Mocha 4 Jackson, an 8 base pair deletion in *Ap3d1*

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Mutation (allele) symbol: *Ap3d1*$^{mh\text{-}4J}$

Mutation (allele) name: mocha 4 Jackson

Gene symbol: *Ap3d1*

Strain of origin: (B6;129P2-*shsn*/J x BALB/cByJ)F2

Current strain name: STOCK *Ap3d1*$^{mh\text{-}4J}$/GrsrJ

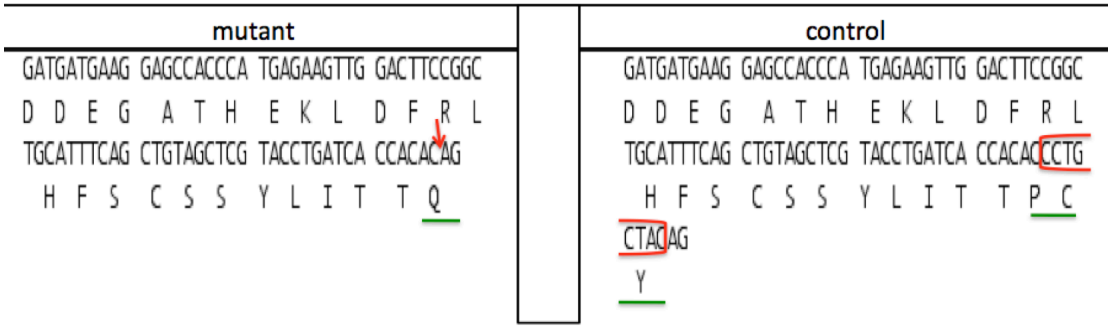
Stock #022088 (JaxMice.jax.org)

Origin and Description:
A spontaneous, recessive mutation was discovered at The Jackson Laboratory by Michelle Curtain in the F2 population of a mapping outcross of B6(129P2)-*shsn*/GrsrJ bred to BALB/cByJ. Homozygotes display a smaller body size, head tilt, constant quivering, gauntness and overall frailty. These homozygotes are noticeable by 2 weeks of age by their smaller body size compared with littermates. Because the strain background of this mutant subline is homozygous for albino, any variation in pigmentation that may have been caused by this mutation was not detected. Both female and male homozygotes live a normal life span, but male homozygotes have failed to breed and female homozygotes breed poorly. A routine pathological screen[1] of one male mutant at 15 weeks of age found no sperm in the epididymis. Hearing assessments by auditory-evoked brainstem response testing[2] (ABR) of two homozygotes at 3.5 weeks of age showed severe hearing loss in both.

Genetic Analysis:
Mutant animals were outcrossed to 129S1/SvlmJ mice to establish heritability. No affected mice were found in the F1 generation. Intercrossing of unaffected F1 animals resulted in affected F2 animals, indicating a recessive mode of inheritance. Affected F2 mice were generated for linkage analysis and fine mapping. Using standard SNP protocols, linkage analysis for this mutation was completed in the Fine Mapping Laboratory at The Jackson Laboratory. This mutation mapped to Chromosome 10, between MGSCv37 positions 67,317,776 bp and 92,128,703 bp.

Mapping data demonstrated linkage to Chromosome 10 and whole exome sequencing was used to identify candidate mutations in the mapped region. Briefly, genomic DNA was enriched for coding sequence by hybridization-based capture with probes representing 54 Mb of annotated coding sequence. The enriched DNA was then sequenced using the Illumina HiSeq high throughput sequencing platform. An eight-nucleotide deletion was found at position 80,171,948-80,171,955 on Chromosome 10 in adaptor-related protein

complex 3, delta 1 subunit (*Ap3d1*). Primers were generated that produce a 458 base pair product spanning the predicted mutation: *Ap3d1* Left (TCACCATTCAGAGCATCGTC) and *Ap3d1* Right (ACAGGCACACAGGGTAGTCC). Sequence analysis of genomic DNA from two mutants with that of two unaffected animals confirmed the eight-nucleotide deletion at Chr10: 80,171,948-80,171,955 (MGSCv37) in *Ap3d1*. This 8 base pair deletion is predicted to cause a frameshift beginning at amino acid position 1098.



A portion of the protein coding sequence of Ap3d1 with the control DNA sequence and its amino acid translation on the right, and the Ap3d1$^{mh\text{-}4J}$ mutant DNA sequence and its translation on the left. An arrow indicates the eight-nucleotide deletion in the mutant sequence and a red box in the control sequence presents those eight nucleotides. The mocha 4 Jackson mutation is predicted to cause a frameshift beginning at amino acid position 1098.

This is the fourth spontaneous mutation in the *Ap3d1* gene to occur at The Jackson Laboratory and it has been designated mocha 4 Jackson, *Ap3d1*$^{mh\text{-}4J}$. Although the pigment dilution has not been confirmed, the phenotypes of reduced fertility, head tilt, smaller body size, tremor, and diminished hearing are consistent with the phenotypic findings of the original mocha mutation. This mutation provides another *Ap3d1* mutant model for Hermansky-Pudlak Syndrome 2.

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[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.