Sharkey, a new mutation in the *Sostdc1* gene

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Mutation (allele) symbol: *Sostdc1*^{shk}

Mutation (allele) name: sharkey

Gene symbol: *Sostdc1*

Current strain name: B6(NOD)- *H2*^{g7} *Sostdc1*^{shk}/J

Stock #005717 (jaxmice.jax.org)

Phenotype categories: teeth, hearing

Origin and description

This recessive mutation arose spontaneously in 2003 in one of The Jackson Laboratory research colonies. It arose on a predominantly C57BL/6J strain that was congenic for the NOD-derived *H2*^{g7} and for a T Cell Receptor transgene with an unmapped insertion site. The transgene was removed from this strain, but *H2*^{g7} remains. Mice homozygous for the sharkey (*shk*) mutation display supernumerary incisors. The colony is maintained by sibling matings between a homozygote parent and a heterozygote.

A mouse homozygous for the sharkey mutation showing extra incisors that require routine trimming

Pathology

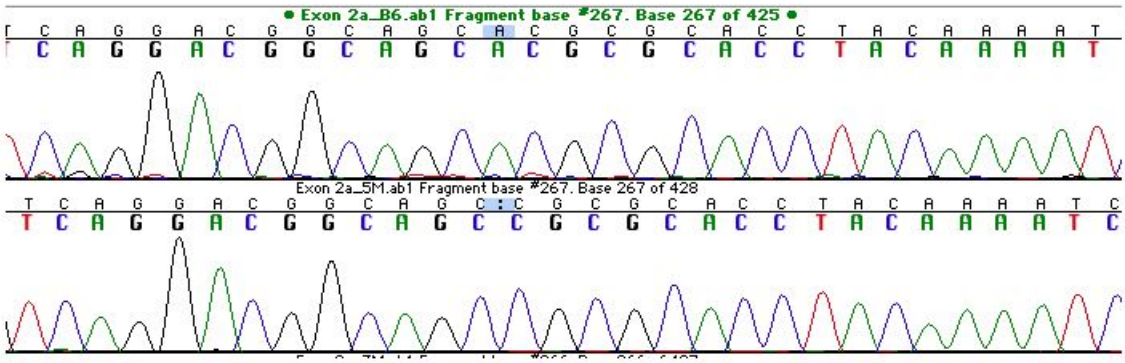
Using our standard pathological screen, tissues from three male mutants and one male heterozygote were prepared for histopathological examination. The screen revealed no lesions in any somatic tissues of two mutants ages 4 and 17 weeks old. A 15-week-old mutant had otitis media. A closer exam of the teeth in the mutants revealed that there are separate roots for these extra teeth. One mutant and one control were assessed for hearing loss by auditory brainstem response (ABR)[1]. The four-month-old mutant had severe hearing loss whereas the control had normal hearing. A one-year-old mutant was deaf and a littermate control had normal ABR thresholds. A clinical eye exam revealed no abnormalities in either mutants or controls.

Genetic analysis

A cross was performed between a homozygous *shk* female and a CAST/Ei male. F1 progeny

were intercrossed and 59 mutant F2 animals were genotyped for mapping studies. Using the standard mapping protocol of the Mouse Mutant Resource the mutation was mapped to a region on Chromosome 12 between *D12Mit146* and *D12Mit271* where the *Sostdc1* gene is located.

PCR primers were designed to amplify each exon of the *Sostdc1* gene using IDT Technologies. PCR products were sequenced at The Jackson Laboratory core sequencing facility. The sequence of each exon was then compared to the mouse sequence publicly available at ExonPrimer.html. A base pair deletion was detected in exon 2 of the *Sostdc1* gene resulting in a frame shift mutation with a premature stop codon.



Comparison of sequence chromatograms showing the base pair deletion in *Sostdc1* exon 2 highlighted in blue, wildtype sequence on top and homozygous *shk*/*shk* sequence on the bottom.

Biological Characterization

20 of 39 offspring from eight homozygote by heteozygote matings were mutants, just over the 50% expected ratio. Strict homozygote matings produce all affected pups (100% penetrance). All mutants have supernumerary incisors, specifically an extra pair of upper and lower incisors that erupt posterior to the normal incisors. But there is variability in the phenotype. Some mutants' supernumerary incisors grow slower while others' teeth exhibit faster growth and need to be trimmed back more frequently. When this strain was crossed to CAST/Ei for mapping, affected mice from the cross only got upper supernumerary incisors indicative of modifiers specific to strain background.

The upper palate also displayed unusual patterning. The control had symmetrical patterning while the mutants had different degrees of disorganized formation.

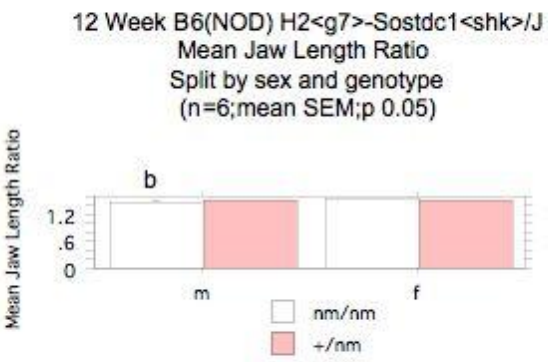
A. DEXA Analysis of Whole Body BMD and Body Composition

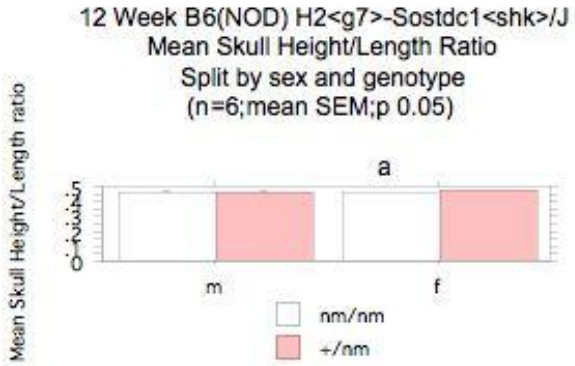
DEXA analyses were performed using the PIXImus densitometer (PIXImus, LUNAR, Madison, WI) which provides skeletal and body composition values. Bone Mineral Density (BMD, g/cm2), Bone Mineral Content (BMC, g/cm2), total body mass (g), lean mass (g), fat mass (g), and percent fat were collected for six male and six female mutants and controls (+/*shk)* at twelve weeks of age (see Table 1). Values for homozygous *shk* mice were not different between genders. However, there were significant differences in control mice in whole body BMD, BMC, lean, total mass and Skull BMD/Body BMD, which seem to be based on gender (see graphs). Overall, *shk* mutants grow normally to twelve weeks of age in spite of repeated tooth trimming in some of the mutants. Normal grain was supplemented with ground diet of the same formulation.

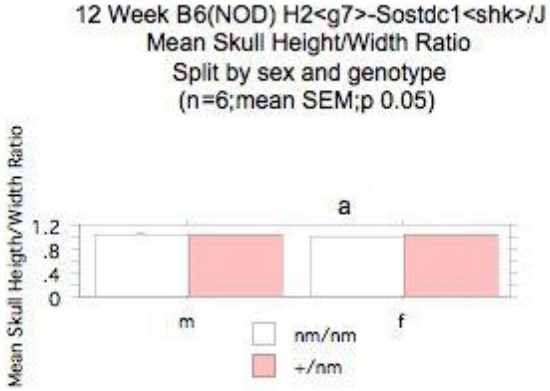
Table 1: PIXImus Densitrometric Measurements of Twelve-Week-Old B6(NOD) H2^{g7}-*Sostdc1*^{shk}/J (n=6; mean ± SEM; p [ab]p≤ 0.05)

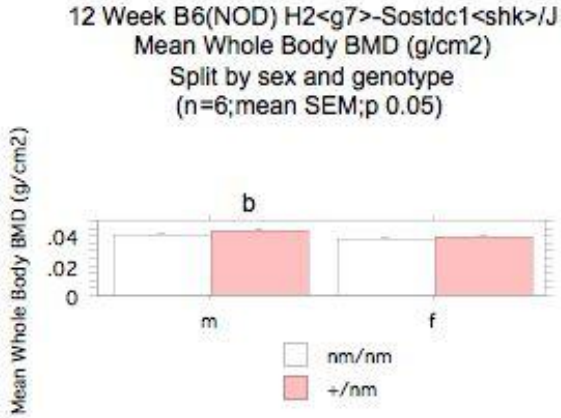
Measurements	Male *shk/shk*	Male *+/shk*	Female *shk/shk*	Female *+/shk*
Whole Body BMD (g/cm2)	0.0399 ± 0.00132	0.0434 ± 0.00109[b]	0.0379 ± 0.00069	0.0395 ± 0.00087
Whole Body BMC (g)	0.323 ± 0.0247	0.364 ± 0.0119[b]	0.301 ± 0.0145	0.320 ± 0.0145
Whole Body Fat (g)	3.1 ± 0.34	4.3 ± 0.46	2.9 ± 0.38	3.5 ± 0.36
Whole Body Lean (g)	16.3 ± 1.30	19.1 ± 0.65[b]	13.3 ± 0.46	13.9 ± 0.48
% Fat	16	18	18	20
Total Mass (g)	19.38 ± 1.641	23.40 ± 0.904[b]	16.25 ± 0.443	17.40 ± 0.588
Skull BMD (g/cm2)	0.1020 ± 0.00304	0.1045 ± 0.00117	0.1008 ± 0.00209	0.1036 ± 0.00319
Skull BMC (g)	0.227 ± 0.0135	0.232 ± 0.0029	0.203 ± 0.0082	0.223 ± 0.0109
Skull BMD/Body BMD	2.5685 ± 0.08930	2.4168 ± 0.05175[b]	2.6603 ± 0.04864	2.6266 ± 0.06971

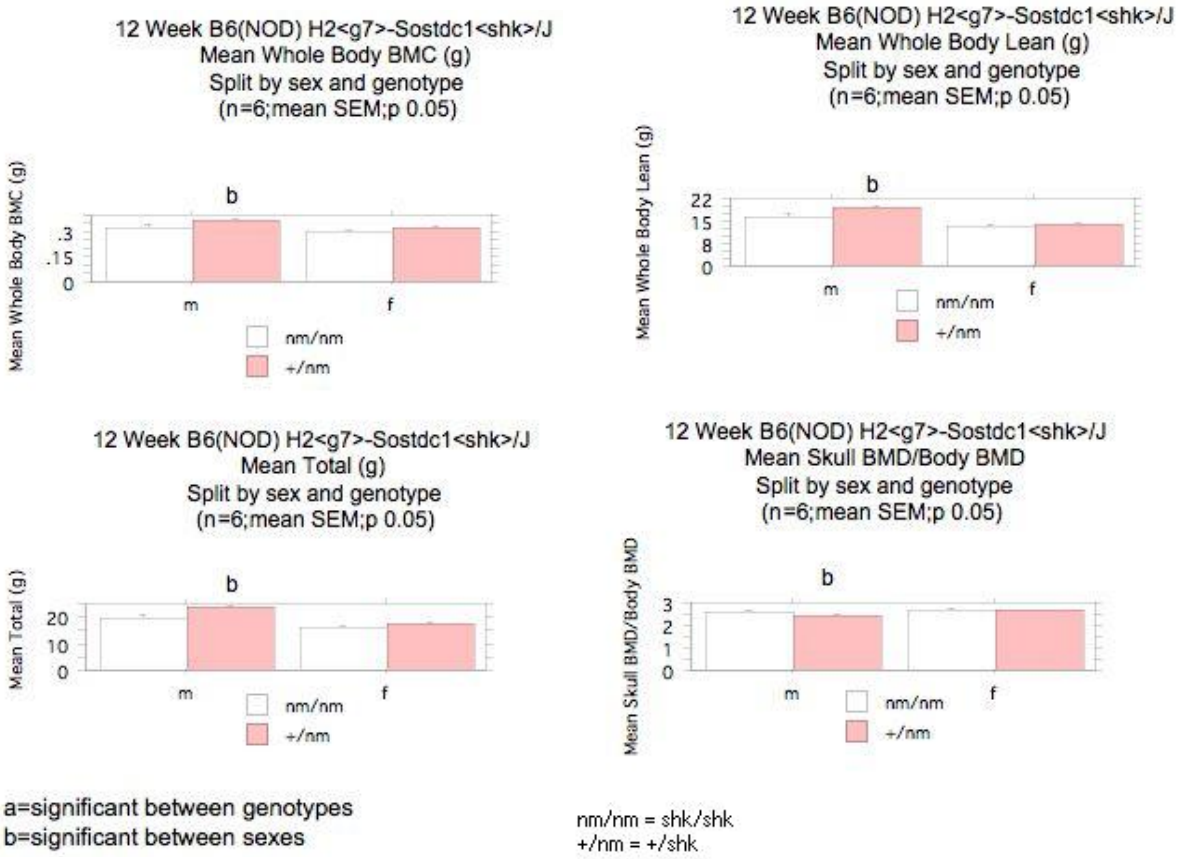
a=significant between genotypes
b=significant between sexes











a=significant between genotypes
b=significant between sexes

nm/nm = shk/shk
+/nm = +/shk

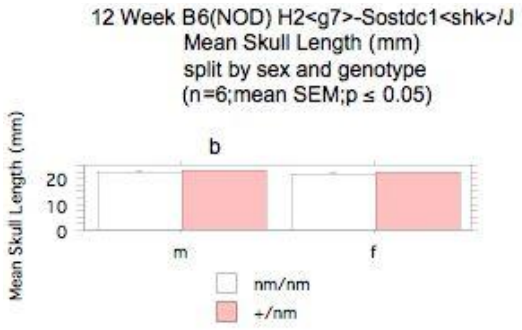
B. Skeletal and Craniofacial Morphology

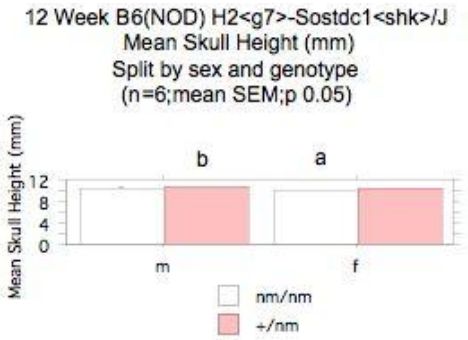
Skulls of six male and six female homozygous mutants and six male and female controls were collected at twelve weeks of age, prepared by incomplete maceration in potassium hydroxide, stained with alizarin red, and stored in undiluted glycerin (Green, 1952). Morphological measurements of the skull were also made using digital calipers (Stoelting, Wood Dale, Ill) with previously established landmarks[2] (Table 2). Female *shk* mutants had significantly shorter skull height, lower jaw length, and less height to length and height to width ratios than controls. Male *shk* mutants differed from controls only in skull width. The only significant gender difference with the sharkey mutation is the ratio between upper and lower jaw, which is greater in females. In controls, males had longer and wider skulls and longer lower jaws (see graphs).

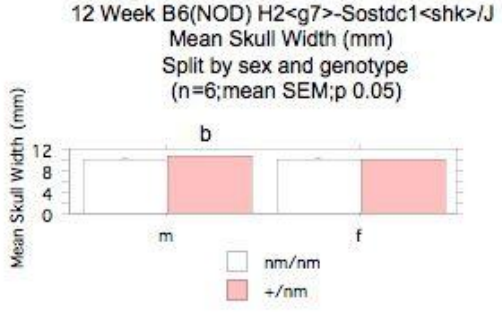
Table 2: Digital Caliper Measurements and Calculated Ratios of Twelve Week Old B6(NOD)-H2g7 *Sostdc1*^{shk}/J Skulls Stained with Alizarin Red (n=6; mean ± SEM; $^{ab}p \leq 0.05$)

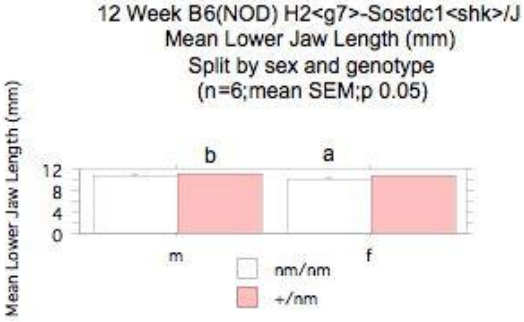
Measurements	Male *shk/shk*	Male *+/shk*	Female *shk/shk*	Female *+/shk*
Skull Length (mm)	22.37 ± 0.366	23.07 ± 0.149[b]	21.8 ± 0.247	22.23 ± 0.202
Nose Length (mm)	15.64 ± 0.244	15.77 ± 0.131	15.34 ± 0.120	15.47 ± 0.140
Skull Height (mm)	10.35 ± 0.296	10.70 ± 0.124b	9.94 ± 0.107[a]	10.37 ± 0.078
Skull Width (mm)	10.10 ± 0.126[a]	10.52 ± 0.051[b]	10.05 ± 0.119	10.11 ± 0.036
Inner Canthal Distance (mm)	5.90 ± 0.098	6.14 ± 0.046	5.88 ± 0.104	6.04 ± 0.076
Upper Jaw Length (mm)	15.68 ± 0.324	16.46 ± 0.148	15.73 ± 0.187	16.04 ± 0.146
Lower Jaw Length (mm)	10.68 ± 0.172	11.03 ± 0.052[b]	10.16 ± 0.176[a]	10.61 ± 0.080
Jaw Length Ratio	1.47 ± 0.032[b]	1.49 ± 0.012	1.55 ± 0.016	1.51 ± 0.018
Skull/Nose Length Ratio	1.43 ± 0.012	1.46 ± 0.012	1.42 ± 0.012	1.44 ± 0.018
Skull Height/Length Ratio	0.46 ± 0.009	0.46 ± 0.004	0.46 ± 0.002[a]	0.47 ± 0.004
Skull Length/Width Ratio	2.21 ± 0.016	2.19 ± 0.012	2.17 ± 0.023	2.20 ± 0.023
Skull Height/Width Ratio	1.02 ± 0.026	1.02 ± 0.009	0.99 ± 0.009[a]	1.03 ± 0.011

a=significant between genotypes
b=significant between sexes









nm/nm=*shk*/*shk*; +/nm=+/*shk*

Acknowledgements

We would like to thank Bob Giggey for originally identifying the mutant, Heping Yu for ABR analysis, Norm Hawes for clinical eye evaluation, Coleen Kane and Dr. Roderick Bronson for pathological screening and Leona Gagnon for her guidance in molecular analyses.

Protocols

[1] **ABR thresholds** in mice are determined using a semi-automated computer system (Intelligent Hearing Systems, Miami, Florida). Subdermal needle electrodes are inserted at the vertex and ventrolaterally to both ears of anesthetized mice. Specific auditory stimuli from 10-100 dB SPL are delivered binaurally through plastic tubes from high frequency transducers. ABR thresholds are obtained, in an acoustic chamber, for clicks and for 8, 16, and 32 kHz pure-tone pips.

[2] **Standard Protocols and Procedures of the JAX Craniofacial Resource**

Mouse Colony Maintenance

Craniofacial Resource mice are housed in 51 square inch polycarbonate boxes, on bedding composed of sterilized shavings of Northern White Pine, under 14:10 hour light: dark cycles. A diet of autoclaved NIH 31 (6% fat diet, Ca:P of 1.15:0.85, 19% protein, vitamin and mineral fortified; Purina Mills International, Richmond, IN) and water acidified with HCl to achieve a pH of 2.8-3.2 (which prevents bacterial growth) are freely available. Mouse colony maintenance and use is reviewed and approved by The Jackson Laboratory Institutional Animal Care and Use Committee and is in accordance with The National Institutes of Health guidelines for the care and use of animals in research.

PIXImus Densitometry

PIXImus scans (PIXImus, LUNAR, Madison, WI) which provide skeletal and body composition data such as Bone Mineral Density (BMD, g/cm2), Bone Mineral Content (BMC, g/cm2), body mass (g), lean mass (g), fat mass (g), and % fat mass, are completed on groups of 6 male and 6 female 12 week old mutant and control mice. The skulls and bodies are scanned separately to provide independent data on skull BMD and BMC and body BMD and BMC. The PIXImus small animal densitometer (DEXA) has a resolution of 0.18 x 0.18 mm pixels and is equipped with software version 1.46. The PIXImus is calibrated routinely with a phantom utilizing known values, and a quality assurance test is performed daily. The variability in precision for measuring total body BMD is, less than 1%, and approximately 1.5% for specialized regions such as the skull. The correlation between PIXImus BMD measurements of 614 lumbar vertebrae compared to peripheral quantitative computerized tomography (pQCT) measurements was found to be significant ($p<0.001$; $r=.704$) (Donahue, 1999),

Faxitron X-rays

X-rays at 5X magnification of the skull and at 3X magnification of the body of a male and female mutant and control at 12 weeks of age are obtained using a Faxitron MX20 cabinet X-ray (Faxitron X-Ray Corp., Wheeling, IL. USA) and Kodak Min-R 2000 mammography film (Eastman Kodak Co., Windsor, CO, USA). X-rays are then analyzed to determine the specificity of the skeletal phenotype.

Skull Preparation

Skulls of 6 male and 6 female mutants and controls are collected at 12 weeks of age, prepared by incomplete maceration in potassium hydroxide, stained with alizarin red, and stored in undiluted glycerin (Green, 1952). During the collection process, right ear pinnae are measured with digital hand calipers (Stoelting, Wood Dale, IL, USA).

Hand Caliper Skull Measurements

Seven measurements taken with hand held digital calipers are used routinely to define skull morphology at the Lab's craniofacial resource. These measures have a high degree of accuracy and precision in our hands and are able to discriminate differences between mutant and control skull characteristics. Our linear measures have been added to those illustrated by Dr. Joan Richtsmeier in her paper characterizing craniofacial differences in mouse models of Down Syndrome using three dimensional anatomical landmarks (Richtsmeier, 2000. Dev. Dyn. Feb; 217(2):137-45). Skulls are cleared with potassium hydroxide and stained with alizarin red dye in preparation for caliper measurements to be taken.

Skeletal Preps

In many cases whole skeletons of mutant and control mice are cleared in 1% KOH, stained with alizarin, stored in glycerin (Green, 1952) and then evaluated for skeletal malformations. Malformations found can indicate that the craniofacial phenotype is part of a greater syndrome.

Data Analysis

Hand caliper skull measurements and PIXImus skeletal and body composition data are evaluated using StatView

4.5 software (Abaccus Cary, NC) for Macintosh computers. Differences are considered significant when $p < 0.05$.

Molecular Mapping

Molecular mapping is completed using DNA extracted from tail or spleen through a Hot Sodium and Tris (HotSHOT) protocol (Truett, 2000). Primer pairs (MapPairs, Research Genetics, Huntsville Ala.) of microsatellite markers are used to establish and refine the initial mapping location. PCR products are visualized and scored via gel electrophoresis and ethidium bromide staining. Linkage analysis and recombination frequencies are calculated via the Map Manager computer program (Manly, 2001) and compared to known and predicted gene location data and marker location data published in the Celera and Ensembl genome databases and the Mouse Genome Informatics (MGI) website.

Sequencing

Primers are designed using published cDNA sequences from the Ensembl or Celera mouse genome databases. DNA is amplified through PCR and separated via gel electrophoresis. The resultant products are then removed and purified with QIAquick Gel Extraction Kits (Qiagen, Inc., Valencia, CA, USA) and sequenced using an Applied Biosystems 373A DNA Sequencer and an optimized DyeDeoxy Terminator Cycle Sequencing method.

References

- Donahue, L.R., Rosen, C.J., Beamer, W.G. (1999) *Comparison of Bone Mineral Content and Bone Mineral Density in C57BL/6J and C3H/HeJ Female Mice by pQCR (Stratec XCT 960M) and DEXA (PIXImus)*. Thirteenth International Mouse Genome Conference. Philadelphia, PA.
- Green, M.C. (1952) *A rapid method for clearing and staining specimens for the demonstration of bone.* The Ohio Journal of Science 52(1):31-33. January 1952
- Manly, K.F., Cudmore, R.H. Jr., Meer, J.M. (2001) *Map Manager QTX, cross-platform software for genetic mapping*. Mamm. Genome 12:930-932.
- Richtsmeier JT, Baxter, LL, Reeves, RH. (2000) *Parallels of craniofacial maldevelopment in Down syndrome and Ts65Dn mice*. Dev. Dyn. Feb;217(2):137-45.
- Truett, G.E., Heeger, P., Mynatt, R.L., Truett, A.A., Walker, J.A., Warman, M.L. (2000) *Preparation of PCR-quality mouse genomic DNA with hot sodium hydroxide and Tris (HoSHOT)*. Biotechniques 29;52-54.