Cauliflower ear-short ear 7 Jackson, a remutation to *Bmp5*

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Mutation (allele) name: cauliflower ear-short ear 7 Jackson

Mutation (allele) symbol: *Bmp5*$^{cfe\text{-}se7J}$

Gene symbol: *Bmp5*

Strain of Origin: C.129S7-Gt(ROSA)26Sor/J

Current Strain Name: C.129S7-Gt(ROSA)26Sor-*Bmp5*$^{cfe\text{-}se7J}$/J

Stock Number: 005420 (JaxMice.jax.org)

Phenotype Category: craniofacial

Discoverer: Phillip Russ

Origin and Description

We report here a new recessive mutation that arose spontaneously in the C.129S7-Gt(ROSA)26Sor/J colony, which we have named cauliflower ear-short ear 7 Jackson (*cfe-se7J*). The recessive mutation was discovered at the Jackson Laboratory in 2000 in an Induced Mutant Resource colony. The homozygous mutation always affects both ears and is characterized by small, round ear pinnae with ridges along the perimeter. The stock is currently maintained by mating homozygotes to heterozygotes of the opposite sex. Both males and females are fertile; females have normal litter sizes and lactate frequently. The strain was tested for penetrance by mating two homozygous mice and was found to be 100% penetrant. The *cfe-se7J* mutation was mapped to Chromosome 9 between the markers *D9Mit11* and *D9Mit259*. A negative complementation test with homozygous *Bmp5*^{se}*/Bmp5*^{se} mice from the Sea/GnJ strain determined that this new mutation is a remutation to *Bmp5*. Because of the ruffled ear pinnae of *cfe-se7J/cfe-se7J* mice compared to the smooth ear pinnae in *Bmp5*^{se}*/Bmp5*^{se} mice, we named this remutation "cauliflower ear-short ear 7 Jackson."

Genetic Analysis

The *cfe-se7J* allele is inherited as a recessive mutation as shown by traditional breeding experiments. For linkage analysis, a CAST/Ei male was mated to a homozygous *cfe-se7J/cfe-se7J* female. F1 hybrids were then intercrossed to produce F2 progeny. There were no visible mutants seen in the F1 generation (0/16) and 24% of F2 progeny were mutants (40/166). F2 progeny were observed for the cauliflower ear phenotype, and spleens and tail tips of affected mice were collected and stored at -70 C for subsequent DNA typing to map the mutation. DNA was extracted from the tail tips by a standard hot sodium and Tris (HotSHOT) procedure (Truett, et al., 2000) and polymerase chain reaction was carried out with MIT primer pairs (MapPairs, Research Genetics, Huntsville Ala.). A genome scan began with markers near the *Fgfr3* gene on Chromosome 5 because it displays defects within the inner ear, and the *Bmp5* gene on Chromosome 9. Linkage of *cfe-se7J* on Chromosome 9 was first detected with marker *D9Mit164* located at 37.0 cM. Twenty-one DNA samples were then typed for additional markers

until results showed linkage at 0% recombination with *D9Mit11* at 48 cM (Mouse Genome Database, JAX). A direct test for allelism confirmed *cfe-se7J* to be a remutation in the *Bmp5* gene.

Biological Characterization

A. DEXA Analysis of Whole Body BMD and Body Composition

Whole body weight assessed by PIXImus densitometry (GE LUNAR, Madison, WI) was not statistically significant different between mutants and controls in either females or males. Tail, femur, back and spine length were measured on six mutants and six controls in each sex, in order to determine a skeletal index (Table 1). Back length was measured from the first cervical vertebrae to the inferior surface of the ischial tuberosity. Spine length was measured from the first cervical vertebrae to the inferior surface of the fifth lumbar vertebrae. There were no significant differences found between mutants and controls. Ratios of tail length : back length, femur length:back length and spine length:back length were calculated (Table 2) and no significant differences were found. Whole body and skull x-rays of two male and two female mutants and controls were developed. After examination, there were no abnormalities found. Whole body bone mineral density (BMD) and whole body bone mineral content (BMC) assessed by PIXImus (Table 3) were less in both female and male mutants than in controls. BMD was statistically significant in both males and females (Figure 1), however BMC was not significant in either gender. There were no significant differences found with whole body lean and whole body fat between mutants and controls. However among homozygous *cfe/cfe* mice, females have significantly less fat and lean than males.

B. Craniofacial Morphology

Skulls of six male and six female mutants and 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). During the collection process, right ear pinnae were measured with digital hand calipers (Stoelting, Wood Dale, I11). Morphological measurements of the skull (Table 4) were also made using digital calipers (Stoelting, Wood Dale, Ill) with previously established landmarks (Richtsmeier, 2000). Both male and female mutants have shorter skull lengths as compared to controls (Figure 2) and were found to be statistically significant. Female mutants have statistically significant shorter nose lengths than controls (Figure 3). Male mutants have shorter nose lengths than controls, however, differences are not significant. Male *cfe-se7J/cfe-se7J* mice have a greater skull height than male controls (Figure 4) and differences were statistically significant. In contrast, female *cfe/cfe* mice have a lower skull height as compared to controls but differences are not significant. There is no difference in skull width among male and female mutants and controls. The upper and lower jaw lengths of both male and female mutants are shorter than controls (Figures 5 and 6). In females, the upper jaw length is not significantly different, but the lower jaw length is. In males, the upper jaw length is statistically significant and the lower jaw length is not. Finally, measurements of the right ear pinna were significantly lower in mutants than controls for both males and females (Figure 7).

In female mutants and controls, the ratio skull length to nose length is statistically significant (Figure 8), however it is not between male mutants and controls. The ratio skull height to skull length is significant in males but not in female mutants and controls (Figure 9). The ratio upper jaw to lower jaw in male and female mutants and controls is not statistically significant. Both male and female mutants and controls show statistically significant ratios between skull length to skull width (Figure 10). Finally, the skull height to skull width ratio is significant in male mutants and controls but not in females (Figure 11).

Table 1: Skeletal Index Measurements of Twelve Week C.129S7-Gt(ROSA)16Sor-*Bmp5*$^{cfe\text{-}se7J/}$J Mice (n=6, mean +/- SEM)

Measurements	Female +/?	Female Bmp5$^{cfe\text{-}se7J}$/*Bmp5*$^{cfe\text{-}se7J}$	Male +/?	Male Bmp5$^{cfe\text{-}se7J}$/*Bmp5*$^{cfe\text{-}se7J}$
Tail Length	9.008±.241	8.442±.283	8.717±.079	8.433±.120
Femur Length	1.492±.049	1.517±.017	1.492±.037	1.467±.033
Back Length	6.717±.087	6.667±.131	6.992±.066	7.000±.043
Spine Length	4.892±.093	4.842±.080	5.183±.059	5.292±.045

Table 2: Ratios of Skeletal Index Measurements of Twelve Week C.129S7-Gt(ROSA)26Sor-*Bmp5*$^{cfe\text{-}se7J}$/J (n=6, mean)

Measurements	Female +/?	Female Bmp5$^{cfe\text{-}se7J}$/*Bmp5*$^{cfe\text{-}se7J}$	Male +/?	Male Bmp5$^{cfe\text{-}se7J}$/*Bmp5*$^{cfe\text{-}se7J}$
Tail Length : Back Length	1.341	1.270	1.247	1.205
Spine Length : Back Length	.728	.727	.742	.756
Femur Length: Back Length	.223	.228	.213	.210

Table 3: PIXImus Densitometric Measurements of Twelve Week Old C.129S7-Gt(ROSA)26Sor-*Bmp5*$^{cfe\text{-}se7J}$/J (n=6, mean +/- SEM)

Measurements	Female +/?	Female Bmp5$^{cfe\text{-}se7J}$/Bmp5$^{cfe\text{-}se7J}$	Male +/?	Male Bmp5$^{cfe\text{-}se7J}$/Bmp5$^{cfe\text{-}se7J}$
Whole Body BMD (g/cm2)	.046±.002	.043±.001 [b]	.045±.002	.044±.001 [b]
Whole Body BMC (g)	.421±.038	.394±.119	.447±.055	.422±.031
Whole Body Fat (g)	2.750±.428	2.633±.408	3.267±.997	3.383±.354
Whole Body Lean (g)	14.367±1.583	13.683±1.303	18.117±1.359	17.533±.866
Body Weight (g)	17.083±.779	16.300±.672	21.383±.522	20.917±.357

*b = p-value <.05

Table 4: Digital Caliber Measurements of Twelve Week C.129S7-Gt(ROSA)26Sor-*Bmp5*$^{cfe\text{-}se7J}$/J skulls stained with Alizaren Red (n=6, mean +/- SEM)

Measurements	Female +/?	Female Bmp5$^{cfe\text{-}se7J}$/*Bmp5*$^{cfe\text{-}se7J}$	Male +/?	Male Bmp5$^{cfe\text{-}se7J}$/*Bmp5*$^{cfe\text{-}se7J}$
Skull Length	22.7071±.345	22.003±.409 [b]	22.875±.288	21.993±.762 [b]
Nose Length	15.457±.208	14.542±.478 [b]	15.338±.304	14.790±.536
Skull Height	10.158±.182	10.042±.251	10.122±.251	10.437±.135[b]
Skull Width	10.238±.083	10.215±.041	10.432±.116	10.462±.064
Upper Jaw Length	16.528±.346	16.027±.556	16.677±.387	15.830±.458 [b]
Lower Jaw Length	10.580±.151	10.305±.116 [b]	10.668±.213	10.443±.170
Right Ear Pinna	14.353±.225	9.010±.986 [b]	14.215.±.601	9.592.±.758 [b]
Skull length:Nose length Ratio	1.469	1.514[b]	1.492	1.487
Skull height:length ratio	.447	.456	.442	.475[b]
Upper:Lower Jaw Ratio	1.562	1.556	1.516	1.563
Skull length: width ratio	2.218	2.154[b]	2.194	2.102[b]
Skull Height : width ratio	.992	.983	.970	.998[b]

*b = p-value <.05

Figure 1: BMD in *Bmp5*$^{cfe\text{-}se7J}$/*Bmp5*$^{cfe\text{-}se7J}$ Male and Female Mice Compared to Controls

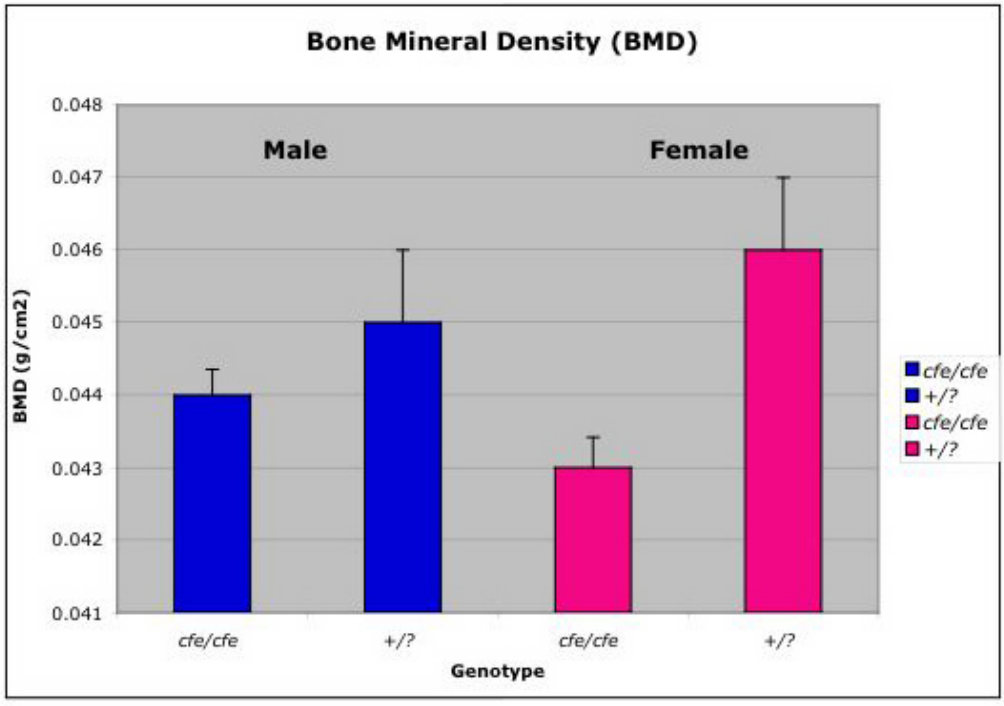


Figure 2: Skull Length in *Bmp5*$^{cfe\text{-}se7J}$/*Bmp5*$^{cfe\text{-}se7J}$ male and Female Mice Compared To Controls

Figure 3: Nose Length in *Bmp5*$^{cfe\text{-}se7J}$/*Bmp5*$^{cfe\text{-}se7J}$ Male and Female Mice Compared to Controls

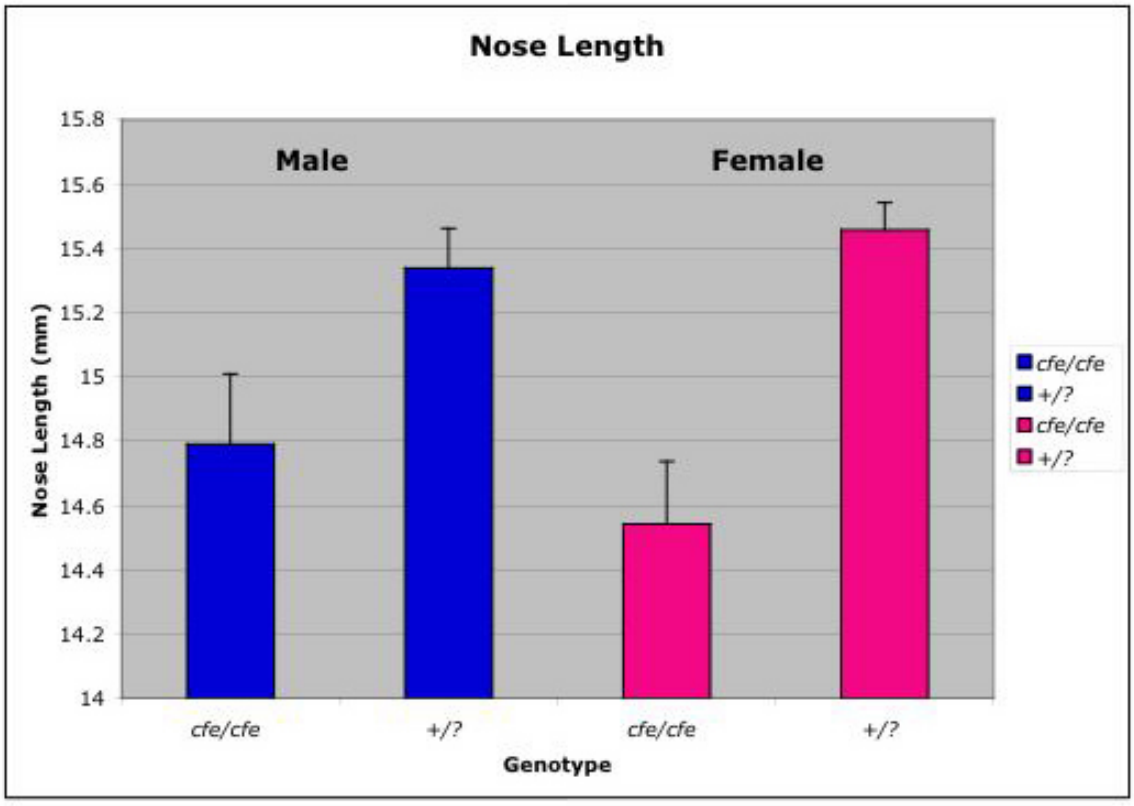


Figure 4: Skull Height in *Bmp5*$^{cfe\text{-}se7J}$/*Bmp5*$^{cfe\text{-}se7J}$ Male and Female Mice Compared to Controls

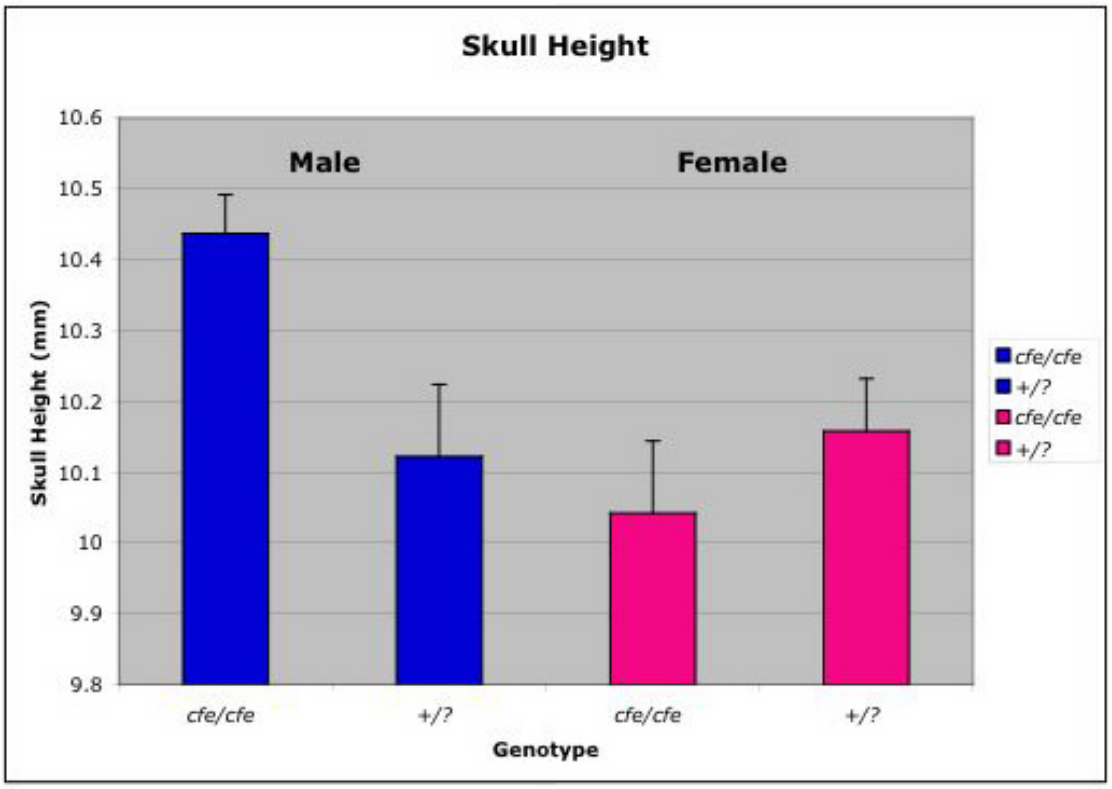


Figure 5: Upper Jaw Length in *Bmp5*$^{cfe\text{-}se7J}$/*Bmp5*$^{cfe\text{-}se7J}$ Male and Female Mice Compared to Controls

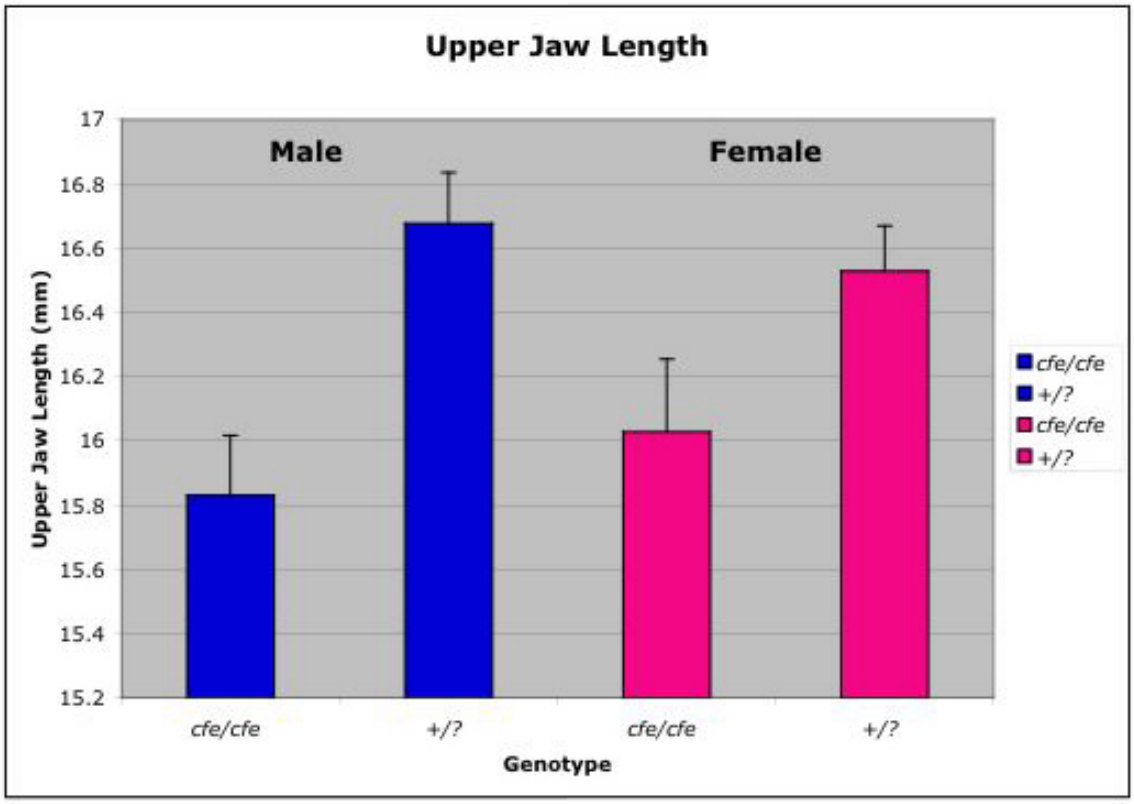


Figure 6: Lower Jaw Length in *Bmp5*$^{cfe\text{-}se7J}$/*Bmp5*$^{cfe\text{-}se7J}$ Male and Female Mice Compared To Controls

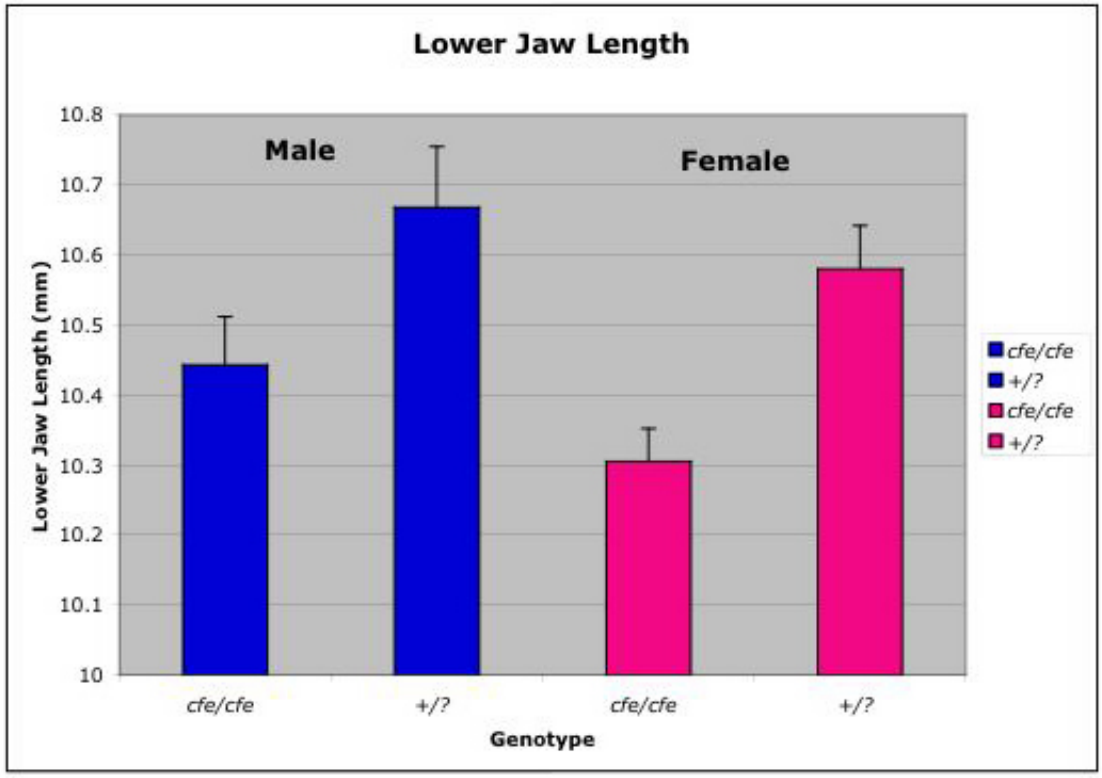


Figure 7: Right Ear Pinna in *Bmp5*$^{cfe\text{-}se7J}$/*Bmp5*$^{cfe\text{-}se7J}$ Male and Female Mice Compared to Controls

Figure 8: Skull Length to Nose Length Ratio in *Bmp5*$^{cfe\text{-}se7J}$/*Bmp5*$^{cfe\text{-}se7J}$ Male and Female Mice Compared to Controls

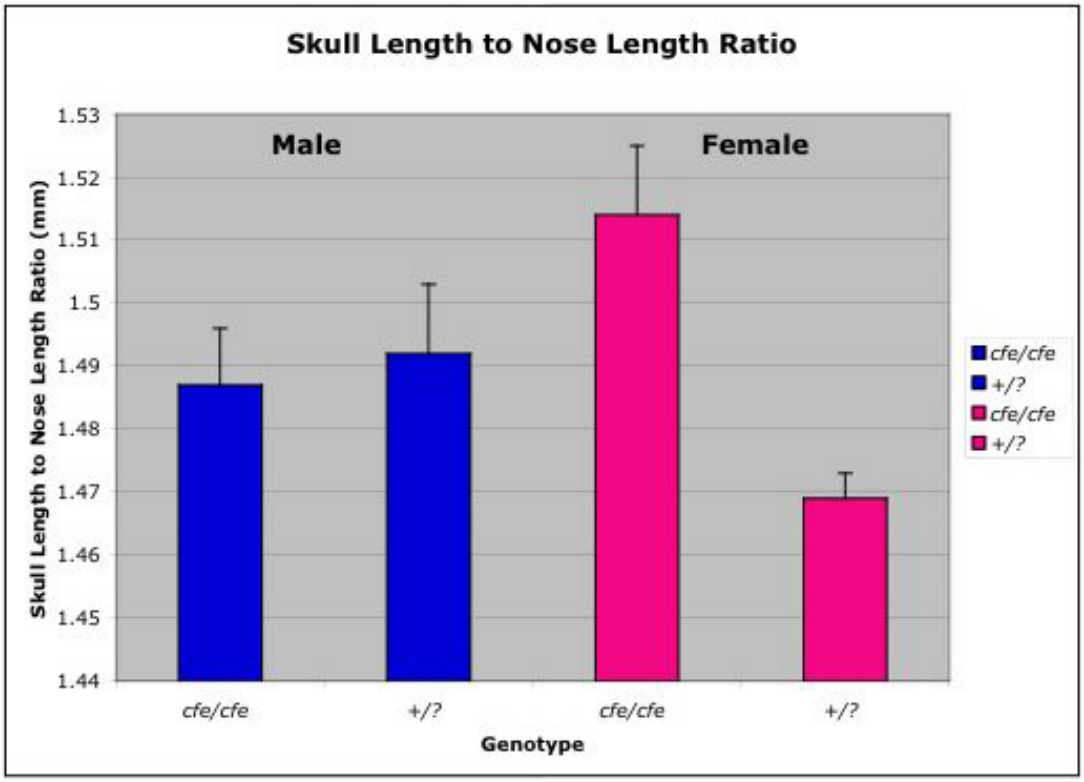


Figure 9: Skull Height to Skull Length Ratio in *Bmp5*$^{cfe\text{-}se7J}$/*Bmp5*$^{cfe\text{-}se7J}$ Male and Female Mice Compared to Controls

Figure 10: Skull Length to Skull Width Ratio in *Bmp5*$^{cfe\text{-}se7J}$/*Bmp5*$^{cfe\text{-}se7J}$ Male and Female Mice Compared to Controls

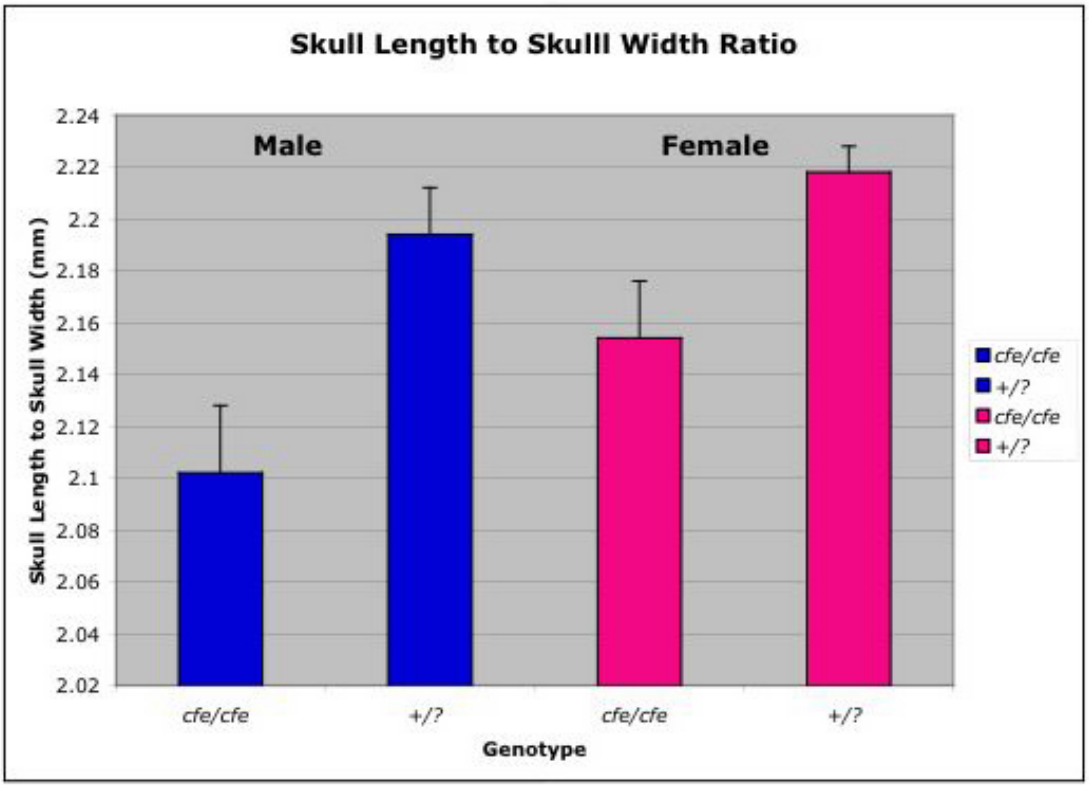
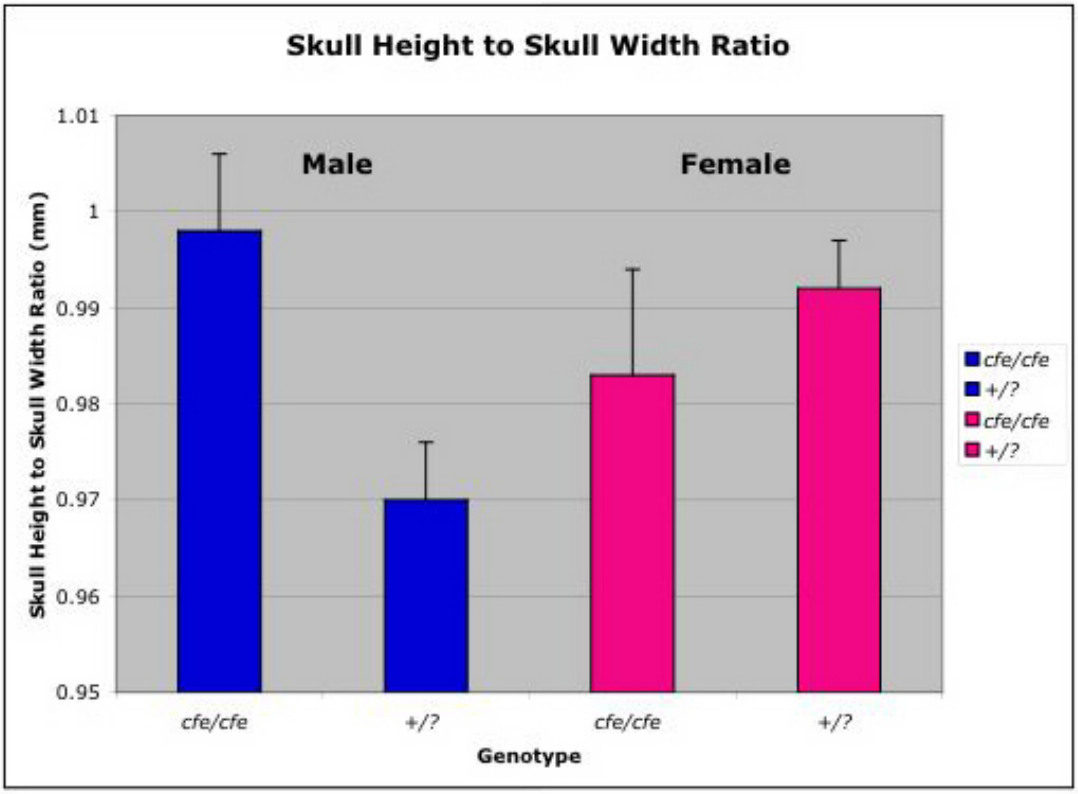


Figure 11: Skull Height to Skull Width Ratio in *Bmp5*$^{cfe\text{-}se7J}$/*Bmp5*$^{cfe\text{-}se7J}$ Male and Female Mice Compared to Controls



C. Hearing tests
Hearing was assessed by ABR threshold analysis (Zheng et al. 1999) with two mutants and one control mouse at three months of age. The ABR results showed that both mutants and control mice have normal hearing.

D. Eye Examination
Two three-month old mutant and control male mice were examined by a slit lamp and indirect ophthalmoscope. Some mice showed an early onset of severe cataract, which may be progressive. Otherwise, eyes are normal.

Pathology
One male control and one female mutant were perfused at six weeks of age via cardiac infusion of Bouin's fixative following admission of anesthesia. No lesions were found in any major organ.

Discussion
Bone morphogenetic protein (BMP) is a family of highly conserved, secreted proteins that affect differentiation, axis formation, growth control, and sexual development (Massague, 1990). It was found that mice carrying the recessive short ear mutation displayed defects within one of the eight *Bmp* genes (Kingsley, 1992). Studies have shown that the classical short ear mutation presents with a nonsense mutation within the coding region of the *Bmp5* gene (King, 1994). Therefore, BMP5 plays a key role in skeletal patterning and soft tissue development (King, 1994).

Phenotypically, mice with the short ear mutation develop characteristic skeletal defects, including reduction of the external ear, loss of several small bones, alterations in size or shape of the xiphoid process, reduction of ventral processes at the sixth cervical vertebrae and deletion of one pair of ribs (Green and Green, 1946; Green, 1951,1968). Measurements of the skull show that short ear mice have a wider skull and a shorter nose than wild types (Kingsley, 1994). There are also a variety of soft tissue anomalies, including an increased frequency of misplaced ovaries, hydrotic kidneys, lung cysts, liver granulomas, and neuromuscular tail kinks that occur on inbred strains (Green, 1968). Various phenotypes have been reported for the short ear mutation, which suggests modifier genes are present in different mouse strains (Green, 1957).

The cauliflower ear mutation presents with a shortened external ear but with ruffles along the edges of the ear pinnae. This short ear allele is viable within this congenic strain and shows typical Mendelian ratios. There are no differences in body size or shape between the cauliflower mutant and wild type. There were no differences in the skeletal index measurements, including length of the femur, tail, back and spine. Finally, we have not found in our $Bmp5^{cfe\text{-}se7J}/Bmp5^{cfe\text{-}se7J}$ mice any skeletal or soft-tissue abnormalities with routine pathological inspection.

The whole body bone mineral density and whole body bone mineral content is less in mutants than controls due to a disruption in skeletal patterning within the bones of BMP5 deficient mice. However, whole body BMD is significant and BMC is not, which is a result of the small sample size and the high standard error of measurement found within the BMC data. The skull length and nose length and the upper and lower jaw lengths of the *cfe-se7J/cfe-se7J* mice are shorter than controls in both genders. Skull length is significant in both genders, however, nose length is significant in females only. There is a significant difference in the lower jaw of females and the upper jaw is significant in males only. Male mutants have a greater skull height than female mutants and controls. There were no differences found with skull width between mutants and

wild types. The skull height to skull length and skull height to skull width ratios are significant in males but not females. The skull length to nose length ratio is significant in females and not males. Both males and females have a significant skull length to skull width ratio. However, upper jaw and lower jaw ratios are not significant in either gender. Therefore, *cfe-se7J/cfe-se7J* mice of both genders display a shorter skull length, smaller ear pinnae, and shorter upper and lower jaws than controls. Females have a shorter nose length and a shorter skull height, while males have a greater skull height than females. Although the bodies are well proportioned in this strain, the skull measurements are different between male and female mutants and controls.

The morphological differences seen between the original short ear mutation and *cfe-se7J* remutation may be due to differing genetic backgrounds. Further exploration of potential modifying genes contributed by various inbred backgrounds could help to elucidate the functional pathways of BMP5 protein and its role in skeletal and soft-tissue abnormalities. This could be of great use to determine the mechanisms responsible for human EPS (ear, patella, short stature) syndrome, which could be the human equivalent of BMP5 deficiency in the mouse (Lacombe, 1994).

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