Helicopter Ears: A Structural Mutation

Authors: Michelle M. Curtain and Leah Rae Donahue

Source of Support: This research was supported by NIH-NEI grant EY015073 to Leah Rae Donahue, PI.

Mutation Symbol: *He*

Mutation Name: Helicopter Ears

Strain of Origin: B6129SF2/J

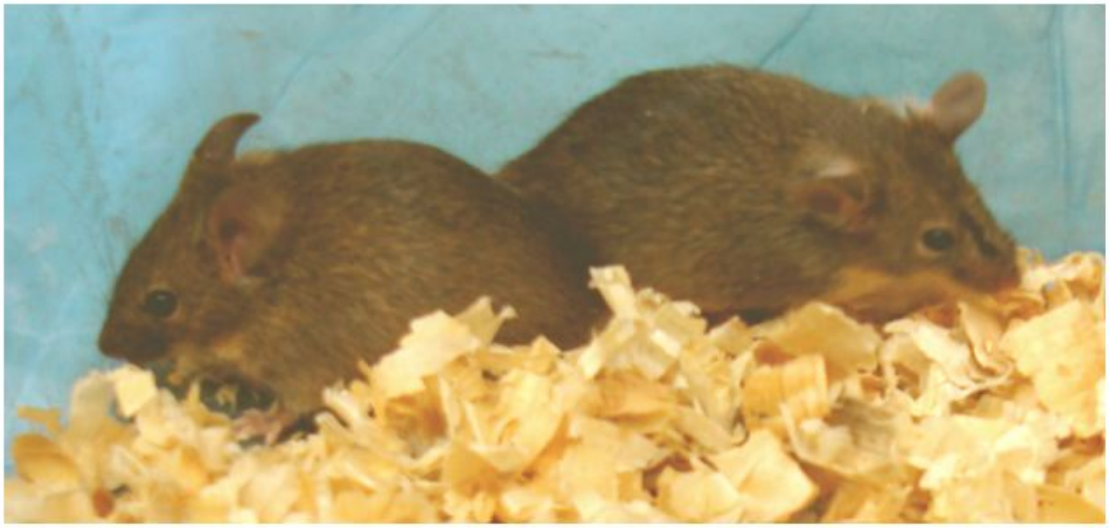
Current Strain Name: B6;129P2-*He*/J

Stock #007947 (jaxmice.jax.org)

Phenotype Category: craniofacial, skeletal

Abstract
We report here an ear pinnae mutation along with a skeletal phenotype.

Origin and Description
Helicopter Ears (*He*) was discovered in a production colony of B6129SF2/J (Stock #101045) mice in April 2006. This new spontaneous, dominant mutation is characterized by ear pinnae angled outward from the head rather than upward. Mutants also have smaller bodies than littermate controls. Male and female heterozygotes are fertile even though mutants exhibit smaller genitals.



A helicopter ears heterozygote is shown on the right and a control littermate is seen on the left.

Genetic Analysis
The *He* mutation was proven to be a dominant mutation when affected mice arose in the first generation of a *+/He* by C57BL/6J mating. Using the standard mapping protocol of The Mouse Mutant Resource, the *He* mutation was mapped by mating a female *+/He* with a CAST/Ei inbred male. Affected F1s were then backcrossed to another CAST/Ei. Affected N2 mice were collected and tissue was stored at -70 degrees C. DNA was

extracted from either spleen or tail tips using standard phenol extraction methods. Polymerase chain reaction was done with MIT or Research Genetics primer pairs (MapPairs, from Research Genetics, Huntsville, Ala., or from Integrated DNA Technologies, Coralville, Ia.). Linkage cross analysis of 20 *+/He* mice showed no recombination near the centromere of Chromosome four. The three markers in this non-recombinant region are *D4Mit316* at 5.2Mb, *D4Mit235* at 8.3Mb and *D4Mit181* at 9.5 Mb. The first recombination is at 24.5 Mb at marker *D4Mit105* with 5% (1/20). Recombination increased at 45.1 Mb (*D4Mit5*) to 17.6% (3/17).

A preliminary look at homozygote viability was done. We mated *+/He* to a CAST/Ei inbred mouse and collected DNA from neonate and embryonic F2s. DNA from a one-week-old litter consisted four +/+ and eight *+/He*. DNA from E 14.5 typed for three wild types, five heterozygotes and two homozygotes. Results indicate that *He/He* are not born but are still in utero at E14.5 though more numbers would be needed to confirm homozygote lethality.

Biological Characterization

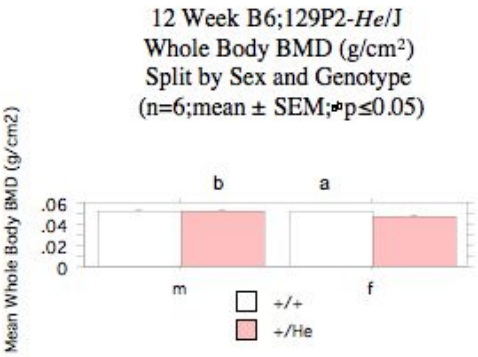
-DEXA analysis of whole body aBMD and body composition of twelve-week-old mice:

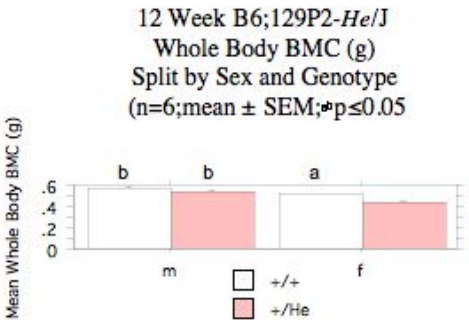
Three-month-old male and female mutant and controls were collected and whole body, areal bone mineral density (aBMD), bone mineral content (BMC) and body composition (lean, fat and % fat mass) were assessed by PIXImus densitometry[1]. Overall *+/He* females differed significantly from *+/He* males and +/+ females. Female mutants had significantly less whole body aBMD and BMC, fat, total mass, skull aBMD and BMC. The composition and skeletal phenotype appears to mainly affect females.[2]

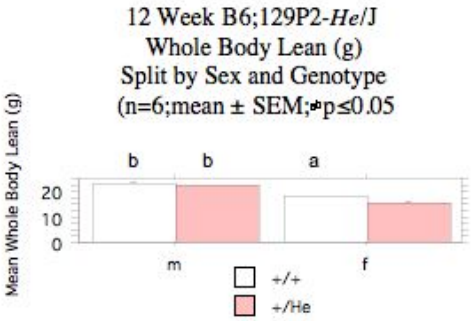
Table 1:PIXImus Densitrometric Measurements of Twelve-Week-Old B6;129P2-*He*/J Mice (n=6; mean±SEM; $^{ab}p \leq 0.05$)

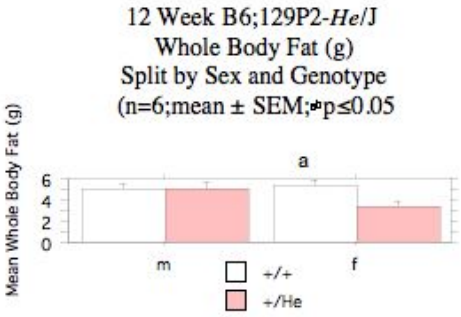
Measurements	Male *+/He**	Male +/+	Female *+/He*	Female +/+
Whole Body BMD (g/cm^2)	0.0515 ± .0.00119^b	0.0522 ± 0.00049	0.0471 ± 0.00108^a	0.0519 ± 0.00111
Whole Body BMC (g)	0.528 ± 0.0255^b	0.566 ± 0.0110^b	0.429 ± 0.0197^a	0.514 ± 0.0247
Whole Body Lean (g)	22.1 ± 0.50^b	23.1 ± 0.34^b	15.2 ± 0.91^a	17.8 ± 0.48
Whole Body Fat (g)	5.0 ± 0.59	5.0 ± 0.41	3.4 ± 0.52^a	5.3 ± 1.47
Total (g)	27.08 ± 0.982^b	28.3 ± 0.563^b	18.57 ± 1.417^a	23.02 ± 1.830
% Fat	18	18	18	23
Skull BMD (g/cm2)	0.1153 ± 0.00230	0.1176 ± 0.00050^b	0.1164 ± 0.00334^a	0.1245 ± 0.00322
Skull BMC (g)	0.288 ± 0.006	0.296 ± 0.0034	0.268 ± 0.0127^a	0.304 ± 0.0098
Skull BMD/Body BMD (g/cm^2)	2.2437 ± 0.04396^b	2.2541 ± 0.02245^b	2.4701 ± 0.05033	2.3976 ± 0.09164

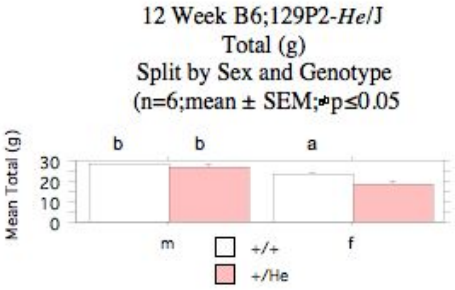
*n=5

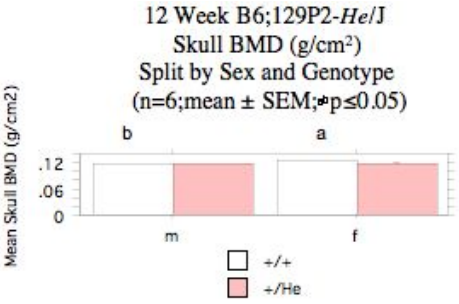


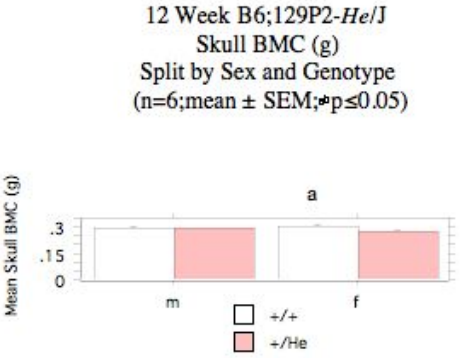


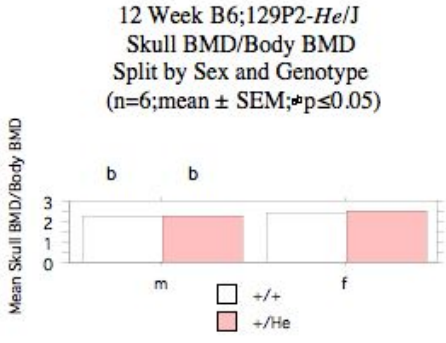












-Craniofacial morphology of twelve-week-old mice:

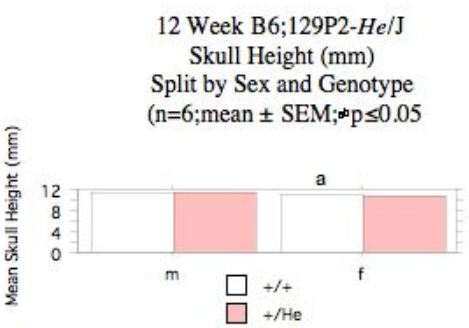
Three-month-old mutant and control skulls were collected from both genders and were prepared by incomplete maceration in potassium hydroxide, stained with alizarin red, and stored in undiluted glycerin (Green, 1952). Morphological measurements of the skull were made using digital calipers (Stoelting, Wood Dale, Ill) with previously established

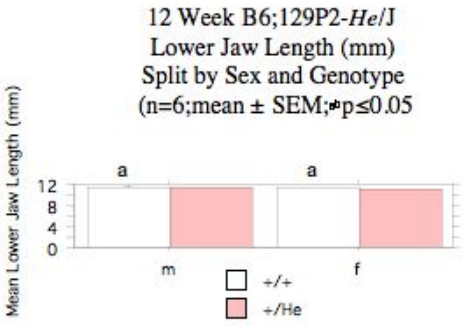
landmarks (Richtsmeier, 2000)[1]. Female +/*He* had significantly less skull height and lower jaw length than female controls. Male mutants had significantly lower jaw length compared to male controls. Ear pinna was significantly reduced in both male and female mutants compared to same sex controls.

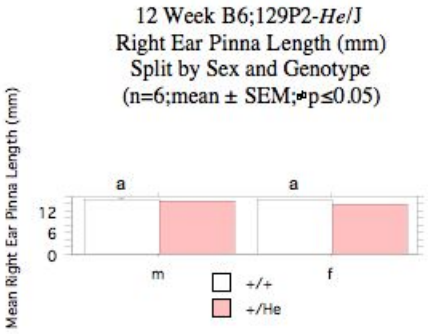
Table 2: Digital Caliper Measurements and Calculated Ratios of Twelve-Week-Old B6;129P2-*He*/J of Skulls Stained with Alizarin Red (n=6; mean±SEM; $^{ab}p \leq 0.05$)

Measurements	Male +/*He**	Male +/+	Female +/*He*	Female +/+
Skull Length (mm)	23.81 ± 0.078	23.74 ± 0.085	22.79 ± 0.191	23.28 ± 0.314
Nose Length (mm)	16.24 ± 0.153	16.13 ± 0.057	15.24 ± 0.215	15.63 ± 0.309
Skull Height (mm)	11.23 ± 0.097	11.19 ± 0.155	10.55 ± 0.138[a]	10.98 ± 0.1047
Skull Width (mm)	10.68 ± 0.078	10.82 ± 0.099	10.38 ± 0.105	10.70 ± 0.286
Inner Canthal Distance (mm)	6.20 ± 0.141	6.17 ± 0.069	5.89 ± 0.078	6.10 ± 0.240
Upper Jaw Length (mm)	16.95 ± 0.177	17.20 ± 0.146	16.38 ± 0.131	16.79 ± 0.382
Lower Jaw Length (mm)	11.21 ± 0.031[a]	11.47 ± 0.086	10.88 ± 0.117[a]	11.23 ± 0.193
Jaw Length Ratio	1.51 ± 0.016	1.50 ± 0.021	1.51 ± 0.008	1.50 ± 0.018
Skull/Nose Length Ratio	1.47 ± 0.013	1.47 ± 0.009	1.50 ± 0.012	1.49 ± 0.017
Skull Height/Length Ratio	0.47 ± 0.004	0.47 ± 0.007	0.46 ± 0.004	0.47 ± 0.005
Skull Length/Width Ratio	2.23 ± 0.022	2.20 ± 0.022	2.20 ± 0.017	2.18 ± 0.070
Skull Height/Width Ratio	1.05 ± 0.014	1.03 ± 0.007	1.02 ± 0.005	1.03 ± 0.023
Ear Pinna Height (mm)	14.52 ± 0.307[ab]	15.29 ± 0.047	13.73 ± 0.165[a]	15.08 ± 0.291

*n=5







[a] +/+ vs. +/*He* within sex
[b] male vs. female within genotype

Pathology

Using the standard pathology screen of The Mouse Mutant Resource, no lesions were seen in a three-month-old male mutant. Ear pinnae were examined in a nine-month-old female mutant and were histologically normal. Hearing was assessed by auditory brainstem response (ABR) on males and females. Three mutants and four controls at 10-weeks-old had normal hearing; a different set of four mutants and two controls were all normal at two to three-months-old. A clinical eye exam of three mutants and four controls of both sexes revealed no abnormalities, and an electroretinography (ERG) on a 54-week-old male mutant was normal.

Discussion

The Helicopter ear mutation affects both sexes equally in regard to the pinnae phenotype, however with regard to the skeleton and body composition phenotype, females are more affected. Hearing is not impaired.

Acknowledgements

We would like to thank Jo-Lyn Harper for discovering this mutation; Heping Yu and Chantal Longo-Guess for ABR analysis; Norm Hawes for clinical eye evaluation; Coleen Kane for preparation of tissues for histological assessment; Rod Bronson, Ph.D, for pathological evaluation and Patricia Ward-Bailey for manuscript assistance and web publication.

[1]Standard Protocols and Procedures of The Jackson Laboratory Craniofacial Mutant 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 website.

Sequencing

Primers are designed using published cDNA sequences from the Ensemble 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.*