Pale ear 7 Jackson, a spontaneous mutation in *Hps1* arising on the CAST/EiJ background

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Mutation (allele) symbol: *Hps1*$^{ep\text{-}7J}$

Mutation (allele) name: pale ear 7 Jackson

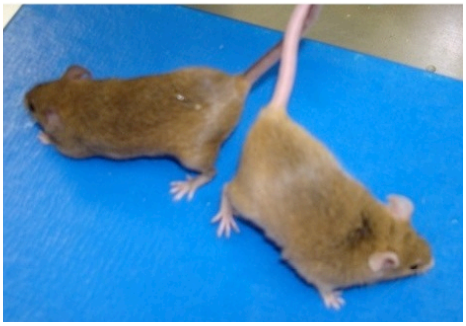
Gene symbol: *Hps1*

Strain of origin CAST/EiJ

Current strain name CAST/EiJ-*Hps1*$^{ep\text{-}7J}$/GrsrJ

Stock#: 016113 (jaxmice.jax.org)

Phenotype categories: pigmentation



A homozygous *Hps1*$^{ep\text{-}7J}$ on the right compared with a CAST/EiJ control on the left

Origin and description

A spontaneous mutation that causes diluted pigmentation was discovered at The Jackson Laboratory in 2008 in the wild-derived CAST/EiJ colony. This mutation makes normally agouti-colored CAST/EiJ mice appear similar in color to brown agouti mice, with pale ears and tail. Mutant females and males are both fertile, and litter sizes, although small, are consistent with that expected for the wild-derived CAST/EiJ genetic background. The average litter size when intercrossing mutant with non-mutant sibling is 4.45 pups per litter, which is not significantly different from CAST/EiJ. Out of 38 litters with 169 pups, 73 were mutant, 5 were born dead, and 23 were missing and 2 were found dead before wean age.

Genetic Analysis

A mutant was outcrossed to a C57BL/6JEiJ and none of the F1 hybrid offspring had the diluted coat color, proving this to be a recessive mutation. The F1 hybrids were intercrossed to generate an F2 population, which had both mutant and unaffected mice for linkage analysis. This mutation was mapped to Chromosome 19 between 27710000 bp and 60050000 bp (MGSCv37). Whole exome sequencing[1] revealed a unique single nucleotide variant of C to A in Chromosome 19 position 42,852,629 (MGSCv37) and 42,778,139 (GRCm38). This variant is in exon 3 of the *Hps1* gene, and is predicted to cause a nonsense mutation, resulting in a premature stop codon at residue 34 of the 704 amino acid protein (transcript ID ENSMUST00000162004). This finding from the exome sequencing data was confirmed using primers designed to produce a 518 base pair product spanning the predicted mutation: Hps1F (ACAGGAACCCCAGAAGGACT) and Hps1R (GCCAAGCTTTTCAGATGGAG). Sanger sequence analysis of PCR product from genomic DNA samples from 4 heterozygous mice and 4 mutant mice confirmed the

presence of a single-nucleotide polymorphism from C to A at position 42,778,139 (GRCm38). This new mutation was therefore designated pale ear 7 Jackson, *Hps1*$^{ep\text{-}7J}$.

Pathology

Auditory-evoked brainstem response analysis of a homozygote at 102 days of age showed very good hearing indicating that this mutation does not adversely impact hearing. A routine pathological screen of 2 homozygous females and one heterozygous female showed no lesions at 14 weeks of age. Eye examinations showed a male heterozygote and a male homozygote had normal eyes at 3 months of age.

Discussion

We report a new mutation in *Hps1* named pale ear 7 Jackson that affects the overall coat color of the mouse and causes an increased percentage of stillborn and pre-wean lethality. The outward phenotype mirrors the original pale ear mutation, but hematological assessment was not performed. This point mutation, resulting in the introduction of a premature stop codon at residue 34 of this 704-residue protein, is predicted to result in a null allele. Mutations in *Hps1* provide models for Hermansky-Pudlak syndrome. Because the CAST/EiJ strain has proven very problematic with current cryopreservation techniques, and a sperm freeze from homozygous males had very poor recovery, this mutation will only be available as DNA once this strain is removed from the shelf.

Acknowledgements

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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[1] 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[2]. Following these analyses, re-sequencing of additional mutant and unaffected samples was performed to validate and determine the most likely causative mutation.

1. www.genomequest.com
2. www.sanger.ac.uk/resources/mouse/genomes