A remutation to achondroplasia in the MRL/MpJ inbred background: *Npr2*$^{cn\text{-}3J}$

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Mutation symbol: *Npr2*$^{cn\text{-}3J}$

Mutation name: achondroplasia 3 Jackson

Gene symbol: *Npr2*

Strain of origin: MRL/MpJ-*Fas*^{lpr}/J

Current strain name: MRL/MPJ-*Npr2*$^{cn\text{-}3J}$/GrsrJ

Stock #013252 (jaxmice.jax.org)

Phenotype categories: craniofacial, growth/size, development

Abstract
A new recessive mutation, achondroplasia 3 Jackson, arose spontaneously and has been identified as a remutation of the *Npr2* gene by its similar phenotype with other *Npr2* mutants, map position on Chromosome 4, a failure to complement in a direct test for allelism with the *Npr2*$^{cn\text{-}2J}$ mutation, and Sanger sequencing based determination of the causative mutation.

Origin and Description
A spontaneous mutation causing a disproportionate dwarf phenotype was identified by Wanda Taylor in 2006 in a production colony of MRL/MpJ-*Fas*^{lpr}/J mice at the Jackson Laboratory. Mice homozygous for this new mutation exhibit shorter body length than littermates, bellies resembling a squared lump, short noses, short limbs and short tails (see photo on MGI allele detail page). Female homozygotes breed poorly and produce small litters. Male homozygotes are not able to breed but cryopreserved sperm function satisfactorily in *in vitro* fertilization.

Genetic Analysis
Using our standard mapping procedure, homozygotes were mated with CAST/EiJ producing only non-affected F1 progeny and proving that this mutation is recessive. F1 progeny were then intercrossed and generated affected F2 animals for linkage analysis. The genetic map position for this new mutation was determined in the Fine Mapping Laboratory at The Jackson laboratory using their single nucleotide polymorphic (SNP) genotyping protocol. This mutation mapped to Chromosome 4 with a SNP-defined critical interval extending from NCBI 37 position 25216761 bp to NCBI 37 position 58850394 bp and concordant with a single SNP at NCBI 37 position 44506628 bp.

Because this map position and phenotype suggested *Npr2* as a candidate gene, a direct allele test was set up by mating a mouse heterozygous for this new mutation with an $Npr2^{cn\text{-}2J}$ heterozygote (stock#004200). This mating produced 9 progeny of which 3 expressed the mutant phenotype, proving this new mutation to be a defect in *Npr2*. This new mutation was, therefore, named achondroplasia 3 Jackson, $Npr2^{cn\text{-}3J}$.

The molecular basis of this mutation has been defined. Primers were designed to amplify sequence from *Npr2* exons. Primers were generated that produce a 547 base pair product spanning the entirety of exons 19 and 20; primer exon 19-20 forward (TACATGGATGCTGGGAACTG) and primer exon 19-20 reverse (AAGAATGGGGCACTTCAGG). Sequence analysis of mutant genomic DNA compared to wild type genomic DNA identified a single nucleotide transition from G to A at 18,293 in exon 19. This mutation would result in a predicted amino acid change of alanine to threonine.

Pathology

The disproportionate dwarfing, with shortened legs and face, is consistent with the defects in skeletal development found in other *Npr2* mutants. A routine pathological screen of two $Npr2^{cn\text{-}3J}$ homozygotes at 20 weeks of age, showed mild hydrocephalus. One homozygote had an atrophic lobe of liver with an old hemorrhage in it. This was not a significant lesion. Chronic otitis media and mild degeneration of the knee joint were also found and these were also deemed not significant.

Hearing as assessed by auditory-evoked brainstem response testing (ABR) of two mutants at 5 months of age and one mutant and one control at 2 months of age, showed that mutants are deaf and that the control has elevated thresholds at some frequencies. Although ABR patterns are not clear, thresholds seem significantly elevated in mutant mice.

The eyes of two mutants at 14 weeks of age and one mutant at 18 weeks of age were tested by electroretinograph (ERG). One mutant showed cataract at 14 weeks age and the other mutants showed normal eyes.

Acknowledgements

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References

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