Two spontaneous mouse mutations in the *Ap3b2* gene

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Source of Support: This research was supported by grants RR001183 and 0D010972 to the Mouse Mutant Resource (Leah Rae Donahue, PI), OD011163 to Models of Neurological Disease (Laura Reinholdt, PI) and by the Cancer Center Core Grant CA34196 awarded to The Jackson Laboratory

Mutation (allele) symbols: *Ap3b2*^{m1J} & *Ap3b2*^{m2J}

Gene symbol: *Ap3b2*

Strain Names:
C57BL/6J-*Ap3b2*^{m1J}/FrkJ Stock #017796
B6;129S6-*Ap3b2*^{m2J}/GrsrJ Stock #008113

Phenotype categories: Neurological

Ap3b2^{m1J} Origin and Description:

A recessive spontaneous mutation was identified in a C57BL/6J colony in the laboratory of Dr. Wayne Frankel at The Jackson Laboratory. Homozygotes display hyperactivity, a slight vibration or shakiness, and have tonic-clonic seizures beginning at two to three months of age. Heterozygotes have no obvious phenotype and do not usually have seizures. This mutation was cloned through exome sequencing and found to be a 2 base pair deletion at Chromosome 7 base pair 88,618,348 - 9, which is a GT deletion in exon 15 of adaptor-related protein complex 3, beta 2 subunit (*Ap3b2*). This is predicted to cause a frameshift and stop codon at amino acid 572 of this 1082 residue protein. Western blot analysis failed to detect AP3B2 protein in samples from homozygotes and detected approximately half the normal level of AP3B2 protein in samples from heterozygotes, proving this a null allele.

Ap3b2^{m2J} Origin and Description:

A recessive spontaneous mutation was identified in a mouse from strain B6;129S6-*Epha2*^{tm1Jrui}/J (stock #006028) at The Jackson Laboratory. Homozygotes have tonic-clonic seizures by eight to ten weeks of age. The colony was backcrossed once to C57BL/6J, the *Epha2*^{tm1Jrui} mutation was subsequently bred out, and the seizure phenotype persisted unchanged. Both female and male homozygotes breed and have a normal life span.

A routine pathological screen[1] of one female at the age of 12 weeks, two females at 16 weeks and one male at 29 weeks revealed only a dilated bladder in the 16-week-old female and no other significant lesions. The brain, spinal cord, nerves and muscles all appeared normal by histology. The eyes of three mutants at eleven weeks of age were examined by ophthalmoscopy and found to be normal. The eyes of one mutant at eleven

weeks of age were tested by electroretinography and found to be normal. Hearing, assessed by auditory-evoked brainstem response testing[2] (ABR), of two mutants at approximately three months of age showed a slightly elevated threshold at 32kHz, but normal thresholds at all other frequencies.

To determine the chromosomal location of the mutation, homozygous mutants were mated to DBA/2J. The F1 progeny were sibling intercrossed and DNA was isolated from affected F2 offspring for use in linkage analysis. This mutation mapped distal to *D7Mit201* (74.3 Mb) and proximal to *D7Mit301* (91.5 Mb). Exome sequencing was used to identify candidate mutations in the mapped region. Analysis indicated a single nucleotide variant of G to A at Chromosome 7: 88,618,348 (MGSCv37), which is in exon 12 of *Ap3b2*. Primers were generated that produce a 246 base pair product spanning the predicted mutation: *Ap3b2* forward (GGACACTTCTCCAGGCAAAC) and *Ap3b2* reverse (TGGGGGTGTTACAGCCTTAG). Sanger sequence analysis of additional genomic DNA samples from homozygotes, heterozygotes, and wild-type controls indicated that this variant allele segregated with the phenotype. This single nucleotide variant is predicted to result in the nonsense mutation R435*, so is also predicted to be a null allele.

Acknowledgements:
We thank Michelle Crispo for discovering the *Ap3b2*^{m2J} mutant, Roderick Bronson and Coleen Kane for pathological screening, Bo Chang for eye examinations and Chantal Longo-Guess for ABR testing.

[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] 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.