Pendred syndrome model, *pdsm*, a spontaneous mouse mutation in the *Slc26a4* gene with associated inner ear abnormalities.

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Mutation (allele) symbol: *pdsm*

Mutation (allele) name: Pendred syndrome model

Gene symbol: *Slc26a4*

Strain of origin: BXA7/PgnJ

Current strain name:BXA7/PgnJ-*Slc26a4*^{pdsm}/J

Stock #006816 (jaxmice.jax.org)

Phenotype categories: neurological/behavioral: motor capabilities/coordination/ movement anomalies/deafness/head bobbing

Origin and Description

The recessively inherited spontaneous mouse mutation Pendred syndrome model (*pdsm*) occurred in the inbred BXA-7/PgnJ mouse strain. The mutant phenotype and inner ear pathology is very similar to that of the mouse strain previously described with a targeted disruption of *Slc26a4* (*Pds*) gene (Everett LA., et al 2001). *pdsm* mutants are identified at weaning age (3 weeks) by their overt head bobbing and circling behavior and occasional head tilt. The mouse colony is maintained by sister/brother mating of a heterozygous (*+/pdsm*) female with a homozygous (*pdsm/pdsm*) mutant male.

A routine pathological screen[1] performed on three mutants and two controls (15-37 weeks), detected inner ear abnormalities including reduced or absent otoconia, hair cells and spiral ganglion cells. Serial cross-sections of the inner ears from a *pdsm/pdsm* mutant and a *+/pdsm* control (12 weeks) mice confirmed these abnormalities as well as revealed a malformation of the tectorial membrane, degeneration of the organ of Corti and a displacement of Reissner's membrane enlarging the scala media (fig 1). Whole mount preparations of the inner ears showed a reduction of otoconia in the utriclar and saccular maculae (fig 2), and a variable but always reduced number of cochlear turns, or Mondini malformation, in *pdsm/pdsm* mutants (fig 3).

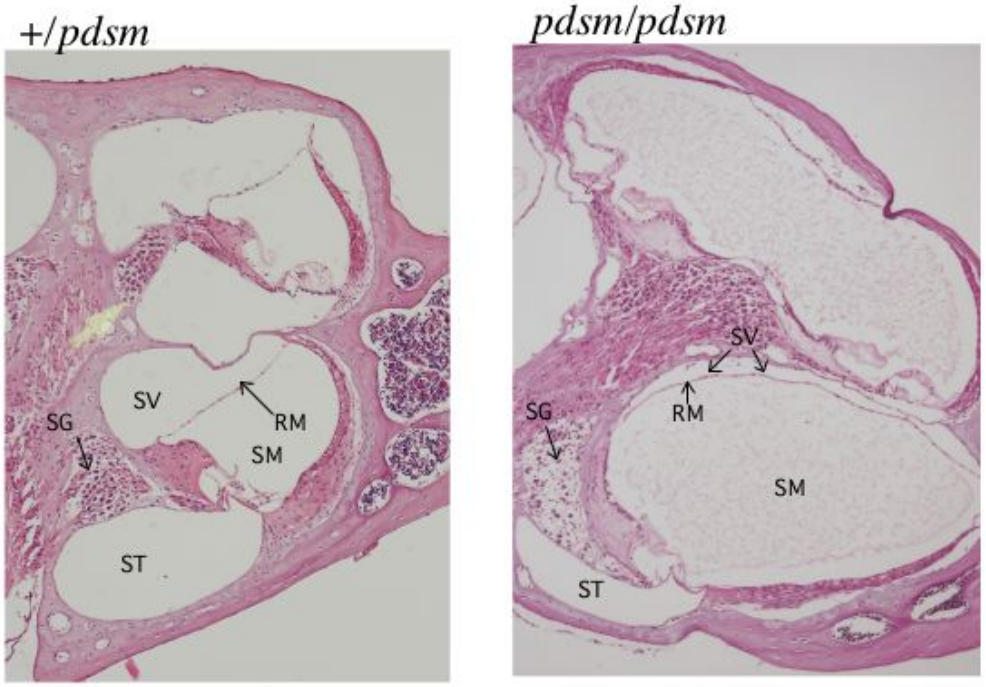

Fig 1. H&E stained cross-sections (4μm) of inner ears from a +/*pdsm* (left) and a *pdsm*/*pdsm* (right) adult. Arrows indicate the spiral ganglion (SG). Reissner's membrane (RM), scala tympani (ST), scala media (SM) and scala vestibule (SV).

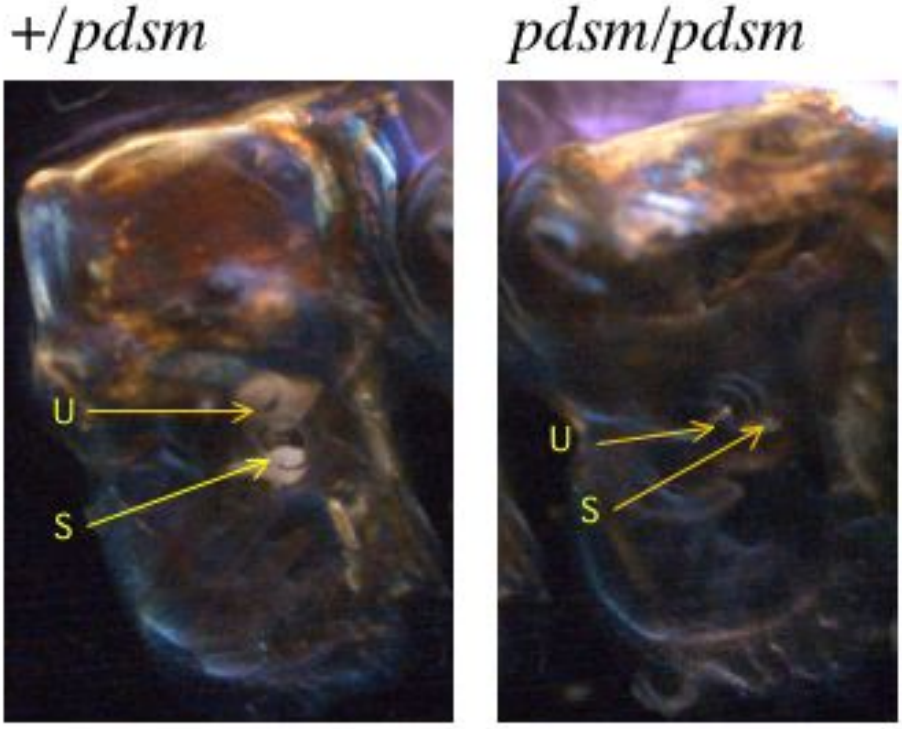

Fig 2. Whole mount preparation of inner ears from a +/pdsm control mouse (left) and a pdsm/pdsm mutant (right) viewed by polarized light microscopy. Arrows indicate the utricle (U) and saccule (S) within the vestibule. Note the reduction, but not absence of otoconia (shite structure) within the macula of mutant mice.

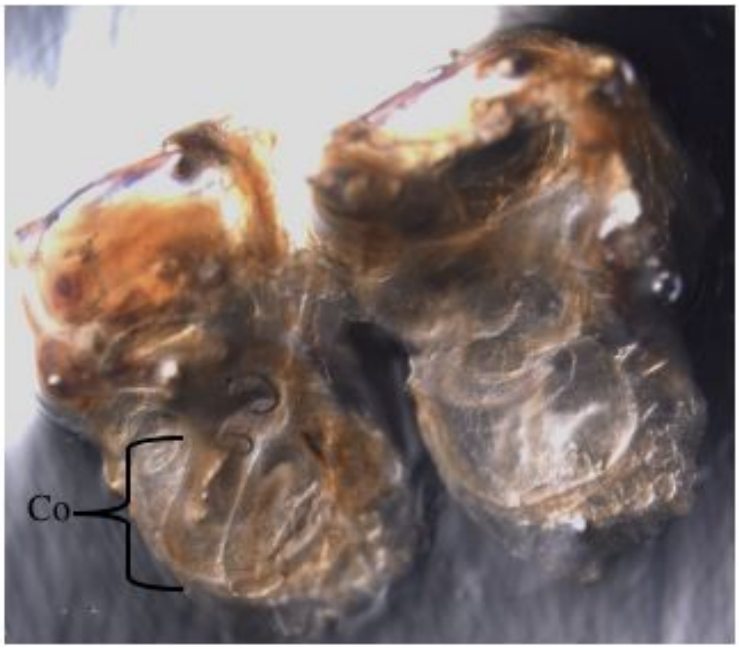

Fig 3. Whole mount preparation of inner ears from a +/*pdsm* control mouse (left) and a *pdsm*/*pdsm* mutant (right) viewed by non-polarized light microscopy. The bracket indicates the cochlea (Co). Note the shorter length and reduced number of turns, but increased endolymph volume in the mutant mouse cochlea.

Eight mutant mice and six controls were assessed for hearing by auditory brainstem response (ABR)[2] at 29 and 80 days of age. Homozygous mutants were completely deaf with no response to the loudest test stimuli (100dB SPL), while the control littermates retained good hearing.

Partial testicular atrophy was observed in the male mutants evaluated (n=2). Further evaluation of the testis and sperm from two additional mutants revealed that the total concentration and motility of sperm were greatly reduced. However, enough normal sperm must be produced because male fertility in the inbred colony is not significantly altered.

Since human Pendred syndrome patients commonly have thyromegaly due to a lack of sufficient thyroid hormone, the *pdsm* mice were evaluated for T4 (μg/dl) levels via blood serum chemistry analysis. *pdsm* mice did not display any signs of hypothyroidism as determined from either the blood serum chemistry (n=22) or histological analysis (n=5) of the thyroid gland at any age examined (4-53 weeks).

Genetic Analysis

An intercross was performed with CAST/EiJ mice and 48 mutant F2 animals were analyzed. Using our standard mapping practice, the mutation was mapped to a region of chromosome 12 between markers *D12Mit136* (30.7 Mb) and *D12mit154* (39.6 Mb). Included in this region is the gene *Slc26a4 (*32.1 Mb, position based on NCBI build 36), already identified as the gene responsible for Pendred syndrome in humans (Everett, et al., 1997). This gene was considered a likely candidate because a targeted disruption of *Slc26a4* in mice causes a similar phenotype (Everett et al., 2001).

In order to evaluate the new mutation at the DNA level, PCR primers were designed to amplify each exon of the *Slc26a4* gene, and PCR products were sequenced at The Jackson Laboratory core sequencing facility. The entire coding sequence, except for a small GC-rich region of exon 2, was then compared to the mouse sequence publicly available. A single base change from T>A was detected in exon 7 in mutant mouse DNA. This base change modifies the amino acid codon from TGT (cysteine) to TGA, a premature stop codon (fig 4).

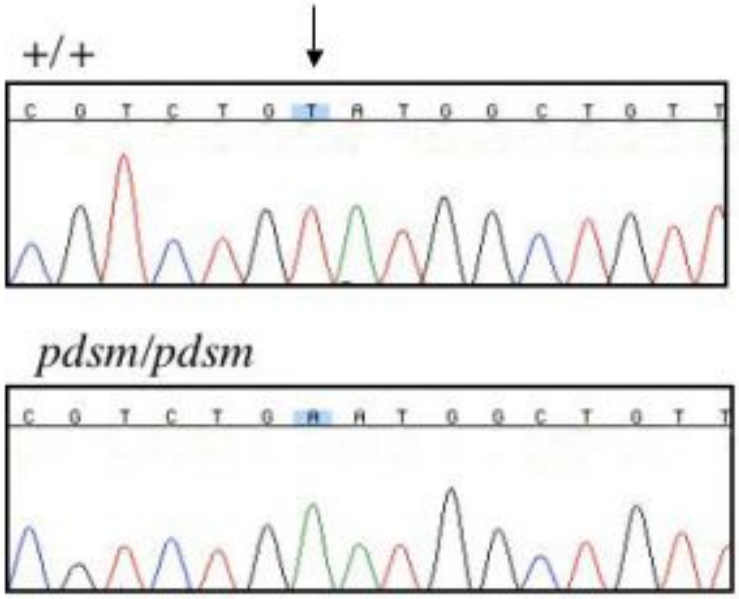

Fig 4. DNA sequence chromatogram from control (top) and *pdsm*/*pdsm* mutant mouse DNA. The blue highlighted base indicated the single base change mutation from T>A altering the amino acid codon from TGT (cysteine) to TGA (stop).

Acknowledgements
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References

Everett LA, Glaser B, Beck JC, Idol JR, Buchs A, Heyman M, Adawi F, Hazani E, Nassir E, Baxevanis AD, Sheffield VC,Green ED. Pendred syndrome is caused by mutations in a putative sulphate transporter gene (PDS). Nat Genet. 1997 Dec;17(4):411-22

Everett LA; Belyantseva IA; Noben-Trauth K; Cantos R; Chen A; Thakkar SI; Hoogstraten-Miller SL; Kachar B; Wu DK; Green ED, Targeted disruption of mouse Pds provides insight about the inner-ear defects encountered in Pendred syndrome. *Hum Mol Genet* 2001 Jan 15;10(2):153-6

[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.

[2]**ABR thresholds** in mice are determined using a semi-automated computer system (Intelligent Hearing Systems, Miami, Florida). Subdermal needle electrodes are inserted at the vertex and ventrolaterally to both ears of anesthetized mice. Specific auditory stimuli from 10-100 dB SPL are delivered binaurally through plastic tubes from high frequency transducers. ABR thresholds are obtained, in an acoustic chamber, for clicks and for 8, 16, and 32 kHz pure-tone pips.