A new skeletal mutation named semi-dominant compacted skeleton that maps to Chromosome 5

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Mutation (allele) symbol: *Scs*

Mutation (allele) name: semi-dominant compacted skeleton

Gene symbol: *Scs*

Strain of origin: C3H/HeDiSnJ-*Dscam*^{2J}/GrsrJ

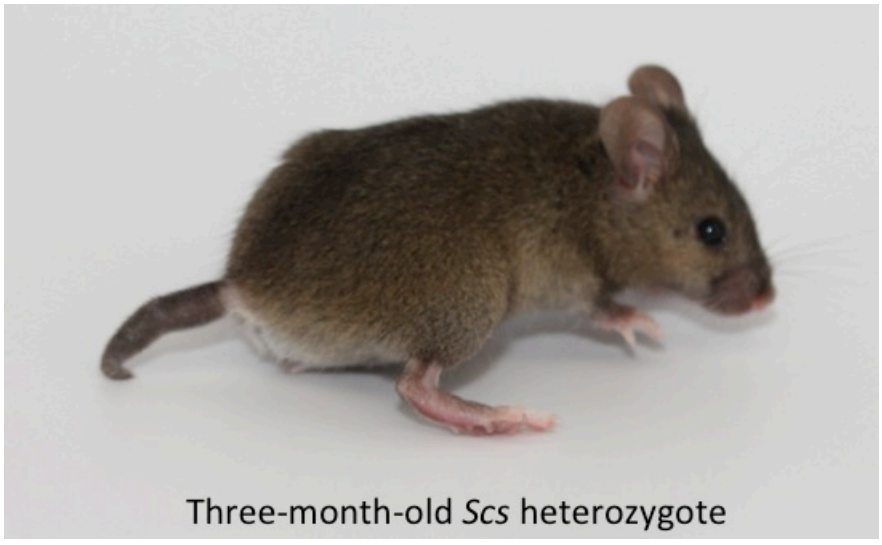
Current strain name: C3H/He-*Scs*/GrsrJ

Stock #016196 (jaxmice.jax.org)

Phenotype categories: developmental, skeletal

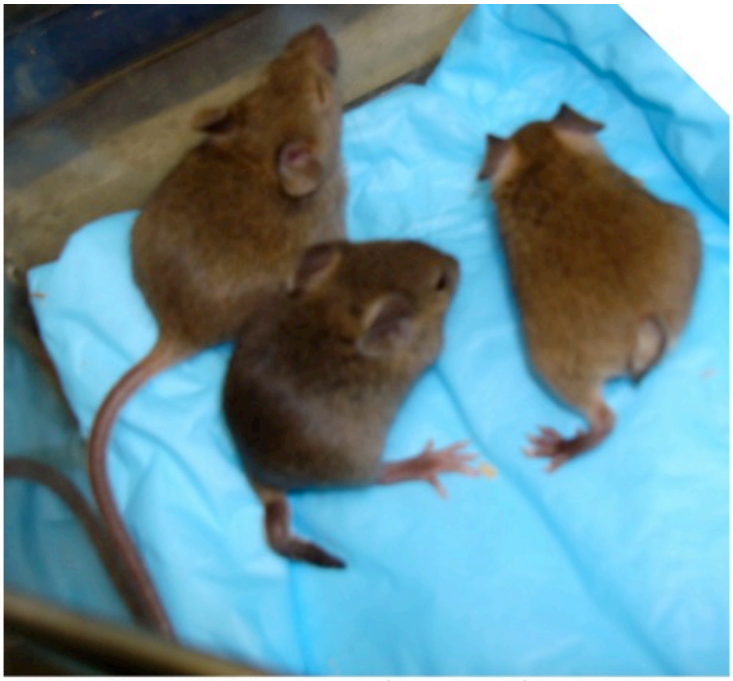
Abstract:
We have identified and characterized a spontaneous, semi-dominant mouse mutation that causes reduced tail length and compacted body. This mutation, named semi-dominant compacted skeleton (*Scs*), has been mapped to Chromosome 5 between *D5Mit325* and *D5Mit101*.

Origin and Description:
A spontaneous mutation was identified in 2004 at The Jackson Laboratory in the strain C3H/HeDiSn-*Dscam*^{2J}/GrsrJ. This new mutation was initially identified by its short tail and compacted body, and the phenotype of the *Dscam*^{2J} homozygote was absent.



This mutation was proven semi-dominant by outcrossing a mutant to C57BL/6J and

finding mutants and unaffected siblings in the F1 hybrid population. When obligate heterozygous mutants from this F1 population were intercrossed, the F2 population was found to include 3 phenotypes, presumably reflecting +/+, heterozygous, and homozygous genotypes. Compared with the wild-type siblings the affected F2 mice have compacted bodies and shortened tails with variability in both the tail length and the degree of foreshortening of the body. The less severe phenotype, presumed heterozygous, has a somewhat stumpy and sometimes kinked tail approximately three quarters of an inch in length and a compacted body in which it appears that the axial skeleton is compressed, but the appendicular skeleton does not appear clinically affected. The more severe phenotype, presumed homozygous, has a very short, stumpy tail approximately half an inch or less in length, and the compacted body is sometimes more severely compressed in the trunk than that observed in the mutants with intermediate tail length.



From left to right: (C57BL/6J x C3H/HeDiSnJ)F2 background +/+, *Scs*/+ with short tail and compact body, *Scs*/*Scs* with shorter tail and compact body

The more severe phenotype was proved homozygous by recovering only mutant offspring from outcrossing to C3H/HeJ; the less severe phenotype was proved heterozygous by recovering both mutant and wild-type offspring from an outcross to C3H/HeJ. We have named this mutation semi-dominant compacted skeleton (*Scs*). Heterozygous *Scs* mice generally have normal fertility and lifespan. One homozygous female mated to a C3H/HeJ male produced 5 litters with a total of 29 mutant offspring and 1 stillborn pup. Thus, female homozygotes can breed and there is no indication of incomplete penetrance. Outcrosses of heterozygous mutants to CAST/EiJ or C3H/HeJ or C57BL/6J all produced approximately 50% mutant offspring, consistent with Mendelian expectation, with no modifiers blatantly impacting the phenotype. These assessments were all performed after this mutant subline had been backcrossed twice to C3H/HeJ to ensure that the *Dscam*^{2J} mutation had been bred out. This strain was subsequently

maintained by intercrossing heterozygotes and wild-type siblings.

Genetic Analysis:
This mutation was mapped using a cross to CAST/EiJ that placed this mutation on Chromosome 5 between *D5Mit325* and *D5Mit329*. Whole exome sequencing[A] was performed on a heterozygous mutant genomic DNA sample but did not reveal any candidate mutations in the critical region with standard SNP and INDEL calling methods. Further genomic analysis is required to determine the genetic basis of this mutation; this may include whole genome sequencing or RNA sequencing methods.

Pathology
X-rays and alizarin preparations of one five-week-old and two eleven-week-old homozygous females showed severe shortening of the axial skeleton; however the appendicular skeleton appeared unaffected. Compression of cervical, thoracic, lumbar and sacral vertebrae resulted in an overall shorter body length and an extremely short tail. Not only were the vertebral bodies compressed, but they were also misshapen and irregularly aligned with intervertebral discs sometimes missing or herniated. Misaligned vertebrae were often fused, causing the spine to be crooked and to appear jumbled. Ribs were often bifurcated or fused. Heterozygotes, one at five weeks of age and one at eleven weeks of age, were similarly, although less severely, affected.

Auditory brainstem response assessment on one homozygote at thirty-nine days of age, one homozygote at eighty-one days of age, and one homozygote and one heterozygote at 4 months of age all showed normal hearing. Retinal degeneration was found in all mice assessed from this strain, including a homozygote, two heterozygotes and an unaffected littermate; this retinal degeneration is likely attributable solely to the *Pde6b*^{rd1} allele of the C3H genetic background and not the *Scs* mutation. Heterozygous offspring of an outcross to C57BL/6J were found to have normal eyes when assessed by electroretinogram and fluorescein angiography.

Discussion
We report a new semi-dominant mutation named semi-dominant compacted skeleton (*Scs*) that interferes with the proper development of axial skeleton components, but does not appear to impact the appendicular skeleton. LFNG O-fucosylpeptide 3-beta-N-acetylglucosaminyltransferase (*Lfng*), also known as lunatic fringe, is an excellent candidate gene for the *Scs* causative mutation due to its map location on Chromosome 5 and some phenotypic similarities with some *Lfng* mutants. The chemically induced allele zigzag (*Lfng*^{m1Bilr}) causes vertebral fusions, and short, kinked tails[1]. Multiple targeted disruptions of *Lfng* including but not limited to *Lfng*^{tm1Grid} and *Lfng*^{tm1Rjo}, also cause, shortened trunk, abnormal ribs, and shortened tail[2,3]. Transcriptional oscillation of lunatic fringe has been shown to be essential for the formation of somites during development[4], and aberrant *Lfng* gene expression in *Scs* mice would be consistent with the observed phenotype. These changes in expression may result from altered regulatory regions outside of the coding region captured in exome sequencing, and should be detectable in future experiments.

Acknowledgements
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References
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3. Evrard YA, Lun Y, Aulehla A, Gan L, Johnson RL. 1998. Lunatic fringe is an essential mediator of somite segmentation and patterning. Nature 394:377-81.
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[A] Exome Sequencing Protocol
The exome sequencing data referred to in this website were analyzed using tools and workflows provided by Genome Quest1 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 Database2. Following these analyses, re-sequencing of additional mutant and unaffected samples was performed to validate and determine the most likely causative mutation.