Vaginal imperforation; a new mutation on Chromosome 13 causing a reproductive phenotype.

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Mutation (allele) symbol: *vgim*

Mutation (allele) name: vaginal imperforation

Gene symbol: updated to *Lhfpl2* in October 2011

Strain of origin: B6.129S4-*Ttpa*^{tm1Far}/J

Current strain name: B6.Cg-*Lhfpl2*^{vgim}/GrsrJ

Stock #013716 (jaxmice.jax.org)

Phenotype categories: reproductive

Origin and Description

The new recessive vaginal imperforation (*vgim*) mutation arose spontaneously in the B6.129S4-*Ttpa*^{tm1Far}/J mouse strain and was discovered by Sean Sullivan at The Jackson Laboratory. The mutation appears to cause a complete closure of the vagina and the phenotype can be observed when the affected mice are 4-5 weeks of age. The *vgim* adult females exhibit a soft, reducible swelling of the perineum, caused by the absence of a normal vaginal orifice. Their uteri exhibit a build up of a viscous fluid. *vgim* homozygous females can live to adulthood, but are not able to produce offspring.

Other examples of imperforate vagina in mice have been recorded in the literature (See references below) however this mutation has been mapped. *vgim* heterozygous mice have normal life spans and breed normally. The B6.Cg-*vgim*/GrsrJ colony is maintained by ovarian transplantation. The colony has been backcrossed for six generations with C57BL/6J. Recently, the *vgim* colony was genotyped to confirm that the *Ttpa*^{tm1Far} mutation has been bred out, leaving only the *vgim* mutation.

Genetic Analysis

The new *vgim* mutation has recessive inheritance as shown by mating an ovarian-transplanted recipient female mouse to an unrelated male DBA/2J mouse. This mating produced unaffected F1 progeny proving the new mutation to be recessive. The unaffected F1 hybrids were intercrossed, and 52 affected mice were generated for linkage analysis.

Using our standard mapping protocols the *vgim* mutation was mapped to Chromosome 13, between *D13Mit126* (NCBI 37 position 85.4Mb) and *D13Mit145* (NCBI 37 position 96.5Mb). Between those two flanking markers, one recombinant was detected with

D13Mit193 (91.9Mb) and *D13Mit106* (93.5Mb). (100 meioses tested).

Pathology

A routine pathological screen[1] of two homozygous mice at 19 weeks of age showed that the mutants have dilated vaginas and cervices. One homozygote showed abnormal eyes with a thin inner nuclear layer. The other homozygote showed one area of an eye with a thin inner nuclear layer, and also had a cystic uterus. A homozygote at 12 weeks age had normal pituitary glands with no lumps or other abnormalities. The abnormal uteri cannot be attributed to an obvious endocrine problem caused by a pituitary abnormality.

Hearing as assessed by auditory-evoked brain stem response testing of two homozygous mice and one control at 3 months of age showed elevated thresholds in the mutant mice suggesting some hearing impairment, while the control has normal hearing.

The eyes of two homozygous mice and one control mouse at 17 weeks of age were examined by electroretinograph (ERG). The mutant mice showed corneal hazing and protruding eyes with lots of pupil movement. One homozygote showed normal retinas, but the other homozygote showed a normal retina in the left eye, but very thin retinal vessels in the right eye and vascularization in the periphery. The control mouse showed normal eyes.

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Addendum

This mutation was identified as a G to A transition at Chromosome 13: 94944483, predicted to cause a glycine to glutamic acid substitution at amino acid 102. Please see Fairfield et al., 2011 and data in MGI reference J:219039.

References

Eisen EJ *et al.,* A Recessive Mutation Causing Imperforate Vagina in Mice. J Hered 1989 Nov-Dec; 80(6): 478-82.

Ginty, I and Hoogstraten-Miller S, Perineal swelling in a mouse. Lab Anim (NY) 2008, May; 37(5): 196-199.

Fairfield H et al., Mutation discovery in mice by whole exome sequencing. Genome Biol. 2011 Sep 14; 12(9) R86

Sundberg JP, Imperforate Vagina and Mucometra in Mice JAX®NOTES Issue 441, Spring 1990. (jaxmice.jax.org/jaxnotes/archive/441a.html)

[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.