Postaxial hemimelia 2 Jackson: a new point mutation in *Wnt7a* and model of Fuhrmann syndrome

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Mutation (allele) symbol: $Wnt7a^{px\text{-}2J}$

Mutation (allele) name: postaxial hemimelia 2 Jackson

Gene symbol: *Wnt7a*

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Abstract
A recessive mutation was identified that causes sterility and malformation of the appendages, particularly the front digits and front legs. We have found that this mutation is a single nucleotide substitution in the gene wingless-related MMTV integration site 7A (*Wnt7a*), and this mutation has been assigned the allele designation postaxial hemimelia 2 Jackson, $Wnt7a^{px\text{-}2J}$. This mutation provides a new model for Fuhrmann syndrome.

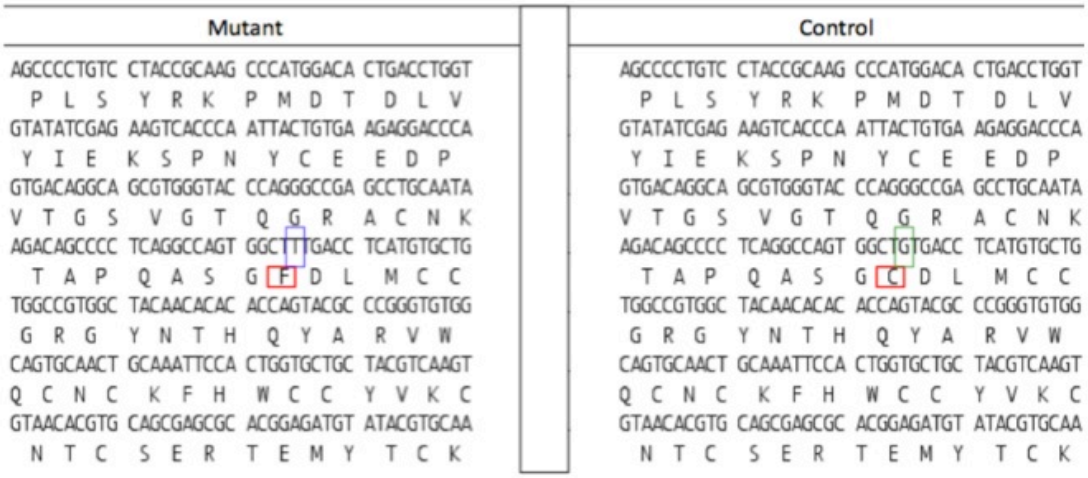
Origin and description
A mutant with odd legs was identified in 2005 at The Jackson Laboratory in the progeny of an ENU mutagenized C57BL/6J male that had been outcrossed to C3HeB/FeJ. Because this mutant did not breed, this mutation was propagated from phenotypically normal siblings that proved to be carriers of the mutation. The forelegs of these mutants are generally more affected than the hind-legs. The humerus bone is generally shorter than normal, and the front legs are set at odd angles to the trunk such that they resemble flippers. The hind-legs are not twisted but when raised by the tail mutants tend to hold the hind-legs together rather then splay them as controls do. The front digits can be twisted, swollen or missing, and rear digits may also be missing or at odd angles. The upper surfaces of both the fore- and hind-paws have dark brown bumps that protrude from the skin, which may be focal thickening of the epidermis.

The nails are normal in appearance. Due to the forelimb deformities, being severely twisted, these mutants have an odd gait, appearing to walk slightly side-to-side. On this mixed B6;C3Fe background the average litter size for 103 litters that produced a total of 797 pups from heterozygous intercrosses was 7.74 pups per litter. Of these 186 were mutant, 14 were missing before phenotypic classification at 1 week, and 1 was born dead. Assuming the missing and born dead were mutant the yield was 25.2% mutants produced; assuming that none of the missing or born dead were mutant the yield was 23.3% mutants produced. Although heterozygotes are fertile and produce the expected Mendelian ratio of homozygotes, to date no homozygote has bred. However, homozygotes live a normal lifespan. This mutant line is maintained by intercrossing progeny tested heterozygous siblings.

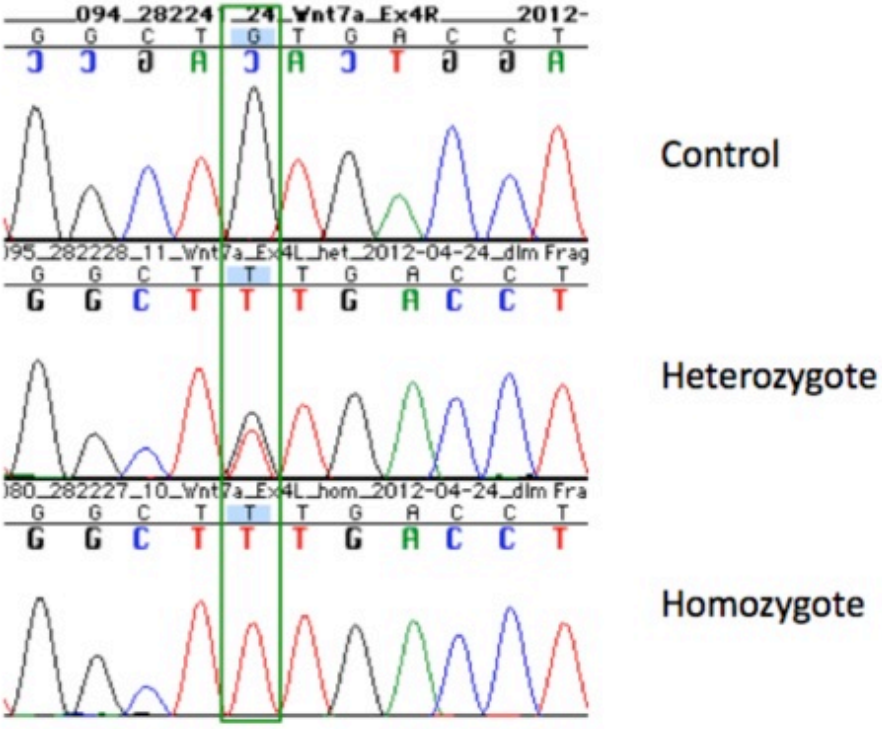
Genetic Analysis
Carriers of the mutation, proven by progeny test, were outcrossed to CAST/EiJ mice and, as expected, no affected mice were found in the F1 generation. Intercrossing of unaffected F1 animals resulted in some affected F2 animals from tested pairs, further proving a recessive mode of inheritance. However, when outcrossed to CAST/EiJ there is a significant reduction in the percentage of mutants produced. Of 197 F2 offspring only 10 were mutant, 4 were found dead and 3 were missing before classification at 1 week. Assuming the missing and dead pups were all homozygotes this is still less than 9% homozygotes born. Thus, the CAST/EiJ background modifies the severity of this mutation to either cause increased embryonic lethality or diminished expressivity to the extent that it causes failure of outward phenotypic detection. The cause of the reduced frequency of mutants remains to be determined; however, because of the consistent severity in homozygotes observed on both the B6;C3Fe genetic background and the intercross with CAST/EiJ we hypothesize that CAST/EiJ contributes to increased embryonic lethality of homozygotes.

Affected F2 mice were used for SNP analysis, which mapped this mutation to Chromosome 6. This mutation was found to map near *Wnt7a* and the phenotype was so similar to that of published *Wnt7a* mutants that we sequenced exons 1 through 4 of *Wnt7a*. Sequence analysis of PCR product from exon 4 generated using primer exon 4 left (CCAGGCTTGCACTTGGC) and primer exon 4 right (TCTTTCCCTGTGAGCATCC) revealed a single nucleotide change from G to T at

Chromosome 6 position 91315982 in *Wnt7a*. This is a missense mutation predicted to change amino acid 304 from cysteine to phenylalanine (see sequence and chromatogram below) in the highly conserved cysteine-rich region toward the carboxy terminus. Complementation testing with the strain C57BL/6J-*Wnt7a*$^{px\text{-}J}$/GrsrJ produced seven affected progeny out of 52 born with one born dead, confirming this as a mutation in *Wnt7a*.



A portion of the protein-coding region of *Wnt7a*. The control DNA sequence and its amino acid translation are shown on the right, and the *Wnt7a*$^{px\text{-}2J}$ mutant DNA and its translation on the left. A single nucleotide transition is enclosed by a blue box in the mutant sequence and a green box in the control sequence. A red box in both the control and the mutant sequences encloses the predicted change from cysteine to phenylalanine in amino acid 304.



Comparison of DNA sequence chromatograms from a *Wnt7a*$^{px\text{-}2J}$ homozygote, heterozygote and wild-type control. The green boxed region corresponds to the green and blue boxed regions shown in the sequence figure.

Pathology
3X whole body x-rays were taken of an 8-week-old male, which showed shortened humerus and both fused and missing digits in the fore-paw, but no defects in the hind-legs or hind-paws. This was confirmed in an alizarin red skeletal preparation of the same mouse. Hearing tests on one female and one male homozygote at five months of age, four homozygotes at one month of age, one female and one male control heterozygote at three months of age and one control at one month of age showed all having normal hearing. Eye examinations on four homozygotes and one heterozygote showed all having retinal degeneration consistent with the phenotype of *Pde6b*^{rd1} homozygosity, which is a strain background characteristic and not attributable to the *Wnt7a* mutation.

Discussion
Fuhrmann syndrome often involves shortened or absent ulna, shortened or bowed radius or femur, aplasia or hyperplasia of the fibula, absence of menses, and poly-, syn-, and oligodactyly. Additionally, hypoplasia of the pelvis, congenital dislocation of the hips, fused knee joints, absent patellae, hypoplasia of the fingers and fingernails, and cleft lip and palate have also been reported. There has also been a report of a Fuhrmann syndrome patient having an abnormal texture on the dorsal surface of the hands with hypoplastic flexion creases (Woods et al., *Am. J. Hum Genet.* 2006; 79:402-408). This *Wnt7a* mutant provides a good model for Fuhrmann syndrome, which has the variation between patients expected of a developmental syndrome of limb patterning. While the *Wnt7a*$^{px\text{-}2J}$ mutation has a greater effect on the forelegs than the hind-legs or pelvis compared with published cases of Fuhrmann syndrome, the sterility and skeletal malformations of the appendages are consistent phenotypes. The darkened epidermal bulges found on the dorsal surface of the paws may be similar in cellular cause to the one reported case with textural change in the dorsal surface of the hands. This is a phenotype not yet reported in mouse *Wnt7a* mutants. The severe limb deficiency of Al-Awadi/Raas-Rothschild syndrome has not been observed in these homozygotes to date. There appears to be a modifier in the CAST/EiJ background that causes increased embryonic lethality. Pursuit of this modifier is beyond the scope of our work.

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