Froggy; a new mutation on Chromosome 13 affecting skull shape and body size

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Source of Support: This research was supported by NIH/NCRR grant RR01183 to the Mouse Mutant Resource and NIH-NEI grant EY015073 to Leah Rae Donahue

Mutation (allele) symbol: *frg*

Mutation name: froggy

Strain of origin: A/J

Current strain name: A/J-*frg*/J

Stock #003485 (jaxmice.jax.org)

Abstract
We report a spontaneous mutation with a shortened face and smaller body that has been mapped to chromosome 13.

Figure 1: *frg*/*frg* mouse on left and littermate control on the right

Origin and Description
The recessive froggy (*frg*) mutation arose in Ed Birkenmeier's research colony at The Jackson Laboratory and was discovered by C. Kelly and A. Williams in 1992. We confirmed a recessive mode of inheritance by mating a mouse homozygous for the *frg* mutation to a C57BL/6J inbred mouse and there were no affected progeny observed in the F1 offspring of this mating. The *frg* mutation has been on an inbred A/J background for 46 generations and is maintained by mating a homozygous female or male to a heterozygous littermate. Heterozygous x heterozygous matings are also performed since homozygous mice are not always dependable breeders. The average litter size is 6 to 7 pups for a *+/frg* x *+/frg* mating. The litter size is much less (3 to 4 pups) when a homozygote is mated to a heterozygote, with about half of these matings being nonproductive.

The froggy mutation affects body size and skull shape. The mutation has varied penetrance ranging from an obvious shortened face to less severe where the length of the face is less affected. Despite this varied phenotype, mutants can always be determined due to their wide set eyes. Both males and females are affected and have normal fertility and life span.

Genetic Analysis

Using the standard mapping protocol of The Mouse Mutant Resource, a linkage cross was set up by mating a female C57BL/6J to a homozygous male *frg/frg*. The heterozygous offspring of this mating were then backcrossed to a *frg/frg* and produced 50 affected N2 mice that were used for linkage analysis. A genome scan confirmed a map location on Chromosome 13. The mutation maps between *D13Mit88* (NCBI 36 position 38.8 Mb) and *D13Mit248* (NCBI 36 position 53 Mb) and is non-recombinant with *D13Mit63* (NCBI 36 position 42.7 Mb), *D13 Mit179* (NCBI 36 position 44.8 Mb), *D13Mit91* (NCBI 36 position 46.9 Mb) and *D13Mit139* (NCBI 36 position 51.8 Mb).

Biological Characterization

Craniofacial morphology of sixteen-week-old mice: Skulls were prepared by incomplete maceration in potassium hydroxide, stained with alizarin red, and stored in undiluted glycerin (Green, 1952). Morphological measurements of the skull (Table 1) were made using digital calipers (Stoelting, Wood Dale, Ill) with previously established landmarks (Richtsmeier, 2000)[1]. Male and female *frg* mutants had significantly shorter skull, nose and upper jaw lengths compared to same sex controls. Mutants had significantly wider inner canthal distance than controls. Male mutants also had significantly less skull height than male controls along with significantly less skull height/width ratio. Skull length to nose length and skull height to skull length ratios were greater in the mutants. Jaw Length ratio and skull length to skull width was significantly less in mutants.

Table 1: Digital Caliper Measurements and Calculated Ratios of Sixteen Week Old A/J-*frg*/J Skulls Stained with Alizarin Red (n=6-10;mean ± SEM;$^{ab}p \leq 0.05$)

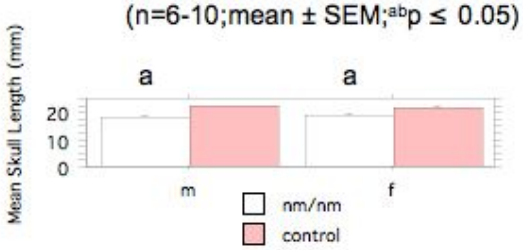
Measurements	Male nm/nm n=7	Male +/nm n=10	Female nm/nm n=6	Female controls* n=6
Skull Length (mm)	17.983±0.664 [a]	22.341±0.167	18.532±0.744 [a]	21.648±0.407
Nose Length (mm)	9.911±0.731 [a]	14.673±0.158	10.487±0.601 [a]	14.173±0.357
Skull Height (mm)	9.817±0.095 [a]	10.186±0.116	10.077±0.132	9.857±0.154
Skull Width (mm)	9.953±0.124	9.839±0.115	9.980±0.082	9.925±0.227
Inner Canthal Distance (mm)	5.936±0.261 [a]	5.378±0.081 [b]	5.940±0.125 [a]	5.085±0.023
Lower Jaw Length (mm)	10.579±0.100	10.607±0.128	10.688±0.099	10.788±0.199
Upper Jaw Length (mm)	11.394±0.821 [a]	15.680±0.168	12.488±0.790 [a]	15.515±0.181
Jaw Length Ratio	1.077±0.076 [a]	1.481±0.026	1.167±0.071 [a]	1.440±0.020
Skull/Nose Length Ratio	1.843±0.070 [a]	1.524±0.013	1.777±0.042 [a]	1.529±0.012
Skull Height/Length Ratio	0.551±0.022 [a]	0.456±0.005	0.549±0.027 [a]	0.456±0.005
Skull Length/Width Ratio	1.805±0.050 [a]	2.272±0.021 [b]	1.857±0.075 [a]	2.182±0.016
Skull Height/Width Ratio	0.988±0.019 [a]	1.036±0.012	1.010±0.014	0.995±0.017

* controls=+/nm and +/? combined

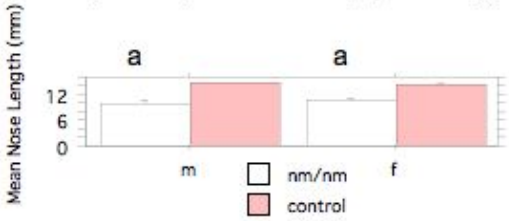
nm/nm=frg/frg
+/nm=+/frg

[a]. p ≤0.05 nm/nm vs. controls within sex
[b]. p≤ 0.05 males vs. females within genotype

16 week A/J-*frg*/J
Skull Length (mm)
Split by Sex and Genotype
(n=6-10;mean ± SEM;$^{ab}p \leq 0.05$)

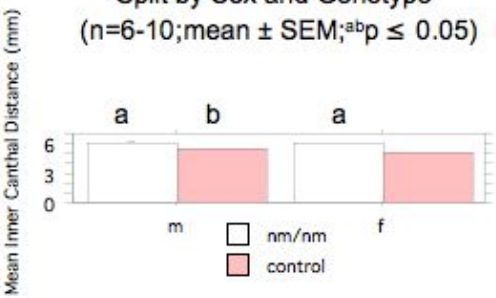


16 week A/J-*frg*/J
Nose Length (mm)
Split by Sex and Genotype
(n=6-10;mean ± SEM;$^{ab}p \leq 0.05$))

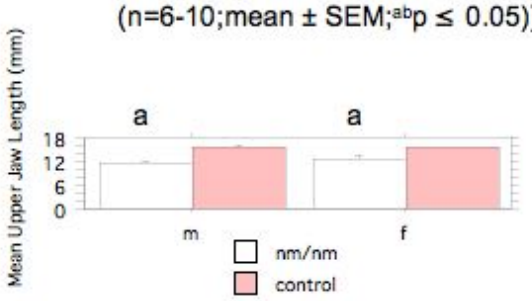


16 week A/J-*frg*/J
Skull Height (mm)
Split by Sex and Genotype
(n=6-10;mean ± SEM;$^{ab}p \leq 0.05$))

16 week A/J-*frg*/J
Inner Canthal Distance (mm)
Split by Sex and Genotype
(n=6-10;mean ± SEM;$^{ab}p \leq 0.05$)

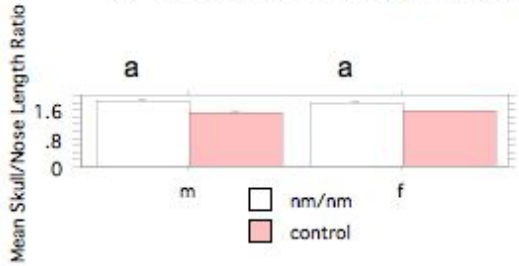


16 week A/J-*frg*/J
Upper Jaw Length (mm)
Split by Sex and Genotype
(n=6-10;mean ± SEM;$^{ab}p \leq 0.05$))

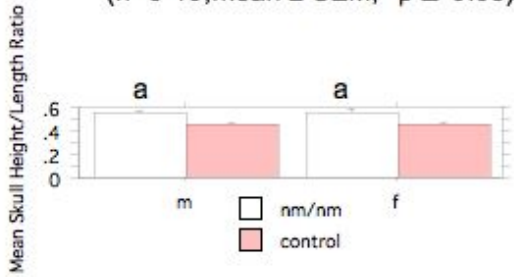


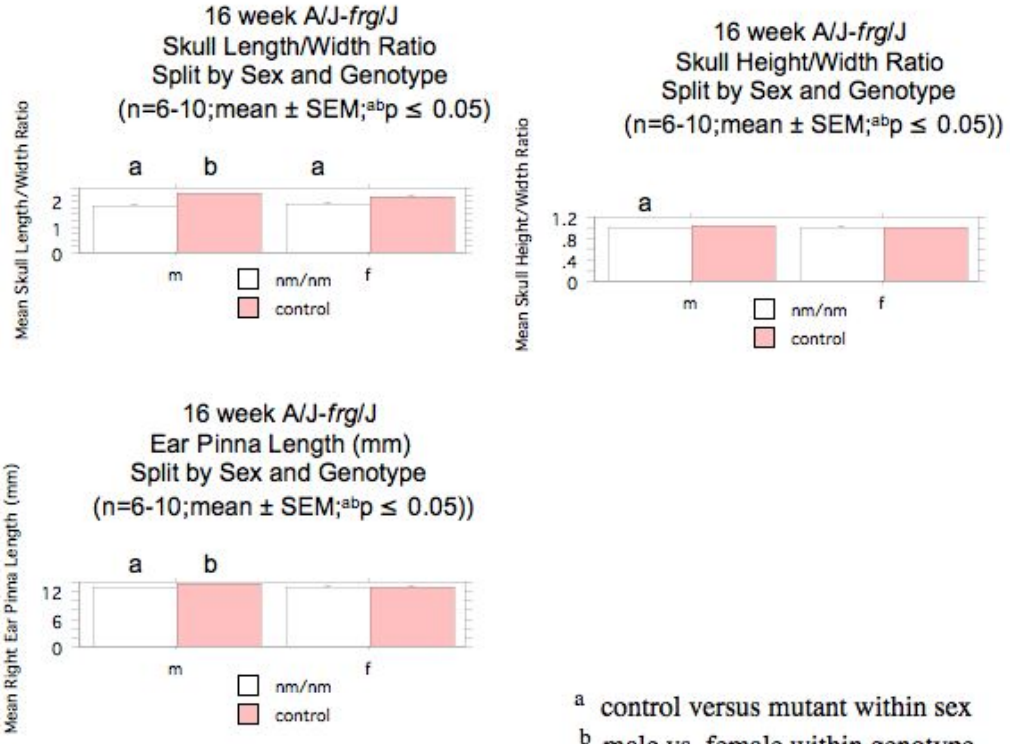
16 week A/J-*frg*/J
Jaw Length Ratio
Split by Sex and Genotype
(n=6-10;mean ± SEM;$^{ab}p \leq 0.05$))

16 week A/J-*frg*/J
Skull/Nose Length Ratio
Split by Sex and Genotype
(n=6-10;mean ± SEM;$^{ab}p \leq 0.05$))



16 week A/J-*frg*/J
Skull Height/Length Ratio
Split by Sex and Genotype
(n=6-10;mean ± SEM;$^{ab}p \leq 0.05$))





[a] control versus mutant within sex
[b] male vs. female within genotype

* controls=+/nm and +/? combined

nm/nm=frg/frg
+/nm=+/frg

DEXA analysis of whole body aBMD and body composition of sixteen-week-old mice: Whole body, areal bone mineral density (aBMD), bone mineral content (BMC) and body composition (lean, fat and % fat mass) was assessed by PIXImus densitometry[1] (Table 2)

Table 2: Hand Caliper Ear Pinna Lengths (mm) and PIXImus Densitometric Measurements of Sixteen Week Old A/J-*frg*/J Exvivo Skulls
(n=6-10;mean ± SEM;[ab]p ≤ 0.05)

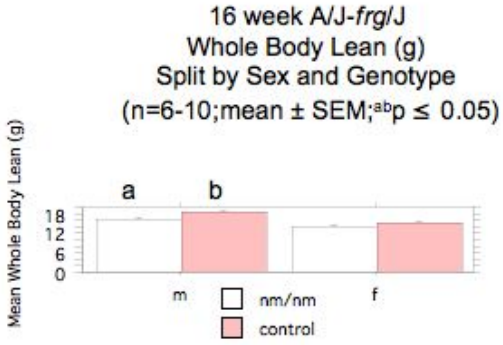
Measurements	Male nm/nm n=7	Male +/nm n=10	Female nm/nm n=6	Female controls* n=6
Right Ear Pinna Length	12.749±0.191 [a]	13.574±0.121 [b]	12.668±0.381	12.950±0.102
Whole Body BMD (g/cm^2)	0.040±0.0003	0.042±0.001	0.041±0.002	0.042±0.0004
Skull BMD/Body BMD	2.527±0.014	2.439±0.053	2.635±0.149	2.459±0.035
Whole Body BMC (g)	0.308±0.009	0.372±0.035	0.290±0.030	0.328±0.011
Whole Body Lean (g)	15.843±0.709 [a]	18.200±0.561 [b]	13.667±0.753	14.850±0.781
Whole Body Fat (g)	2.729±0.256 [a]	4.000±0.394	2.733±0.276	3.517±0.791
Total Mass (g)	18.586±0.929 [a]	22.140±0.887 [b]	16.400±0.887	18.400±1.508
% Fat	14.471±0.845	17.590±1.188	16.667±1.488	18.133±2.658
Skull BMD (g/cm^2)	0.100±0.001 [a b]	0.102±0.0004	0.108±0.002 [a]	0.103±0.001
Skull BMC (g)	0.194±0.004 [a]	0.218±0.005	0.209±0.009	0.211±0.010

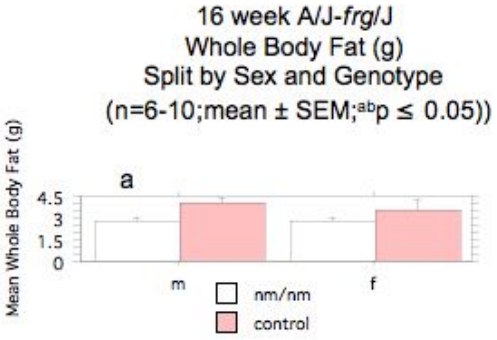
[a]. p ≤0.05 nm/nm vs. controls within sex
[b]. p≤ 0.05 males vs. females within genotype

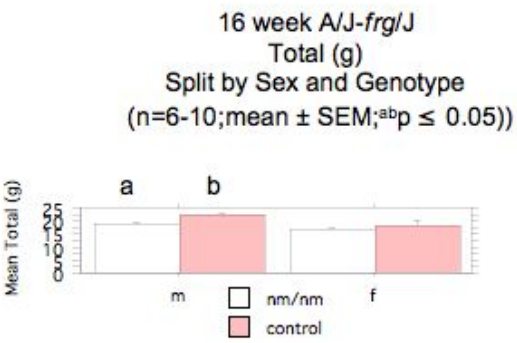
nm/nm=frg/frg
+/nm=+/frg

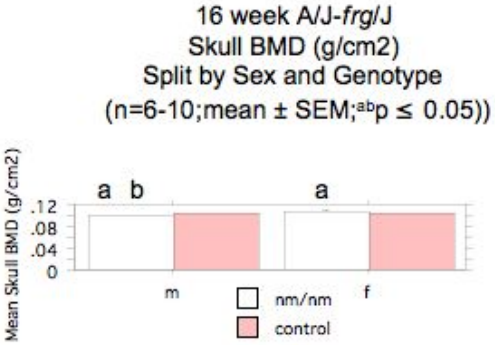
*controls = +/nm and +/? combined

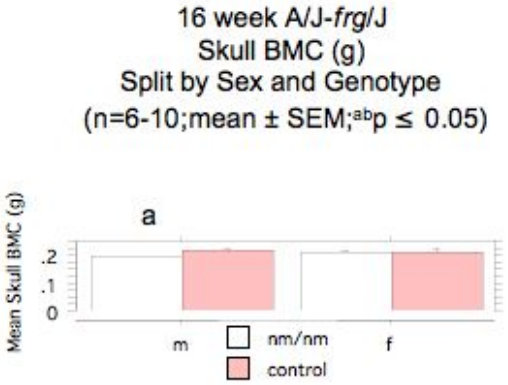
Male and female mutants were both significantly different for skull aBMD with female mutants having significantly more than female controls while male mutants had significantly less than male control and female mutants. All other significant differences within sexes were with males; male mutants had lower whole body fat, total mass, skull BMC, and ear pinnae length.











[a] control versus mutant within sex
[b] male vs. female within genotype
*controls=+/nm and +/? Combined
nm/nm=*frg*/*frg*
+/nm=+/*frg*

Male and female mutants are nearly equal with their short and wide face phenotype. Body composition is more affected in *frg* males than females.

Pathology

A routine pathological screen[2] was done on seven *frg* mice. Two were one-year-old and five were 12-weeks-old; all seven were homozygotes. All these mice showed mild dystrophic muscle due to the A/J background (Ho et al., 2004). Froggy bones were normal with no shortening of leg bones or vertebral bodies. A clinical eye check was done on both affected mice and controls at 18, 22, and 26 weeks and all were normal. Hearing was assessed by auditory brainstem response (ABR) by testing two mutants and one control at three months old. Both genotypes showed severe hearing loss due to the A/J background.

Summary

The *frg* skeletal mutation affects both sexes, however the body composition of males is more affected than females. Hearing is impaired and there is a muscle mutation, but this is due to A/J background.

Acknowledgements

The authors thank Norm Hawes for eye examination, Ronald Hurd for ERG and Heping Yu for ABR testing.

References

Ho M, et al., Disruption of muscle membrane and phenotype divergence in two novel mouse models of dysferlin deficiency. Hum Mol Genet. 2004 Sep 15;13(18):1999-2010

Zheng et al., Assessment of hearing in 80 inbred strains of mice by ABR threshold analysis. Hear Res. 1999 Apr 130(1-2):94-107

Protocols

[1] 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.
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[2]Standard Histology Protocol used in The Mouse Mutant Resource

For fixation of tissues, mice were deeply anesthetized with tribromoethanol (avertin) until they no longer displayed a withdrawal reflex in the hind limbs and then perfused intracardially with Bouin's fixative following a flush of the vasculature with saline solution. After soaking in Bouin's for one week to demineralize bones, tissues were dissected. Six segments of spine with axial muscles and spinal cord in situ, representing cervical, thoracic and lumbar spinal segments, were dissected. The brain was removed and sliced into 6 cross sectional pieces at the levels of olfactory lobes, frontal cortex, striatum, thalamus, midbrain, rostral and caudal medulla with cerebellum. Midsagittal slices of hind leg through the knees were prepared. Slices of basal skull through the pituitary and inner ears were taken. Both eyes, salivary glands and submandibular lymph node, trachea plus thyroid and sometimes parathyroid were removed and cassetted. A longitudinal slice of skin from the back was removed. The thymus, slices of lung, and a longitudinal slice of heart were cassetted. Similarly slices of liver through gall bladder, kidney with adrenal attached, pancreas and spleen were prepared. The stomach was sliced longitudinally to include both squamous and glandular portions. Loops of small intestine from 3 levels and slices of large intestine and cecum were removed, as were slices of urinary bladder. The whole uterus, with ovaries attached, was taken. In males testes were sliced longitudinally. The accessory male organs including seminal vesicles, coagulating gland and prostate were removed en block. Altogether in most cases all tissue fit into a total of 10 cassettes. The cassettes were processed in an automatic tissue processor to dehydrate tissues which were then embedded in paraffin. Six micron sections were cut and stained with hematoxylin and eosin (H&E). Sections of brain and spinal cord in vertebral bones also were stained with luxol fast blue (LFB) for myelin and cresylecht violet (CV) for cellular detail.