Cataracts and Retarded Hair Growth in *rhg*

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Mutation (allele) name: retarded hair growth

Mutation (allele) symbol: *rhg*

Strain of Origin: AKR/J

Current Stain Name: B6Ei;AKR-*rhg*/J

Stock Number: 003544 (jaxmice.jax.org)

Phenotype Category: hair, eye, size

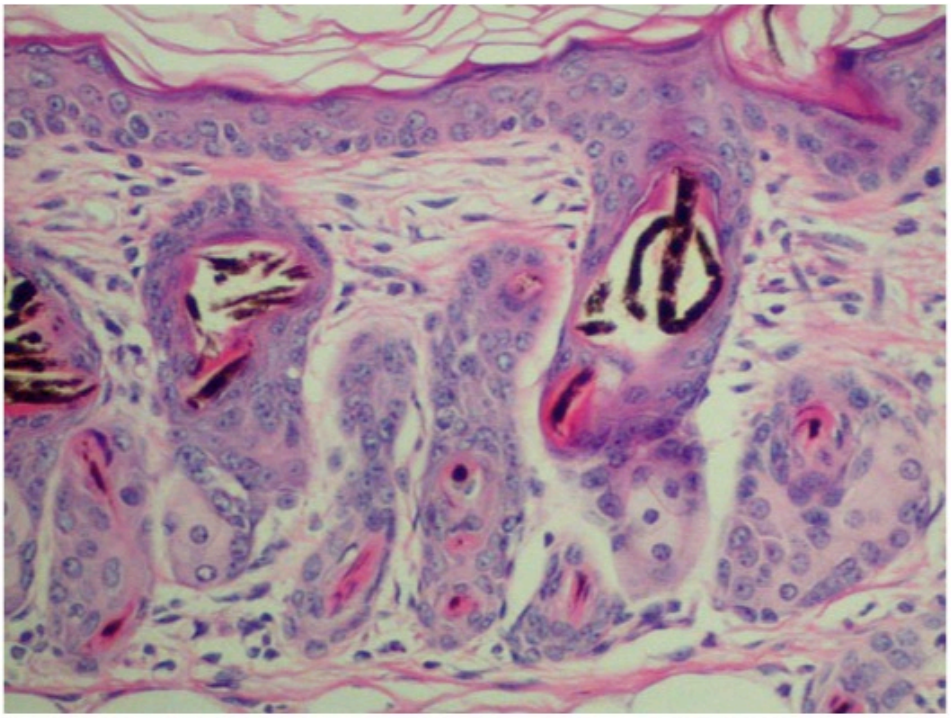
Origin and Description

The retarded hair growth (*rhg*) mutation originally arose on the AKR/J strain background (Fox et al.1978) but due to AKR leukemia, the mutation was transferred onto a C57BL/6JEi background. This autosomal recessive mutation is characterized by smaller mice that have delayed fur development. At birth mutants are the same size as littermates, but at one-week of age mutants are smaller and appear hairless compared to controls. At two weeks of age mutants have no hair compared to littermates who now have a full coat. At three weeks of age mutants are still smaller but are starting to get fur and at four weeks of age fur usually grows in and they begin to catch up in size. After one month, mutants can be identified by the ruffled appearance of their fur (due to the irregular directions and patterns of hair growth). Mutants later can be identified by the white at the tips of their hair.

An *rhg*/*rhg* mutant at 7 days of age is shown on the left with a littermate control on the right.

This strain is maintained by mating a heterozygous female to a homozygous male or the reciprocal as both males and females are fertile. From these matings, mutants comprise 41% of the progeny or just under the 50% expected ratio.

Tissues for histopathological examination[1] were prepared and an *rhg/rhg* female and male exhibited follicular dysplasia. A 13-day-old mutant also had hematopoesis in the liver and the pancreas looked underdeveloped. However, it looks to be an isolated case, as it could not be seen in other mutants. A ten-month-old female *rhg/rhg* had an atrophic thymus. Serum from two-week-old male and female *rhg/rhg* and *+/rhg* were analyzed and mutants had significantly lower levels of albumin, alkaline phosphatase, amylase and thyroid hormone (T4) levels.



Skin section from an *rhg/rhg* mutant showing follicular dystrophy

A clinical eye exam[2] using an ophthalmoscope revealed that *rhg/rhg* mice had cataracts and their *+/rhg* littermates did not. Overall, five *rhg/rhg* and six *+/rhg* mice were examined and ages were 2 months and three-and-half months old.

Auditory Brain Stem Response (ABR) was analyzed in 1 female and male *+/rhg* and 1 female and male *rhg/rhg* littermates at one month of age. They all had normal hearing.

Genetic Analysis

For linkage analysis, an AKR/J mutant was crossed to a C57BL/6JEi control. F1 heterozygotes were intercrossed and 33 mutant F2s were collected. Additionally, a B6Ei;AKR-*rhg*/J mutant was crossed to C3H/HeJ for mapping and 31 mutant F2s were collected and analyzed. *rhg* maps between markers *D7Mit206* at 132 Mb and *D7Mit105* at 135.7 Mb. Based on map position *Sult1a1* at 133.8 Mb was a candidate gene. The PCR-amplified DNA products were sequenced, but no differences were found between mutants and controls.

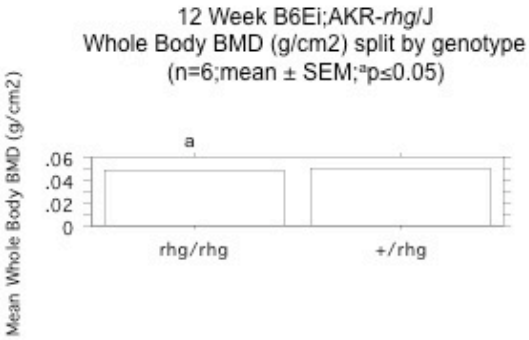
Biological Characterization

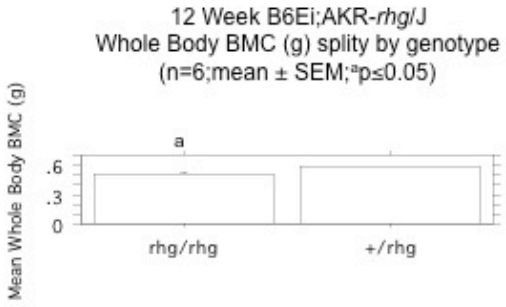
DEXA Analysis of Whole Body BMD and Body Composition

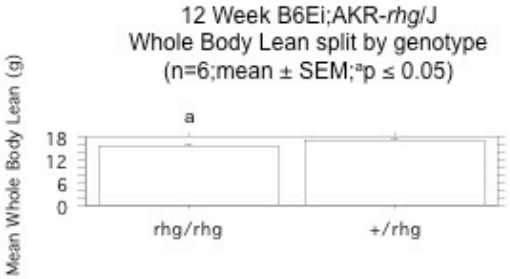
Six female *rhg/rhg* and six female *+/rhg* were collected and assessed by PIXImus densitometry (GE LUNAR, Madison, WI) at 12 weeks of age[3]. Differences in whole Body BMD, BMC and Lean measurements were all statistically significant with female *rhg/rhg* having lower values than those of *+/rhg* females. Skull BMD and BMC measurements were significantly lower in the mutants.

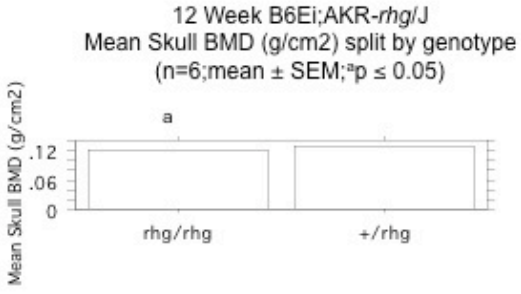
PIXImus Densitometric Measurements of Twelve-Week-Old B6Ei;AKR-rhg/J Females
(n=6; mean ± SEM; $^{a}p \leq 0.05$)

Measurement	rhg/rhg	+/rhg
Whole Body BMD (g/cm2)	0.0481 ± .00081[a]	0.0503 ± 0.00034
Whole Body BMC (g)	0.507 ± 0.0114[a]	0.574 ± 0.0179
Whole Body Lean (g)	15.5 ± 0.46[a]	17.0 ± 0.33
Whole Body Fat (g)	2.4 ± 0.19	2.6 ± 0.17
Total (g)	17.87 ± 0.646	19.55 ± 0.397
% Fat	13	13
Skull BMD (g/cm2)	0.1208 ± 0.00120[a]	0.1278 ± 0.00129
Skull BMC (g)	0.278 ± 0.0067[a]	0.304 ± 0.0068
Skull BMD/Body BMD	2.5140 ± 0.03424	2.5390 ± 0.02749









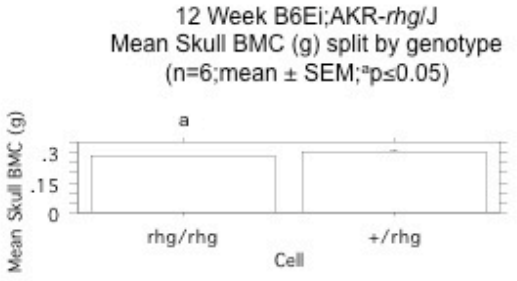
Craniofacial Morphology

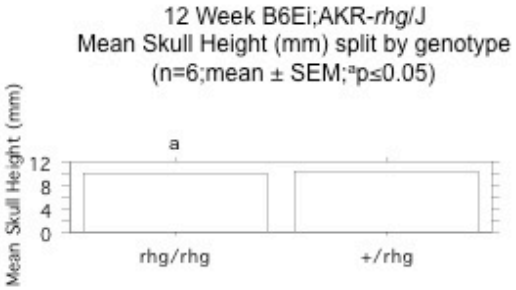
These same six female mutants and control skulls were prepared by incomplete maceration in potassium hydroxide, stained with alizarin red, and stored in undiluted glycerin (Green, 1952). Morphological measurements of the skull were also made using digital calipers (Stoelting, Wood Dale, Ill) with previously established landmarks (Richtsmeier, 2000). During this collection process, each right ear pinna was measured. Skull height and ear pinna length were statistically significant with mutants being less. Also, the skull to nose length ratio was significant (see data below).

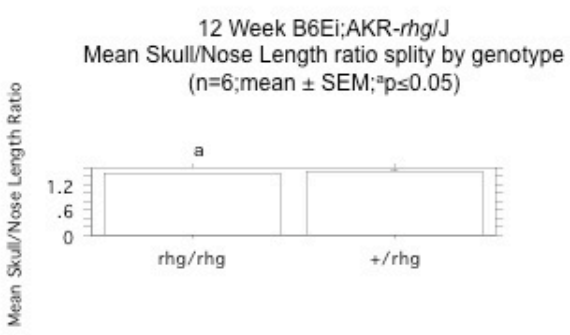
Digital Caliper Measurements and Calculated Ratios of Twelve-Week-Old B6Ei;AKR-*rhg*/J Skulls Stained with Alizarin Red

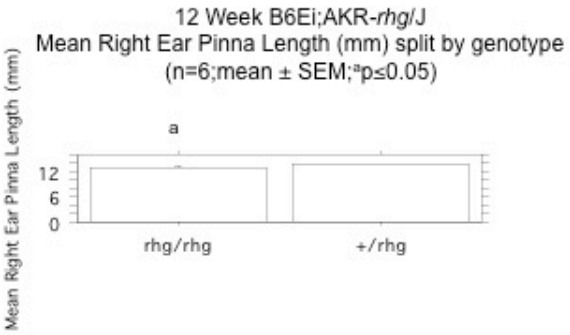
(n=6; mean ± SEM; [a]p ≤ 0.05)

Measurement	rhg/rhg	+/rhg
Skull Length (mm)	22.27 ± 0.293	23.05 ± 0.227
Nose Length (mm)	15.23 ± 0.249	15.13 ± 0.221
Skull Height (mm)	9.90 ± 0.107[a]	10.25 ± 0.089
Skull Width (mm)	10.54 ± 0.131	10.76 ± 0.070
Inner Canthal Distance (mm)	5.82 ± 0.164	6.42 ± 0.295
Lower Jaw Length (mm)	10.41 ± 0.149	10.89 ± 0.184
Upper Jaw Length (mm)	15.95 ± 0.271	16.29 ± 0.165
Jaw Length Ratio	1.53 ± 0.021	1.50 ± 0.022
Skull/Nose Length Ratio	1.46 ± 0.019[a]	1.52 ± 0.014
Skull Height/Length Ratio	0.45 ± 0.006	0.46 ± 0.006
Skull Length/Width Ratio	2.11 ± 0.034	2.14 ± 0.015
Skull Height/Width Ratio	0.94 ± 0.017	0.95 ± 0.010
Right Ear Pinna Length (mm)	13.06 ± 0.128[a]	13.77 ± 0.135









Discussion

Retarded hair growth mutants have follicular dysplasia, delayed overall growth, bone and craniofacial abnormalities and develop cataracts.

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Acknowledgements

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Pathology: Coleen Kane
Pathology Evaluation: Rod Bronson, Ph.D
Colony Management: Louise Dionne

Protocols

[1]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.

[2] Screening procedures of The Jackson Laboratory Eye Mutant Resource

Our screening procedure starts with a primary search for gross eye abnormalities. Mice with a suspected abnormality are followed up with a secondary examination.

Our primary screening procedure includes:

I. We visually inspect for gross eye abnormalities by examining the eyelids, globe, cornea, and iris.

II. Using a Nikon biomicroscope (slit lamp) examination, we check the cornea for clarity, size (bupthalmos vs. microcornea), surface texture, and vascularization. The iris is checked for pupil size, constriction, reflected luminescence, and synechia. The eye is then dilated with 1% atropine and the lens is checked for cataract.

III. We use an indirect ophthalmoscope to examine the fundus for signs of retinal degeneration, such as retinal vessel constriction or retinal pigment epithelial disturbance, or for other problems, such as drusen or deposits or optic nervehead abnormalities.

IV. We use electroretinography (ERG), to test for retinal function loss.

Our secondary screening procedure:

Mice with a suspected abnormality are followed up examination by examining more mice of the same strain and genetically related strains. In addition, this second level screen includes:

(1) electroretinography for suspected retinal problems,
(2) histological check of all eye tissues;
(3) comparison of mice at different ages to determine age of onset of the condition;
(4) comparison of the new mutant's clinical features to those in established mutant eye stocks.

[3] 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 areal bone mineral density (BMD, g/cm^2), bone mineral content (BMC, g), body mass (g), lean mass (g), fat mass (g), and percent 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 aBMD 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 Jackson Laboratory'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 red, 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.

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