Pearl 14 Jackson, a remutation of the *Ap3b1* gene

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Source of Support: The research was supported by NIH/NCRR grant RR01183 to the Mouse Mutant Resource (M.T. Davisson, PI) and Cancer Center Core Grant CA34196.

Mutation (allele) symbol: *Ap3b1*$^{pe\text{-}14J}$

Mutation (allele) name: pearl 14 Jackson

Gene symbol: *Ap3b1*

Strain of origin: B6;129S2-Sele^{tm1Hyn}/J

Current strain name: B6;129S2-Sele^{tm1Hyn}/J-*Ap3b1*$^{pe\text{-}14J}$/GrsrJ

Stock #005532 (**Note**: as of July 27, 2006 available as DNA only from the DNA Resource at the Jackson Laboratory).

Phenotype categories: Coat color

Origin and Description

The *Ap3b1*$^{pe\text{-}14J}$ remutation was discovered by Ralph Bernaquer in a production colony of B6,129-*Sele*^{tm1Hyn}/J mice in Annex 1 at The Jackson Laboratory on June 6, 2001. Mice homozygous for this spontaneous, recessive remutation are recognizable by a diluted gray coat color similar to, but slightly darker than the original pearl allele. The slight darkening of color is most noticeable in the tail and ears when compared to mice homozygous for pearl 11 Jackson (*pe-11J*). Both homozygous males and females breed and live a normal lifespan. The breeding levels of mice carrying the *Ap3b1*$^{pe\text{-}14J}$ mutation are below normal, but are comparable to the breeding levels of the B10.R111-H2^{+}H2T18^{b}/(7INS)Sn-*Ap3b1*$^{pe\text{-}11J}$/+ /J strain.

Genetic Analysis

In order to determine the mode of inheritance, a female, homozygous for this new mutation, was mated to an unrelated C56BL/6J male. No affected offspring were observed in the F1 generation produced from this mating. Mice from this F1 generation were then mated together to produce F2s and in this cross both affected and unaffected animals were produced showing that the mutation is recessive.

A direct test for allelism was set up by mating a female homozygous for the new mutation to a male homozygous for *Ap3b1*$^{pe\text{-}11J}$. This mating produced 2 litters in which all offspring were affected (13/13 animals), proving the new mutation to be an allele of *Ap3b1*^{pe}. The *Ap3b1*^{pe} gene is located at the 47 cM position on Mouse Chromosome 13 (MGD).

Pathology

A routine pathological screen[1] done on one homozygous and one heterozygous mouse showed no lesions. The results of auditory-evoked brainstem response[2] tests showed that a 4 week-old homozygous mutant mouse and a heterozygous littermate both had normal hearing. The eyes of one homozygous mutant showed severe pigment loss in the peripheral retina, both, when examined by an ophthalmoscope, and by histological examination. However, the results of a test on the same animal with an electroretinagram (ERG) were normal. The pigment loss in the retina is not unexpected, since *pe-14J* is a pigment loss mutation.

Acknowledgements

The authors wish to thank Ralph Bernaquer for the discovery of the mutant, Coleen Marden for her excellent technical assistance, and Babette Gwynn for providing the results of the direct test for allelism.

[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.