Humpback: a new mutation on Chromosome 17 causing kyphosis and abnormal muscle phenotypes.

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Mutation (allele) symbol: *hpbk*

Mutation (allele) name: humpback

Gene symbol: *Notch3* (updated Feb 2012)

Strain of origin: C57BL/6J

Current strain name: C57BL/6J-*Notch3*^{hpbk}/GrsrJ

Stock #005330 (jaxmice.jax.org)

Phenotype categories: Skeleton/limbs/neuromuscular

Origin and Description

The humpback (*hpbk*) mutation arose in a C57BL/6J mouse colony that had been mutagenized with N-ethyl-N-nitrosourea (ENU) in the Neuroscience Mutagenesis Facility (NMF) at the Jackson Laboratory. Mice homozygous for the humpback mutation are normal in appearance at weaning age, but then develop a progressive emaciation and curvature of the spine. Mutants are easily distinguished from their wild type littermates at 5 weeks of age. With age, mutant mice become extremely thin and exhibit severe thoracic kyphosis. Sudden death is common in adulthood. Homozygous females are able to become pregnant but are unable to deliver their pups. Homozygous males have been mated with no success, as they all develop paraphimosis. The *hpbk* colony is maintained by breeding hosts of homozygous ovarian transplants to a littermate male and than intercrossing the heterozygous offspring.

Genetic Analysis

Using the Mouse Mutant Resource standard mapping procedures, a mouse homozygous (ovarian transplanted) for the *hpbk* mutation was mated to a mouse of the C3H/HeSnJ strain. The unaffected F1 hybrid mice produced by this cross were then intercrossed to produce affected F2 mice for linkage analysis. This mutation maps to Chromosome 17 between D17Mit46 (NCBI 37 position 25.5 Mb) and D17Mit148 (NCBI 37 position 37.4 Mb). Based on phenotypic similarities and map positioning *Neu1* (NCBI 37 position 35.0 Mb) is considered as a candidate gene. Genomic DNA made from a *hpbk/hpbk* mutant mouse was used to sequence the coding region of *Neu1,* but a mutation was not found.

Pathology

A routine pathological examination[1] of one mutant at 12 weeks age showed extreme kyphosis, scattered small muscle fibers, and neurogenic atrophy in perispinal muscles. At one month of age, three mutants showed mild hydrocephalus and focal myopathy in most muscle groups. At two months of age, two mutants showed neurogenic atrophy, and atrophy in muscles dorsal to the defect in the spine, suggesting the kyphosis is of a myogenic origin. From 4-10 weeks of age, all of four mutants examined exhibited focal myopathy in many muscles and severe muscle loss over the defect in the spine.

Hearing in two mutants at 4 weeks of age and two mutants at three months was assessed by auditory-evoked brainstem response testing (ABR). All had mild hearing loss. Three mutant mice at four weeks of age, one mutant mouse at eight weeks of age and one mutant mouse at twenty weeks of age were tested by electroretinograph (ERG) and with an ophthalmoscope. All showed clinically normal eyes.

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Addendum
The *hpbk* mutation was found to be a G to A point mutation in the spice donor site in intron 31 of *Notch3*. Please see Fairfield et al., Genome Biology 2011, Sept 14; 12(9):R86.

[1]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.