A spontaneous deletion of the *Pou3f4* gene causing inner ear abnormalities.

Leona H Gagnon and Kenneth R Johnson

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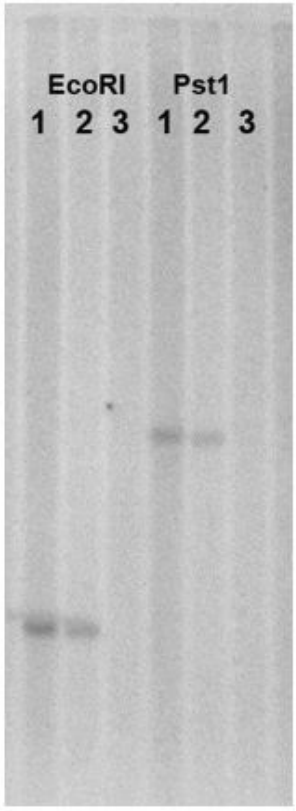
Mutation (allele) symbol: $Pou3f4^{del\text{-}J}$
Mutation (allele) name: *Pou3f4* deletion Jackson
Gene symbol: *Pou3f4*
Strain of origin: C3HeB/FeJ
Current strain name: C3HeB/FeJ-$Pou3f4^{del\text{-}J}$/J
Stock #004406 (jaxmice.jax.org)
Phenotype categories: behavior/neurological/hearing/vestibular/ear phenotype

Origin and Description
The *Pou3f4* deletion-Jackson (*del-J)* mutation arose spontaneously at the Jackson Laboratory in the C3HeB/FeJ inbred mouse strain. Mutant mice are identified by three weeks of age by head shaking and circling behavior. $Pou3f4^{del\text{-}J}$ is a recessive, sex-linked mutation. Both hemizygous mutant males and homozygous mutant females have profound hearing loss, while heterozygous females and wild type controls retain good hearing.

Genetic Analysis
A high-resolution genetic map of the X Chromosome was produced by backcross linkage analysis to refine the location of the new sex-linked mutation now designated $Pou3f4^{del\text{-}J}$. C3HeB/FeJ homozygous mutant females were mated with wildtype CAST/EiJ males. Resulting F1 female hybrids (+/*del*) were then backcrossed to hemizygous C3HeB/FeJ mutant males (*del*/Y). DNA samples from 98 backcross progeny (N2) were typed by PCR with simple sequence length polymorphism (SSLP) primer pairs distributed across the mouse X Chromosome, and the *del* mutation was localized between *DXMit177* (102.0 Mb position, NCBI Build 36) and *DXMit66* (134.8Mb). This region includes the previously identified *Pou3f4* transcription factor gene, which is the mouse ortholog of the human *POU3F4* gene and underlies non-syndromic hearing disorder DFN3. The *Pou3f4* gene consists of a single 2.7 kb exon. The *del* mutation is a large deletion of approximately 100 kb that extends from 69.4 kb before the 5' end of *Pou3f4* to 30.2 kb beyond the 3' end of the gene. The extent of the deletion was estimated by testing for the presence or absence of PCR products in mutant DNA using multiple primer pairs corresponding to known positions in the genomic region surrounding the gene.
We performed Southern blot analysis to confirm deletion of the *Pou3f4* gene in mutant mice. A DNA probe was generated by PCR amplification of genomic DNA from C3HeB/FeJ wildtype (+/+) mice with primers that produce a 596 bp product specific to the coding region of *Pou3f4*: S-probe-F (GACCAGCAAGACGTGAAGC) and S-probe-R (TGAGCAGCGATCTTGTCAAT). The PCR amplified fragment was extracted from an agarose gel, purified with the QIAquick Gel Extraction kit (Qiagen, Inc., Valencia, CA #28604), and labeled with 32P using the Amersham Rediprime II Random Prime

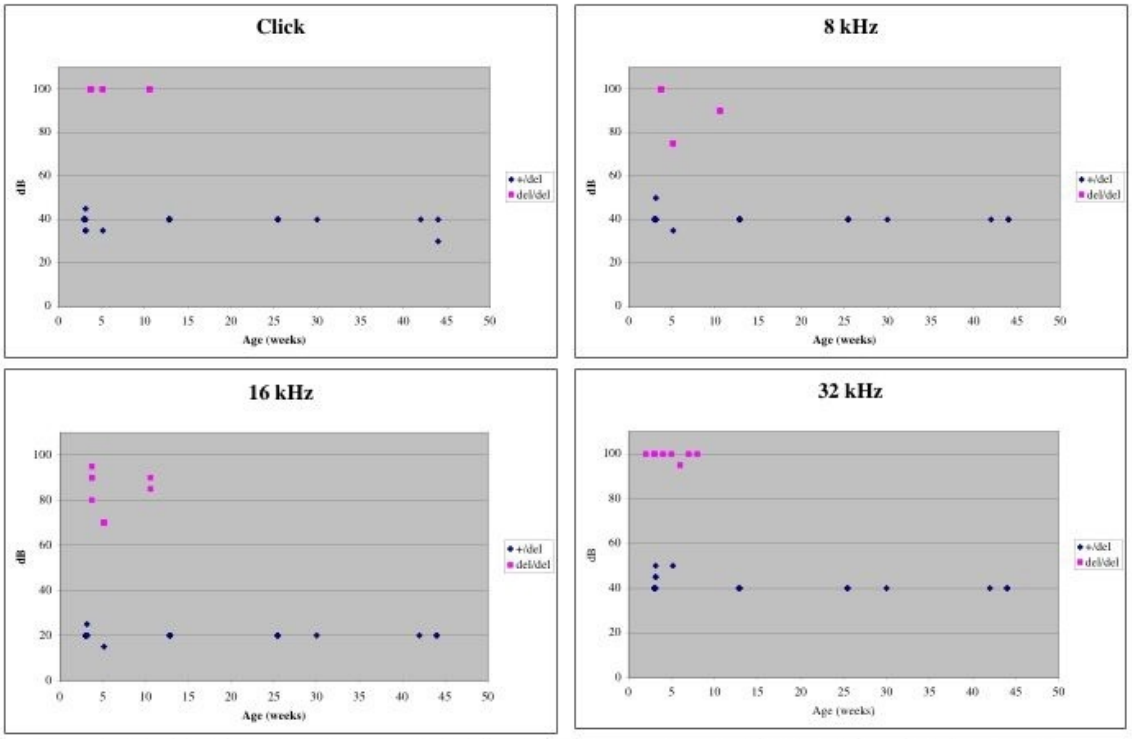
Labeling System (GE Healthcare Bio-Sciences Corp., Piscataway, NJ). The *Pou3f4*-specific probe failed to hybridize to restriction fragments from *del*/*del* DNA while hybridizing successfully to +/del and +/+ DNA, thus confirming deletion of the *Pou3f4* gene in mutant mice.



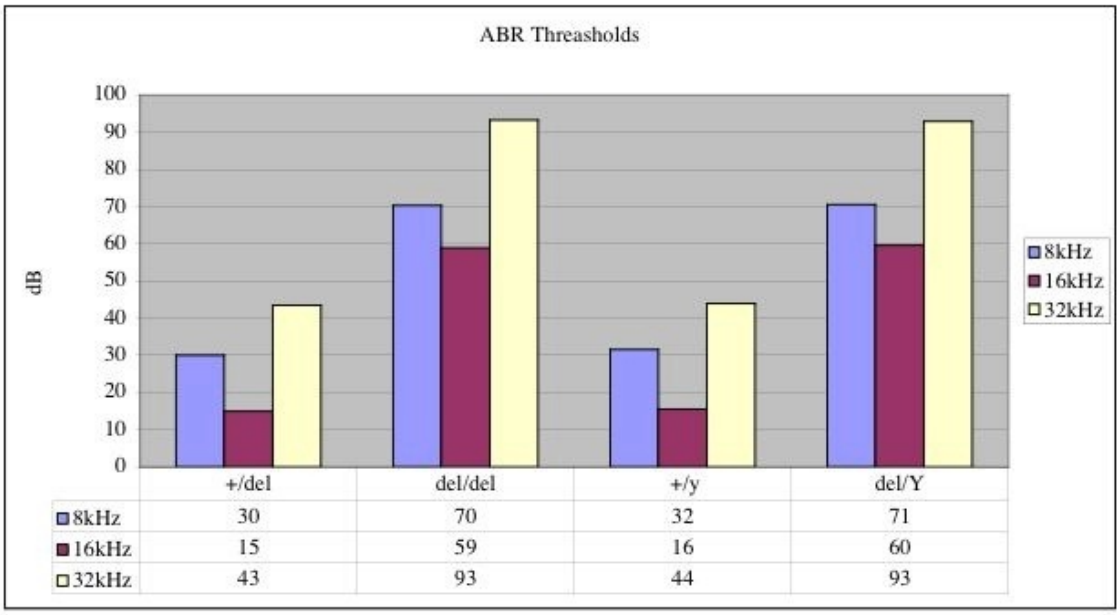
Southern blot analysis. Restriction fragments from *del*/*del* (lane 3) mice are absent as compared to +/+ (lane 1) or +/*del* (lane 2) when genomic DNA is probed with the *Pou3f4* gene coding region.

Pathology

Four mutant mice and two controls from the inbred colony were assessed for hearing by auditory brainstem response (ABR)[1]. All mutants (36-74 days old) were completely deaf with no response to the highest ABR stimulus presented (115 dB), while the controls (one +/*del* female and one +/Y male) retained good hearing. We found no evidence of late-onset hearing loss in heterozygous females as previously described. In fact, 12 obligate female carriers of the del mutation (+/*del)* were assessed by ABR at ages ranging from 3 to 44 months of age, and they retained good hearing, comparable to the inbred control strain C3HeB/FeJ. The C3HeB/FeJ strain is resistant to age-related hearing loss and thus our analysis was not confounded by strain background effects, which could possibly account for the results reported by Xia et al. (2002) who analyzed the mutation on a mixed 129/Sv-C57BL/6 strain background. Our analysis of linkage cross mice also showed that heterozygous females (+/*del*) retained normal hearing equivalent to wildtype males (+/Y) at the 6-8 week test age. Although most mutant females (del/del) and mutant males (del/Y) from the linkage cross lacked observable vestibular dysfunction, the hearing impairment was fully penetrant. The mean ABR thresholds of *del*/*del* and *del*/Y mutant mice were 40-50 dB higher than the mean thresholds of +/*del* and +/Y mice.



ABR thresholds of mutant (*del/del*) and obligate heterozygous control *(+/del)* female mice. Note that *+/del* females aged to 44 weeks show no elevation of hearing threshold as compared to younger mice.

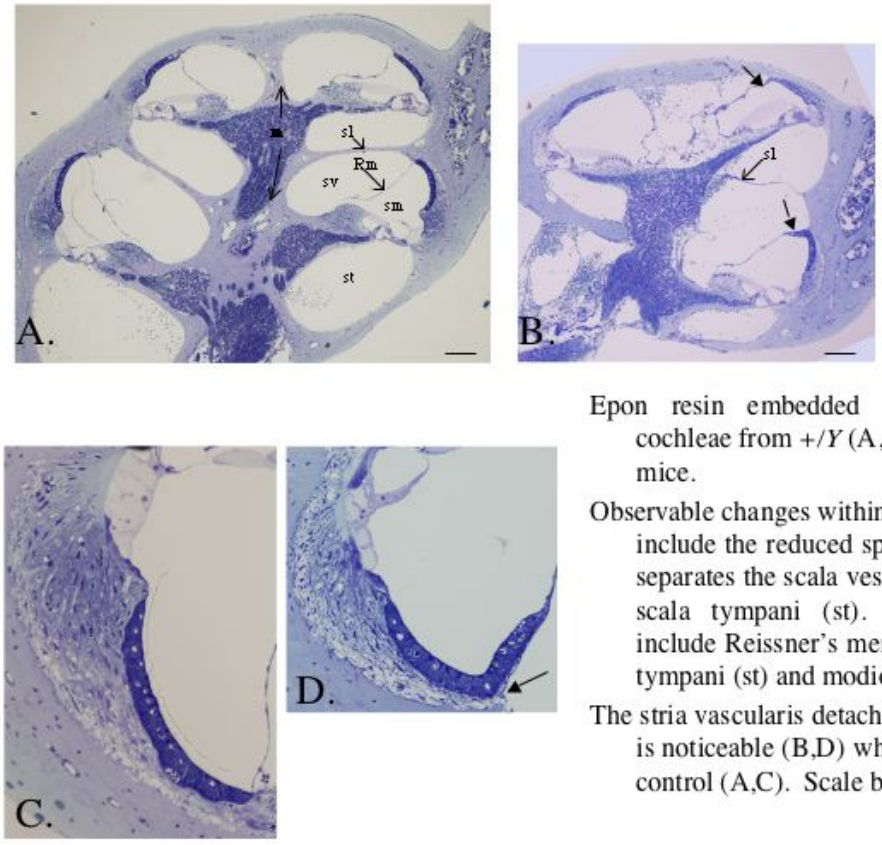


	+/del	del/del	+/y	del/Y
8kHz	30	70	32	71
16kHz	15	59	16	60
32kHz	43	93	44	93

The mean of ABR thresholds of N2 linkage progeny. All progeny were assessed for hearing loss (N=16) +/del; 27 del/del; 28 +/Y and 26 del/Y) between 6-8 weeks of age

A routine pathological screen failed to show any abnormalities in organs from a *del*/Y mouse with the exception of the inner ear. A clinical eye exam had normal results. We examined cross sections from two and five-month old mutant animals to determine if any

morphological changes in the inner ear could be observed. Vestibular structures within the inner ear appeared normal; however, within the cochlea numerous abnormalities were observed. To control for histological artifact and tearing of tissue, inner ears were taken from five *del*/Y and four +/Y control mice and embedded in Epon resin. Mice were anesthetized and perfused intracardially with 5 ml of PBS/0.1% sodium nitrite followed by 5 ml of 4% para-formaldehyde. Cochleae were dissected and then fixed and decalcified in 10% formalin /0.14 M EDTA for 4 -5 d with a daily change of solution. The tissues were then treated with osmium tetroxide, dehydrated in graded alcohols, equilibrated in propylene oxide, and embedded in Epon (Electron Microscopy Sciences, Fort Washington, PA). The embedded cochleae were sectioned at 2 um and stained with 1% toluidine blue. The resulting histological preparations confirmed previously observed pathology reported by others, including an overall hypoplasia of the cochlea, a reduced number of cochlear turns, a failure to fully form the bony structure of the modiolus (especially apparent at the apex), and a consistent detachment of the stria vascualris (particularly in the base and mid portions of the cochlea).



Epon resin embedded cross sections of cochleae from +/*Y* (A, C) and *del*/*Y* (B,D) mice.

Observable changes within the cochlea (A, B) include the reduced spiral lamina (sl) that separates the scala vestibuli (sm) from the scala tympani (st). Other structures include Reissner's membrane (Rm), scala tympani (st) and modiolus (m).

The stria vascularis detachment (solid arrows) is noticeable (B,D) when compared to the control (A,C). Scale bars are 100um.

Acknowledgements

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References

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model of DFN3 nonsyndromic deafness [see comments]. Science 285:1408-1411.

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1 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.