotes at six weeks of age uncovered hearing impairment at higher frequencies. Control C57BL/6J animals had normal hearing. These animals had routine eye examinations done and eyes examined of two homozygotes at two months of age and were found to be normal.

Discussion

The *Foxn1* gene is conserved among vertebrates and encodes a transcription factor with a highly conserved forkhead DNA binding domain. The previously identified $Foxn1^{nu}$ allele is a single base pair deletion within exon 3 resulting in a frameshift mutation. This mutation is predicted to result in an early termination codon upstream of the forkhead domain, normally encoded by amino acids 269-361. $Foxn1^{nu\text{-}2J}$ results in the introduction of 186 novel amino acids, and a premature termination codon within the DNA binding domain. If a stable protein is produced from this transcript, it will be truncated with unique domains. These two molecularly distinct, but similar mutant models may provide unique insight into the function of *Foxn1*, specifically the ability of the protein to function with loss or alteration of the conserved DNA binding domain.

Acknowledgements

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Protocols

[1]Exome Sequencing Protocol

The exome sequencing data referred to in this website were analyzed using tools and workflows provided by Genome Quest 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 Database. Following these analyses, re-sequencing of additional mutant and unaffected samples was performed to validate and determine the most likely causative mutation.

[2]Standard Histology Protocol used in the Mouse Mutant Resource

For fixation of tissues, mice were deeply anesthetized with tribromoethanol (avertin) until they no longer displayed a withdrawal reflex in the hind limbs and then perfused intracardially with Bouin's fixative following a flush of the vasculature with saline solution. After soaking in Bouin's for one week to demineralize bones, tissues were dissected. Six segments of spine with axial muscles and spinal cord in situ, representing cervical, thoracic and lumbar spinal segments, were dissected. The brain was removed and sliced into 6 cross sectional pieces at the levels of olfactory lobes, frontal cortex, striatum, thalamus, midbrain, rostral and caudal medulla with cerebellum. Midsagittal slices of hind leg through the knees were prepared. Slices of basal skull through the pituitary and inner ears were taken. Both eyes, salivary glands and submandibular lymph node, trachea plus thyroid and sometimes parathyroid were removed and cassetted. A longitudinal slice of skin from the back was removed. The thymus, slices of lung, and a longitudinal slice of heart were cassetted. Similarly slices of liver through gall bladder, kidney with adrenal attached, pancreas and spleen were prepared. The stomach was sliced longitudinally to include both squamous and glandular portions. Loops of small intestine from 3 levels and slices of large intestine and cecum were removed, as were slices of urinary bladder. The whole uterus, with ovaries attached, was taken.

In males testes were sliced longitudinally. The accessory male organs including seminal vesicles, coagulating gland and prostate were removed en block. Altogether in most cases all tissue fit into a total of 10 cassettes. The cassettes were processed in an automatic tissue processor to dehydrate tissues which were then embedded in paraffin. Six micron sections were cut and stained with hematoxylin and eosin (H&E). Sections of brain and spinal cord in vertebral bones also were stained with luxol fast blue (LFB) for myelin and cresylecht violet (CV) for cellular detail.