A spontaneous mutation in thyroid stimulating hormone receptor that allows partial fertility in both females and males

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Mutation (allele) symbol: $Tsh^{hyt\text{-}3J}$

Mutation (allele) name: hypothyroid 3 Jackson

Gene symbol: *Tshr*

Strain of origin: CXB10/HiAJ-*smrl*/GrsrJ

Current strain name: CByJ;CXB10- $Tsh^{hyt\text{-}3J}$/GrsrJ

Stock #017010 (jaxmice.jax.org)

Phenotype categories: metabolism, growth/size, hearing, homeostasis

Abstract
We have identified a spontaneous mutation in thyroid stimulating hormone receptor (*Tshr*) that causes diminished body size, increased pre-wean death, and hearing loss. There is decreased fertility, but when backcrossed two generations to BALB/cByJ both male and female homozygotes have successfully bred without hormone supplements.

Origin and Description
A spontaneous mutation that causes significantly diminished body size was identified in the strain CXB10/HiAJ-*smrl*/GrsrJ. These mutants are approximately half the size of their normal littermates and are proportionate dwarfs. They are particularly fragile at approximately three weeks of age and many die at that time. If they survive through wean age they usually survive to adulthood and live a relatively normal lifespan, although there is some premature death in the adult population of mutants. This strain is maintained on a standard rodent 4% chow in a standard mouseroom.

To determine the mode of inheritance a mutant was outcrossed to 129S1/SvImJ, which produced no affected mice in the F1 generation (0 affected of 12 progeny born), proving this mutation recessive. These unaffected F1 hybrid mice were intercrossed and produced a mix of mutant and unaffected F2 offspring: 16 mutant offspring out of 122 total progeny (13.1%) with nine (7.3%) missing prior to wean age. This is lower than the expected 25% mutants, indicative of some embryonic and pre-wean lethality. On the original CXB10 background, surviving female homozygotes often breed, but to date only one homozygous male has bred. Female mutants are fair breeders and may not live a normal lifespan, though some do. The average litter size when intercrossing

heterozygotes on the CXB10 background is 5.05 pups per litter, with 22 affected pups out of 111 pups for 19.8% homozygotes.

Efforts to breed this mutation onto a BALB/cByJ strain background are currently in progress (N2). Both female and male homozygotes have successfully bred with BALB/cByJ and have producing near normal size litters, although homozygotes have produced fewer litters per breeder than normal and approximately half of the small number of homozygotes set up to breed have failed to do so. Heterozygous intercrosses have produced 7.37 pups per litter (118 pups in 16 litters) with 20 mutants in these 118 pups (16.9%).

Genetic Analysis

To map this mutation SNP analysis was done on an F2 population from an outcross to 129S1/SvImJ. This mutation mapped to Chromosome 12 between 80,909,435 bp and 120,327,443 bp (MGSCv37/mm9), an interval that includes the candidate gene thyroid stimulating hormone receptor (*Tshr*).

Whole exome sequencing and single nucleotide variant (SNV) analysis detected a cluster of 4 SNVs with allele frequencies of 0.3-0.63 on chromosome 12 in the final exon of thyroid stimulating hormone receptor (*Tshr*), in a homozygous sample. This lower allele frequency SNP cluster and the similarities in phenotype between affected animals and previously published Tshr mutant alleles, lead to the manual examination of the exome data alignment (BAM) file. This revealed an actual homozygous deletion in the final exon of Tshr, which was subsequently validated by PCR using primers to span the predicted deletion: *Tshr* forward (CGTGGTGTGGTTTGTCAGTC) and *Tshr* reverse (AGCAGCAGAACGAGGACAAT). Sanger sequencing analysis of additional homozygous mutant, heterozygous, and wild type samples confirmed the presence of this intragenic deletion. This 232-nucleotide deletion is predicted to result in a frameshift, which, if translated would introduce 11 novel amino acids, beginning at amino acid 442, prior to a premature stop codon (S442Mfs*10). Since the deletion occurs at the distal end of the transcript, it is possible that mutant transcript would be successfully transcribed, and if so, would result in an altered rhodopsin-like, g-protein-coupled receptor domain at the N-terminus. This is the third spontaneous mutation in the Tshr gene to arise at The Jackson Laboratory and has therefore been designated hypothyroid 3 Jackson, $Tshr^{hyt\text{-}3J}$.

Pathology

A routine pathological screen[a] of a 27-week-old homozygous female and control, a 27-week-old homozygous male and control, and a 28-week-old homozygous male and control revealed in the female homozygote a small area of new bone formation in the hard palate as might result from a healing fracture, in the 27-week-old male homozygote a tooth root abscess, and in the 28-week-old male a lost molar, gum disease, bladder stones, and dilated and somewhat atrophic testicular tubules. The three controls were normal except for otitis media in one. Eye examinations of two homozygotes and two controls at four and a half months of age were all normal. ABR testing of two homozygotes at four and one half months of age showed elevated thresholds at all frequencies tested. ABR testing of two controls at four and a half months of age showed elevated thresholds at only 32kHz.

Discussion

The hypothyroid 3 Jackson mutation provides a new model for congenital nongoitrous hypothyroidism. The original hypothyroid (*Tshr*^{hyt}) mutation, which is a single point substitution that replaces proline at position 556 with leucine, causes reduced fertility, including that in homozygotes on a BALB/cByJ hybrid background. The targeted disruption *Tshr*^{tm1Rmar} on a segregating 129S1/Sv and C57BL/6J background has been reported to result in homozygotes that, if they survive weaning, fail to reproduce without supplemental hormone[1]. This *Tshr*$^{hyt\text{-}3J}$ truncation mutation causes the same diminution of size and hearing impairments already attributed to *Tshr* mutations, and reduced fertility in those that survive to adulthood, but does not make all homozygotes sterile, and fertility does appear to be improved by increasing the contribution of BALB/cByJ to the genetic background. Direct comparisons of the different alleles and the impact of genetic background on the phenotype is outside of the scope of our work, but would be valuable for understanding the comparative usefulness of each allele as a model for hypothyroidism.

Acknowledgements

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References

1) Marians RC et al., Proc Natl Acad Sci USA. 2002 Nov 26;99(24):15776-81.

Footnotes

a) 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.