A new pale lethal mouse mutation (*pale*) has been identified on Chromosome 8

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Mutation (allele) symbol: $Ank1^{pale}$

Mutation (allele) name: pale lethal

Gene symbol: *Ank1*

Strain of origin: C57BL/6J

Current strain name: C57BL/6J-$Ank1^{pale}$/GrsrJ

Stock #009157 (http://jaxmice.jax.org)

Phenotype categories: lethal, color

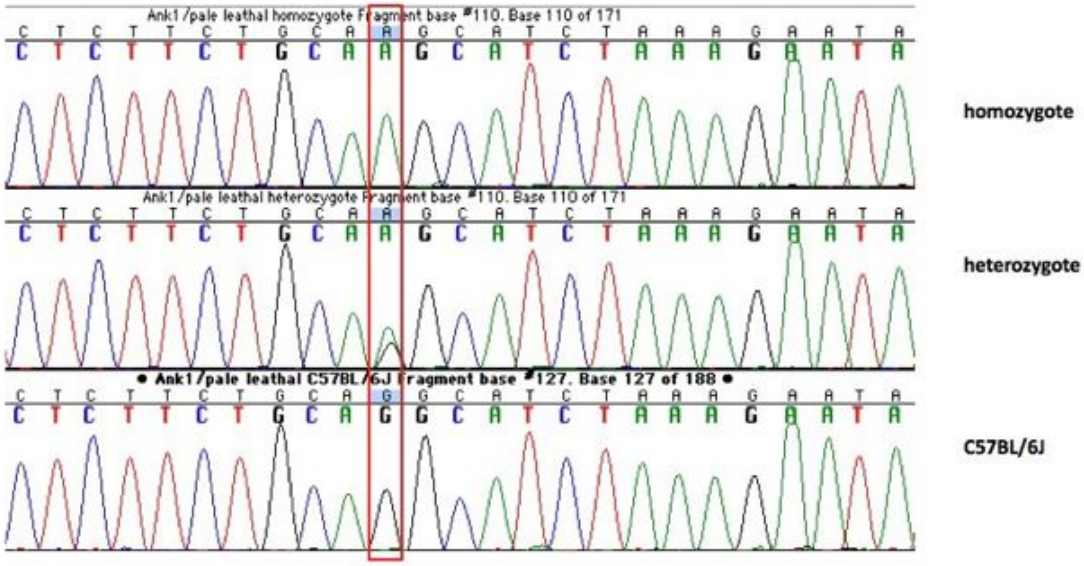
Note: This article was initially posted in late 2009 and was updated in May, 2011 to include the characterization of pale lethal as a mutation in *Ank1*.

Abstract
We have identified a new recessive mouse mutation that causes affected mice to have a pale, light gray skin color at birth and usually death typically by one week of age. Pale lethal (*pale*) was mapped using an intercross with CAST/Ei and was found to be on Chromosome 8. Sequence analysis subsequently showed this to be a point mutation of a splice acceptor site of an exon of erythroid ankyrin 1.

Origin and Description
The pale lethal mutation was discovered in 2006 by Lynda Clay in a colony of inbred C57BL/6J mice within the Mouse Mutant Resource at The Jackson Laboratory. It was observed that some newborn mice had a lighter skin color than their littermates and that the more lightly colored mice subsequently died shortly after birth. Occasionally, a mutant may live past seven days of age and one rare homozygote did live until almost four weeks of age. Those homozygotes dying earlier than one week of age have a pale grey skin color and are somewhat smaller than littermates. The rare ones that live longer than one week do develop the expected nonagouti hair color but remain smaller than littermates until they die. The pale lethal colony is maintained by progeny testing; less than the expected 25% homozygotes are produced in heterozygous matings.

Genetic Analysis
The pale lethal mutation was mapped using an intercross scheme. CAST/Ei females were crossed to a male heterozygote and the F1 progeny produced were progeny tested. Only proven carrier F1 pairs were used to establish the intercross. Eighteen F2

homozygous progeny were utilized for linkage analysis. The *pale* locus was mapped to Chromosome 8 using standard PCR protocols and was positioned between *D8Mit143* (NCBI 37 position 24.9 Mb) and *D8Mit223* (NCBI 37 position 27.8 Mb). Based on phenotype and map location, erythroid ankyrin 1 (*Ank1*) was considered a good candidate gene for this new mutation. Primers were chosen to amplify a 252 base pair product specific to the coding region of *Ank1*: *Ank1* Primer Left (ACTTTACAGGGGACCAG), and *Ank1* Primer Right (GGCTAATGTTGTCTGAGGTC). Sequence analysis of mutant genomic DNA compared with wild type C57BL/6J genomic DNA identified a single nucleotide transition from G to A at 24224235 base pair. This mutation disrupts the consensus AG dinucleotide of the splice acceptor of the adjacent exon.



Comparison of DNA sequence chromatograms of the homozygous *pale lethal* mutant (Top), heterozygote (in the middle) and strain origin C57BL/6J (bottom).

Pathology

Our standard pathology screen[1] of two homozygous males and two homozygous females at 3 days of age revealed no somatic lesions. One male homozygote that lived over three weeks of age had a very enlarged spleen, an abnormal amount of hematopoesis, and poorly developed lymphoid tissue.

Discussion

Pale lethal is a new spontaneous mutation in the erythroid akyrin 1 gene that causes homozygotes to die at a very young age with no apparent lesions but is easily recognizable by its grayish skin color after birth.

Acknowledgements

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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.