An ear pinna mutation in *Fgfr1*

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Mutation (allele) symbol: *Eask*

Mutation (allele) name: ear askew

Strain of origin: BALB/cByJ-*Agtpbp*$^{pcd\text{-}3J}$/J

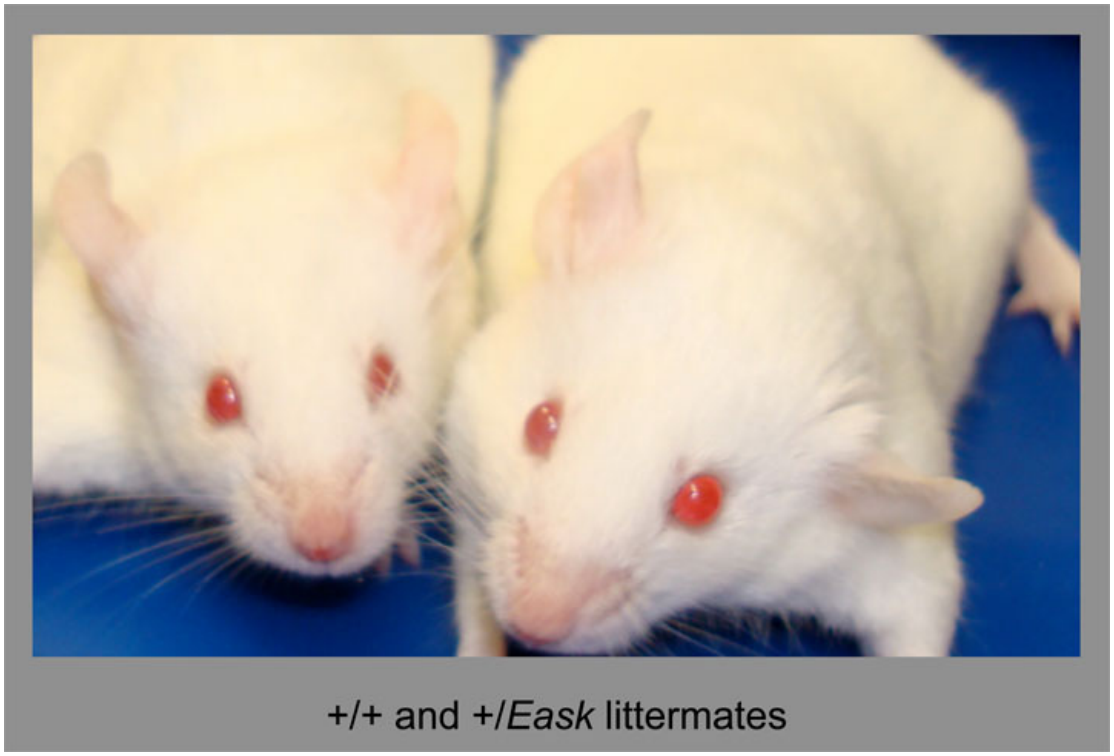
Current strain name: BALB/cByJ-*Fgfr1*^{Eask}/GrsrJ

Stock #005412 (jaxmice.jax.org)

Phenotype categories: craniofacial, hearing

Origin and Description

The ear askew mutation (*Eask*) was found in the BALB/cByJ-*Agtpbp*$^{pcd\text{-}3J}$/J colony at The Jackson Laboratory. The *Agtpbp*$^{pcd\text{-}3J}$ mutation was subsequently bred out of the *Eask* mutant subline without altering the ear askew phenotype. Mutants have low-set ear pinnae, which can be on the right or left side or both. There is a wide range of variability in the phenotype from a very low-set and malformed pinna to more subtle variations with position and shape only slightly affected. *Eask* mutants are viable and fertile. The colony is maintained by mating a heterozygote of either gender to a wild-type sibling.

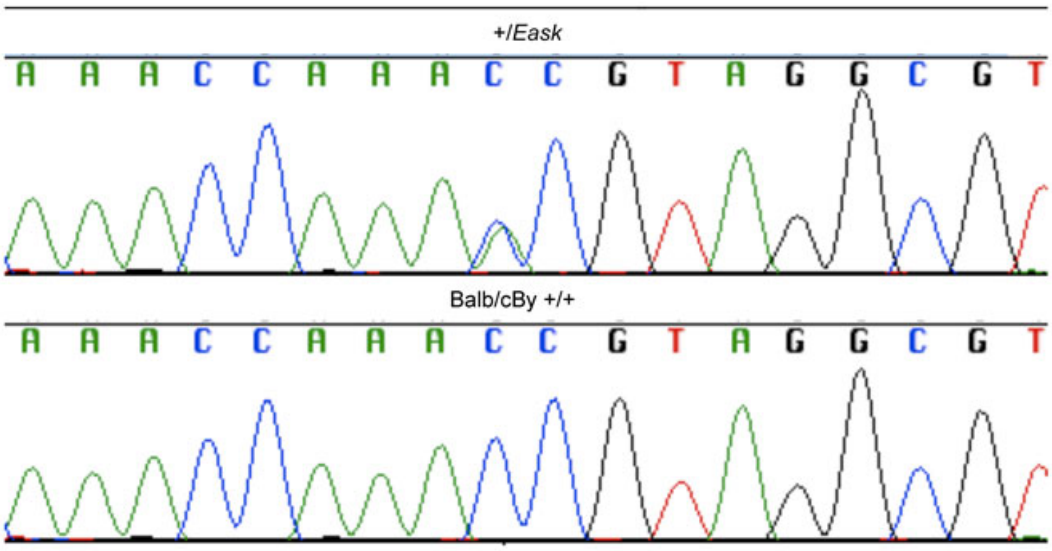


+/+ and +/*Eask* littermates

Genetic Analysis

Eask is inherited as an autosomal dominant mutation, proven by mating an affected mouse from the colony to an unrelated BALB/cByJ. Affected mice were observed in the F1 litter. For linkage analysis a mutant from the colony was mated to a C3HeB/FeJ and affected F1 offspring were mated to BALB/cByJ. Spleen and tail were collected from the resulting N2 offspring. DNA from 90 N2 animals (70 unaffected and 20 affected, as judged by visual phenotyping) was submitted to the Fine Mapping Laboratory at The Jackson Laboratory. The mutation was found to map to Chromosome 8 with flanking markers *D8Mit143* at 24.9 Mb and *D8Mit289* at 29.8 Mb. However 22 out of the 70 unaffected, or 37%, typed as mutants in the non-recombinant region indicating the mutation may have incomplete penetrance and/or that there are cases where apparently normal mice are actually subtly affected mutants whose phenotype is difficult to observe visually. To examine penetrance, three pairs of the phenotypically unaffected N2 mice that typed as affected were mated and they all produced affected offspring, supporting the hypothesis that there are penetrance issues or varying expressivity of the phenotype.

Whole Exome Sequencing[1] revealed a mismatch allele in *Fgfr1* in exon 4, predicted to result in an amino acid change of asparagine to lysine in the 147th amino acid. Using the Primer3 design program (Koressaar T, Remm M(2007) *Bioinformatics* 23(10):1289-91), we confirmed the mismatch with 12 *Eask*/+ mice sequencing as heterozygotes at this locus and 23 BALB/cByJ as wild types that match the published Ensembl sequence.



Whole exome sequencing revealed a mismatch allele on chromosome 8 at 26,668,285 C-->A. This is predicted to result in an amino acid change of asparagine to lysine in the 147th amino acid of *Fgfr1*.

Embryos from *Eask*/+ by *Eask*/+ matings were genotyped at embryonic day (E) 16.5 and E 9.5. Although not enough embryos were sequenced to confirm that homozygotes die

around gastrulation (as is reported in other *Fgfr1* mutants), our findings indicate that there is early lethality in homozygotes, as seven embryos typed as heterozygous, six typed as wild-type and zero typed as homozygotes.

Pathology

Routine pathological screening[2] as done on two nine-week-old *Eask*/+ female mice. The results were normal except for a little inflammation in the ears. Mice were also checked at 19 weeks of age with one female *Eask*/+ and one female +/+ both having mild hydrocephalus.

An ophthalmoscope was used to view the eyes of 2 heterozygotes and 4 wild-type controls that were all three months old (eyemutant.jax.org/screening.html). All eyes were normal.

Hearing was assessed by auditory brainstem response (ABR)[3]. The ABR results show that the threshold is slightly higher (some hearing loss) for the six *Eask*/+ mice than the six controls that were tested. Older mice at six months of age were also tested and the mutants showed severe hearing loss while controls had mild hearing loss.

Discussion

Eask mutants have misplaced and deformed ear pinnae that can affect the left or right side, or both. Mutants also have hearing loss. The *Eask* mutation may have incomplete penetrance and/or a subtle phenotype in some cases.

Acknowledgements

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Belinda Harris: Discovery of the mutation
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Protocols

1. Exome Sequencing Protocol

The exome sequencing data referred to in this website were analyzed using tools and workflows provided by Genome Quest including processes for mapping (HS3), SNP calling and annotation of variants. Our analysis focused on novel variants, which were not positioned in repetitive sequence, had expected allele ratios (>0.95 for homozygous variants and >0.2 for heterozygous variants), and displayed sufficient locus coverage (at least 5X for homozygous variants and 10X for heterozygous variants) for effective mutation discovery. High priority was given to protein coding or splice variants within mapped regions, as well as unique variants that were not found in other exome data sets or in the Sanger Mouse Genomes Database. Following these analyses, re-sequencing of additional mutant and unaffected samples was performed to validate and determine the most likely causative mutation.

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.

3. Auditory-Evoked Brainstem Response (ABR) Thresholds

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. ABR thresholds of all mice and strains tested are entered in spreadsheet files for storage, easy access, and for the production of periodic progress reports. Click-evoked ABR waveforms, obtained at threshold (T) and at T+10, T+20 and T+30 dB or each mouse, are also stored for future reference. Mice of the CBA/CaJ strain are tested periodically as references for normal hearing, and for monitoring the reliability of the equipment and testing procedures.