A Hydrocephalus Mutation with Rhinitis on Mouse Chromosome Eight

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Source of Support: NIH-NEI grant EY015073 to Leah Rae Donahue

Mutation (allele) symbol: *hyrh*

Mutation (allele) name: hydrocephalus with rhinitis

Strain of origin: B10.RIII-*H2*^{r}*H2-T18*^{b}/(71NS)SnJ

Current strain name: B6.Cg-*Hydin*^{hyrh}/J

Stock #006428 (jaxmice.jax.org)

Phenotype categories: craniofacial

Abstract
A spontaneous, recessive hydrocephalus mutation with rhinitis (*hyrh*) has been identified and mapped to Chromosome 8.

Origin and Description

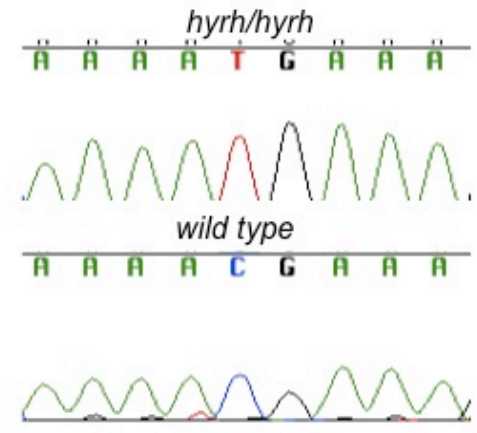
Mice carrying this new mutation were discovered in a colony of B10.RIII-*H2*^{r}*-H2-T18*^{b}/(71NS)SnJ (Stock# 00457) at the Jackson Laboratory. The *hyrh* mutation is now an incipient congenic on C57BL/6J (Stock # 000664) since it has been crossed to this background five times. Homozygotes are recognized by smaller bodies and domed skulls and do not live beyond three weeks, as the hydrocephalic condition is unremitting and only worsens. The B6.Cg-*hyrh*/J colony is maintained by ovarian transplant surgery taking ovaries from an *hyrh/hyrh* female and transplanting them into a C3SnSmn.CB17-*Prkdc*^{scid}/J (Stock # 001131) host female. The female hosts are either mated to a C57BL/6J wild-type male and *+/hyrh* offspring are subsequently mated to produce mutants, or the female hosts are mated to a *+/hyrh* from the colony.

Genetic Analysis

Hydrocephalus with rhinitis (*hyrh*) was determined to be recessive when female *hyrh/hyrh* ovaries were transplanted into a C3SnSmn.CB17-*Prkdc*^{scid}/J female, this host female was then mated to a C57BL/6J male wild type and *hyrh* mutants were observed only in the F2 litters.

Using our standard mapping protocol[1] the *hyrh* mutation maps to Chromosome 8 between markers *D8Mit86* at 108.2Mb and *D8Mit165* at 111.45 Mb. There were no recombinants at *D8Mit151* at 109.9 Mb and *D8Mit316* at 110.5 Mb. In this region at 109.6 Mb is the *Hydin* gene. Due to the chromosomal location and similarity of phenotypes between the *Hydin*^{hy3} mutants and the *hyrh* phenotype, a complementation test was performed. *+/Hydin*^{hy3} mice were bred with *+/hyrh* mice and there were no affected mice from 114 born.

The complete lack of pups observed to develop hydrocephaly after the complementation test led to an initial conclusion that *hyrh* and *Hydin*^{hy3} were not allelic. However sequencing has now confirmed that *Hydin* is indeed the affected gene. Six *hyrh/hyrh* showed a base pair change in exon 67 and six unaffected mice did not have this change. This C to T changes an arginine to a premature stop codon at amino acid 3806 of 5155 (Lance Lee, Ph.D., unpublished data, 2011).



A base pair change was detected in in exon 67 of the *Hydin* gene in an *hyrh/hyrh* mutant resulting in a premature stop codon.

Pathology

A pathological screen[2] of 7 *hyrh* mutants was performed ranging from one to three weeks of age. Two mutants were assessed at one week of age and each was found to have moderate rhinitis but a healthy thymus. However, two-week-old mutants had developed acute rhinitis and an atrophic thymus and two other mutants had hydrocephalus and severe acute rhinitis; age-matched controls had none of these conditions. One mutant at three weeks of age had severe hydrocephalus and rhinitis. Histological sections were prepared from a two week old mutant's middle ear to evaluate for otitis media and infections were not detected.

Discussion

Hydrocephalus with rhinitis (*hyrh*) mutants get rhinitis as early as one week after birth. Their health continues to decline as hydrocephalus develops and rhinitis worsens.

Acknowledgements

We wish to thank Ezra O'Connor for discovering the mice carrying the new *hyrh* mutation; Coleen Kane for preparation of tissues for histological assessment; Rod Bronson, for pathological evaluation, Victoria DeMambro for mapping the mutation, Pat Ward-Bailey for web posting and we thank Lance Lee, Ph.D. of the Sanford School of Medicine of the University of South Dakota for confirming the mutation through sequencing.

Protocols

[1]Standard Mapping Protocol used in The Mouse Mutant Resource

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.

References

Manley KF (1993) A MacIntosh program for storage and analysis of experimental mapping data. Mamm Genome 4: 303-313.

Manly KF, Cudmore RH Jr, and Meer JM (2001) Map Manager QTX, cross-platform software for genetic mapping. Mamm Genome12: 930-932.

Taylor BA, Navin A, and Phillips SJ (1994) PCR-amplification of simple sequence repeat variants from pooled DNA samples for rapidly mapping new mutations of the mouse. Genomics 21: 626-32.

Truett GE, Heeger P, Mynatt RL, Truett AA, Walker JA, and Warman ML(2000) Preparation of PCR-quality mouse genomic DNA with hot sodium hydroxide and Tris (HotSHOT). Biotechniques 29:52-54

[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.