A new spontaneous short snout mutation in *Pfas*

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Mutation (allele) symbol: *Pfas*$^{Sofa\text{-}2J}$

Mutation (allele) name: Short face 2 Jackson

Strain of origin: B6.Cg-*Lep*^{ob}/J

Current strain name: B6(Cg)-*Pfas*$^{Sofa\text{-}2J}$/GrsrJ

Stock #011079 (jaxmice.jax.org)

Phenotype category: craniofacial, coat color

Origin and Description

A spontaneous mutation was identified in the B6.Cg-*Lep*^{ob}/J strain (Stock #000632) at The Jackson Laboratory in November, 2008, and was initially referred to as short nose (*Shn*). Affected mice have a short snout and about 80% of mutants also have a ventral white belly spot that ranges from a few white hairs to a large spot (see photo on MGI allele detail page). This mutation was proven dominant by mating the original male mutant to a C57BL/6J female and obtaining eight mutant offspring out of twelve pups produced. By intercrossing these F1 offspring, wild-type bred with dominant heterozygous mutant, and continuing to sibling inbreed heterozygote with wild-type sibling, the obese allele was bred out of this mutant subline and the phenotypes of the short snout and belly spot persisted unmodified.

Pathology

Routine pathological screening was done on a six-week-old male and control littermate. No distinct lesions were found. Fifty-nine F2 embryos resulting from the intercross of heterozygotes were examined for additional phenotypes between embryonic day 14.5 and embryonic day 17.5. Of these, five exhibited delayed eye development, but correlation with genotype is not available. Ophthalmoscopic assessment of two heterozygotes and two wild-type controls at 6 weeks of age showed no abnormality, while assessment of two heterozygotes and two wild-type controls at approximately 10 weeks of age found that one of the mutants had one small eye and photosensitivity. Hearing was assessed by auditory brainstem response threshold analysis (ABR) in two mutants and two controls each at six weeks of age, 3 months of age, and 111 days of age, and no hearing loss could be attributed to this mutation.

Genetic Analysis

For linkage analysis, a mutant was outcrossed to A/J and affected F1 offspring were then

backcrossed to C57BL/6J. From the resulting N2 population, tissues from thirteen affected and 12 unaffected mice were collected and sent to the Fine Mapping Laboratory at The Jackson Laboratory. This mutation mapped to Chromosome 11 with flanking markers *D11Mit208* at 58.4 MB having 23% recombination and SNP 11-103758588-N having 8% recombination. Whole exome sequencing revealed a single mismatch allele of C to T on Chromosome 11 at position 68,814,092 (NCBI37/mm9), which is within the splice acceptor site at the 3 prime end of intron 6 of the gene phosphoribosylformylglycinamidine synthase (*Pfas*). This is the second spontaneous mutation in *Pfas* identified at The Jackson Laboratory and has thus been designated $Pfas^{Sofa\text{-}2J}$ for short face 2 Jackson.

During development*, de novo* synthesis is the predominant pathway for purine biosynthesis. Inosine monophosphate (IMP), the precursor of purine biosynthesis, is created through a number of enzymatic reactions, the fourth of which is catalyzed by PFAS. Two highly conserved functional domains are present in the PFAS protein: the type 1 glutamine amidotranserase (GATase-1)-like domain and the PurM-like domain. The previously identified $Pfas^{Sofa}$ spontaneous mutation is a 15 base pair deletion creating the predicted in-frame deletion of H1194_G1198del, which resides in the center of the GATase1-like domain, the primary catalytic site of the enzyme. $Pfas^{Sofa\text{-}2J}$ alters the highly conserved splice acceptor site at the 3 prime end of intron 6, which likely results in the total loss of exon 7. We predict that this exon skipping will lead to the loss of amino acids 228-274 which reside in the PurM-like domain, directly within the AIR (aminoimidazole ribonucleotide) synthase-related region. AIR synthase domains are often involved in protein dimerization and ATP binding. Thus, these two molecularly distinct spontaneous mutant models, each on a predominantly C57BL/6J background, may provide unique insight into the function of the PFAS protein and its conserved domains in mammals.

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