Brachypodism 5 Jackson, a mouse model of *GDF5* genetic diseases

Authors: Son Yong Karst, Melissa L. Berry, Laura G. Reinholdt, Heather E. Fairfield, David E. Bergstrom, and Leah Rae Donahue

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Mutation (allele) symbol: $Gdf5^{Bp\text{-}5J}$

Mutation (allele) name: brachypodism 5 Jackson

Gene symbol: *Gdf5*

Strain of origin: C57BL/6J

Current strain name: C57BL/6J-$Gdf5^{Bp\text{-}5J}$/GrsrJ

Stock #021333 (jaxmice.jax.org)

Phenotype categories: skeletal

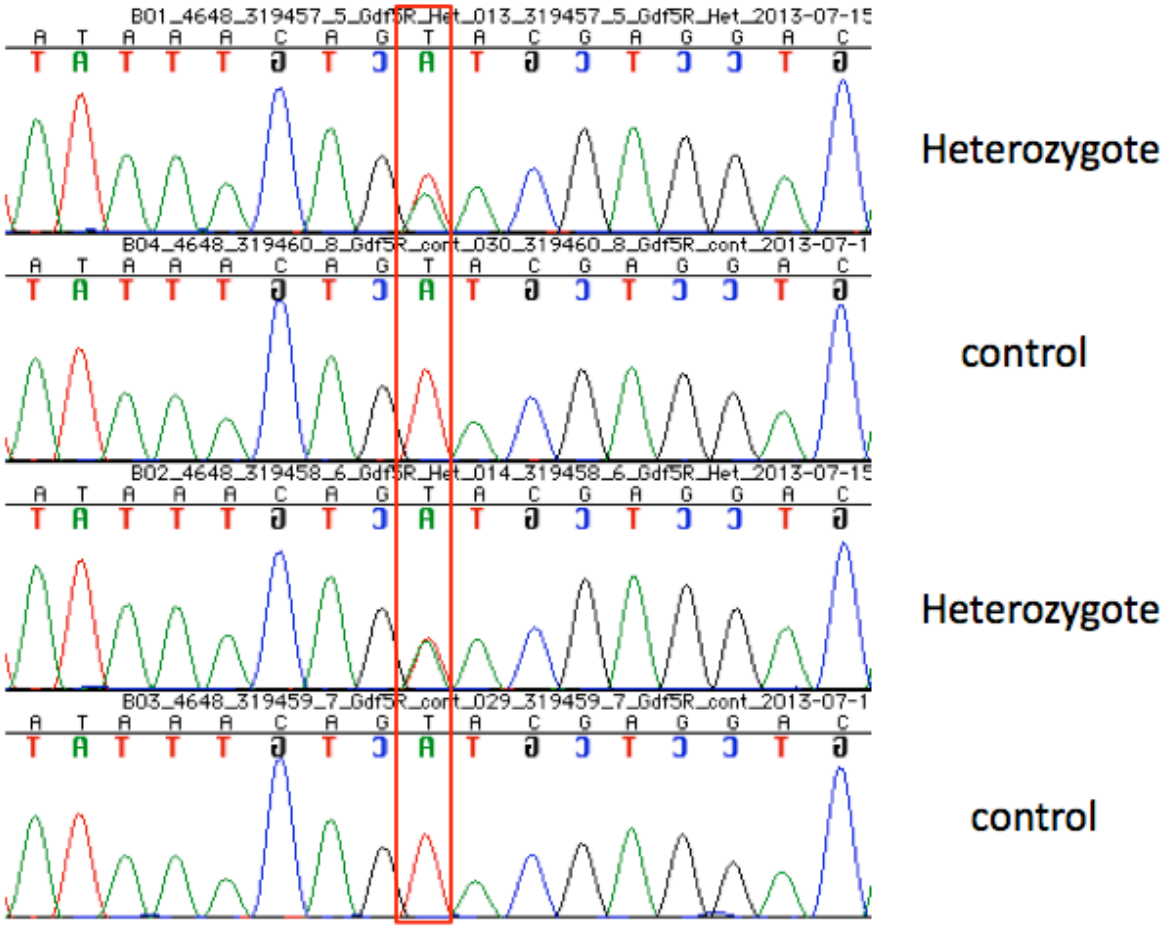
Abstract:
A spontaneous, dominant mutation that causes disproportionate dwarfing with malformed feet and digits has been characterized and identified as a point mutation in *Gdf5*. This mutant, designated brachypodism 5 Jackson ($Gdf5^{Bp\text{-}5J}$), is the second dominant mouse mutation reported in GDF5 and provides a model useful in the study of an array of GDF5 genetic diseases.

Origin and Description:
Jeanette Dyer discovered a spontaneous mutation in the C57BL/6J colony at The Jackson Laboratory in 2011. Affected animals are born with severely shortened digits and malformed limbs, but have normal body length. These characteristics, disproportionate dwarfing with shortened limbs, digits, and underdeveloped feet, are similar to those found in brachypody ($Gdf5^{bp}$) homozygotes. Both male and female mutants are fertile and live a normal lifespan.

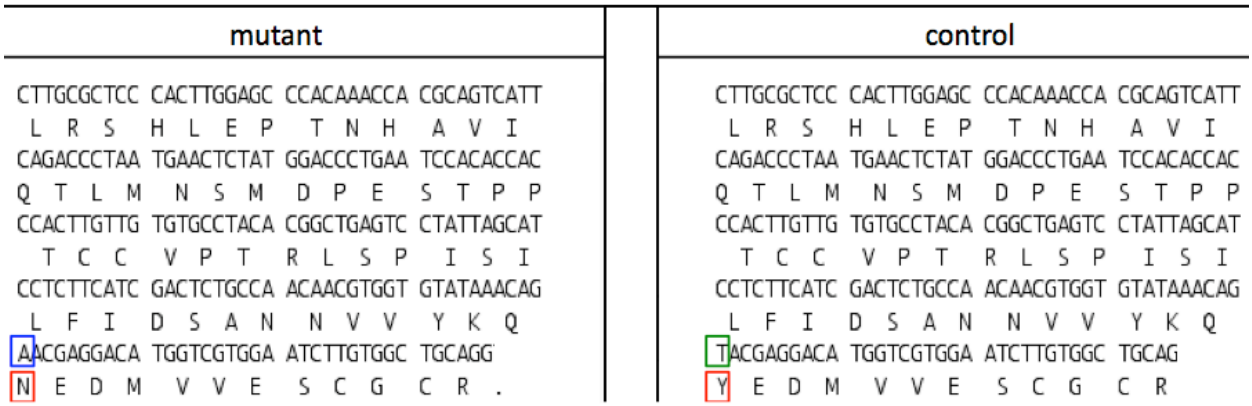
Genetic Analysis:
Mutant animals were outcrossed to FVB/NJ mice to establish heritability and affected mice were found in the F1 generation, proving that this mutation is not recessive. The homozygous phenotype of this mutation remains to be characterized. Backcrossing affected F1 animals to FVB/NJ produced affected F2 animals for linkage analysis and fine mapping. Using standard SNP protocols, linkage analysis for this mutation was completed in the Fine Mapping Laboratory at The Jackson Laboratory. This mutation mapped to Chromosome 2, between 139,603,599 bp and 176,114,999 bp (MGSCv37).

After mapping data demonstrated linkage to Chromosome 2, whole exome sequencing was performed to identify candidate mutations in the mapped region. Briefly, genomic DNA was enriched for coding sequence by hybridization-based capture with

probes representing 54 Mb of annotated coding sequence. The enriched DNA was then sequenced using the Illumina HiSeq high throughput sequencing platform. A single nucleotide polymorphism was found at MGSCv37 position 155,767,317 on Chromosome 2, which is in growth differentiation factor 5 (*Gdf5*). Primers were generated that produce a 484 base pair product spanning the predicted mutation: *Gdf5* Left (GACTGGATCATCGCACCTCT) and *Gdf5* Right (GTTCCTTGGGCAGGAATCTT). Sequence analysis of genomic DNA from two mutants compared to genomic DNA from two unaffected animals confirmed a T to A transversion at MGSCv37 Chromosome 2: 155,767,317 in growth differentiation factor 5 (*Gdf5*). This position is equivalent to Chromosome 2 position 155,941,581 in GRCm38. This is a missense mutation and predicted to change position 484 of this 495 amino acid protein from tyrosine to asparagine (Y484N). This mutation has been designated brachypodism 5 Jackson (*Gdf5*$^{Bp\text{-}5J}$).



Comparison of DNA sequence chromatograms from *Gdf4*$^{Bp\text{-}5J}$ heterozygotes and controls. The red boxed region corresponds to the green and blue boxed regions in the comparative sequence figure.



A portion of the protein coding region of *Gdf5*. The control DNA sequence and its amino acid translation are shown on the right, and the *Gdf4*$^{Bp\text{-}5J}$ 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. The mutation that is predicted to change amino acid 484 from tyrosine to asparagine, enclosed by red boxes in the control and the mutant sequence.

Pathology:
A standard pathology screen performed on three mutant males and three unaffected sibling controls, two male and one female, all at 20 weeks of age. One mutant male had mild hydrocephalus, a common finding in C57BL/6J mice, but aside from the abnormal skeletal development no abnormal pathology was identified. The eyes of one mutant mouse at age 16 weeks were tested by an electroretinogram (ERG) and the results were normal.

Discussion:
This spontaneous point mutation causing an Y484N substitution near the C-terminus of growth differentiation factor 5 results in a non-recessive phenotype of shortened and malformed limbs, feet, and digits.

Mutations in GDF5 cause varying degrees of hypoplasia of the distal limb bones and complex brachydactyly. Of the eight other characterized mouse *Gdf5* mutations, only one is not recessive, *Gdf5*^{Rgsc451}. *Gdf5*^{Rgsc451} is an ENU-induced tryptophan to arginine substitution in amino acid 480, just four residues upstream of the residue mutated in *Gdf5*$^{Bp\text{-}5J}$. *Gdf5*^{Rgsc451} is semi-dominant, causing brachypodism and ankylosis in heterozygotes, while homozygotes display a more severe phenotype, including carpal-tarsal joint fusion, complete fusion of the knee joints, shortened long bones of the hind legs, and early onset degeneration in the elbow joints, resembling osteoarthritis[1]. Of the sequenced mouse mutations, only *Gdf5*^{Rgsc451} has been shown to be a dominant-negative mutation, interfering with wild-type GDF5 protein function. The other mouse mutations, all recessive, are predicted to cause functional null mutations.

In human, dominant GDF5 mutations have been found to cause Hunter-Thomson type acromesomelic dysplasia, multiple synostoses syndrome 2, and some cases of Du Pan

syndrome. Grebe type chondroplasia, proximal symphalangism 1B, and several types of brachydactyly are also attributed to mutations in GDF5, but have recessive inheritance[2]. Specific amino acid substitutions have been traced to particular instances of these human developmental disorders, most of them dominant[2]. By phenotype, *Gdf5*$^{Bp\text{-}5J}$ heterozygotes may provide a useful model for the study of the developmental abnormalities involved in any of these human GDF5 genetic diseases. The phenotype of *Gdf5*$^{Bp\text{-}5J}$ homozygotes, and therefore whether this allele provides a model for osteoarthritis like the *Gdf5*^{Rgsc451} allele, awaits characterization. The heterozygous phenotype of this amino acid substitution provides one more data point supporting a model in which deletion or inactivation of GDF5 yields recessive brachypody mutations, while point substitutions yield dominant negative alleles.

Acknowledgements:
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References:

1. Masuya H. et al., Hu Mol Genet (2007) 16(19) p2366-75

2. Online Mendelian Inheritance in Man, OMIM. Johns Hopkins University, Baltimore, MD. MIM Number:601146: September 8, 2013