A New Spontaneous Mutation Causing Corneal Opacity

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Mutation (allele) symbol: *whe*

Mutation (allele) name: white eyes

Gene symbol: *whe*

Strain of origin: B6.V-*Lep*^{ob}/J

Current strain name: B6.V *Lep*^{ob}-*whe*/GrsrJ

Stock #005624 (jaxmice.jax.org)

Origin and Description

We report a new spontaneous, recessive mutation, named "white eyes" (*whe*), which arose at The Jackson Laboratory in 2003, in the B6.V-*Lep*^{ob}/J colony. The homozygous *whe* mutation usually is expressed bilaterally and is characterized by a small, white spot on the cornea; occasionally, only one eye will be affected. When viewed with a slit lamp and by indirect ophthalmoscope, the eye displays white spots and an atypical growth of blood vessels on the cornea.

Since the obese phenotype, characteristic of *Lep*^{ob}/*Lep*^{ob} homozygotes of the B6.V-*Lep*^{ob}/J strain, continues to segregate in the *whe* colony, the stock is maintained by mating a non-obese male or female *whe/whe* to a non-obese mouse that is heterozygous for *whe*. Both non-obese males and non-obese females are fertile; females have normal litter sizes and lactate normally. The strain was tested for penetrance by mating two *whe* homozygotes and was found to be 100% penetrant (51 pups out of 51 born were affected). The *whe/whe* mutation was mapped to Chromosome 11 with 2.5% recombination at marker *D11Mit71* which is located 6.8 Mb (Ensembl Genome Browser, v33) from the centromere.

Genetic Analysis

White eyes is inherited as a recessive mutation as shown by traditional breeding experiments performed as part of the linkage cross for genetic mapping. For linkage analysis, a C3H/HeJ (Stock #001912) male, without retinal degeneration, was mated to a homozygous *whe/whe* female. F1 hybrids were then intercrossed to produce F2 progeny. There were no visible mutants seen in the F1 generation (0/39) and 21.9% of F2 progeny were mutants (32/146). F2 progeny were observed for the white, opaque color of the eye, and an additional screening of the cornea with a slit lamp and indirect ophthalmoscope

was performed to confirm the mutant phenotype of affected mice. Spleen and tail tip of mutant 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.).

Because of the *whe* eye phenotype, we began by testing for linkage (using pooled DNA from affected F2s) with *Pax6* on Chromosome (Chr) 2, *Tgfa* on Chr 6, *Egfrwa* on Chr 11, and *Bmp4* on Chr 14. No linkage was observed at these four locations. Linkage of white eyes was first detected on Chr 11 at 8.8 Mb from the centromere with marker *D11Mit226* (Ensembl Genome Browser, v33). Twenty-one DNA samples were then typed individually and results showed linkage with 2.5% recombination at marker *D11Mit71*, 6.8 Mb from the centromere (Ensembl Genome Browser, v33). A spontaneous mutation called "gaping lids" is located on Chr 11 at 0.2 cM from the centromere, and homozygotes for this mutation display a phenotype similar to *whe*; however, the gapping lids mutant was not available for a complementation test. Although not confirmed to be the causative gene for gapping lids, Neurofibromin 2 (*Nf2*) found at 0.25 cM was considered a candidate gene (Juriloff, personal communication). Due to the similarity of phenotypes and its chromosomal location, we sequenced the coding regions of *Nf2*, but found no mutation of *Nf2* in DNA from *whe* homozygotes.

Biological Characterization

A. DEXA Analysis of Whole Body aBMD and Body Composition

Whole body areal bone mineral density (aBMD), bone mineral content (BMC) and body composition (lean, fat, and %fat mass) was assessed by PIXImus densitometry (GE LUNAR, Madison, WI, see protocols below[1]). No significant differences were found between mutants and controls in either gender (**Table 1**).

Table 1: PIXImus Densitometric Measurements of Twelve Week Old Mutant and Control Mice of Both Sexes (n=5, mean±SEM).

Measurement	Female (+/*whe*)	Female (*whe*/*whe*)	Male (+/*whe*)	Male (*whe*/*whe*)
Whole Body aBMD (g/cm²)	0.044±0.001	0.044±0.001	0.046±0.001	0.045±0.0002
Whole Body BMC (g)	0.372±0.008	0.374±0.014	0.441±0.017	0.428±0.012
Whole Body Lean (g)	15.617±0.435	14.967±0.622	20.983±0.495	21.2±0.506
Whole Body Fat (g)	4.983±0.373	4.667±0.279	5.433±0.395	5.683±0.584
Total Body Weight (g)	20.6±0.458	19.667±0.772	26.450±0.362	26.867±0.540
Percent Fat (%)	24.3	23.8	20.6	21.1
Skull BMD (g/cm²)	0.113±0.001	0.114±0.002	0.109±0.002	0.108±0.001
Skull BMC (g)	0.306±0.046	0.260±0.007	0.255±0.007	0.250±0.005

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). Morphological measurements of the skull (**Table 2**) were made using digital calipers (Stoelting, Wood Dale, Ill) with previously established landmarks (Richtsmeier, 2000, see protocol this website). Inner canthal

distance (**Figure 1**) and lower jaw length (**Figure 2**) were significantly different in *whe/whe* females compared to control females. No other skull measurements or skull measurement ratios were significantly different for either gender when homozygotes were compared to controls.

Table 2: Digital Caliper Measurements of Twelve Week Old Skulls of Male and Female *whe/whe* and Control Mice (n=6, mean±SEM; a=p value <0.05)

Measurement	Female (+/whe)	Female (whe/whe)	Male (+/whe)	Male (whe/whe)
Skull Length (mm)	22.410±0.197	22.592±0.124	22.657±0.067	22.728±0.106
Nose Length (mm)	15.490±0.120	15.335±0.180	15.587±0.095	15.338±0.214
Skull Height (mm)	9.673±0.106	9.550±0.101	10.098±0.133	10.187±0.030
Skull Width (mm)	10.513±0.115	10.415±0.053	10.595±0.043	10.567±0.052
Inner Canthal Distance (mm)	5.263±0.087	5.595±0.093 [a]	5.413±0.076	5.272±0.046
Upper Jaw Length (mm)	15.903±0.066	15.887±0.142	15.990±0.074	16.080±0.111
Lower Jaw Length (mm)	11.023±0.039	10.737±0.117 [a]	10.968±0.030	11.093±0.069
Skull Length : Nose Length Ratio	1.45	1.47	1.45	1.48
Skull Height : Length Ratio	0.43	0.42	0.45	0.45
Upper Jaw : Lower Jaw Ratio	1.44	1.48	1.45	1.48
Skull Length: Width Ratio	2.13	2.17	2.14	2.15
Skull Height : Width Ratio	0.92	0.92	0.95	0.96

Figure 1: Mean Inner Canthal Distance of Male and Female *whe/whe* and Control Mice (n=6).

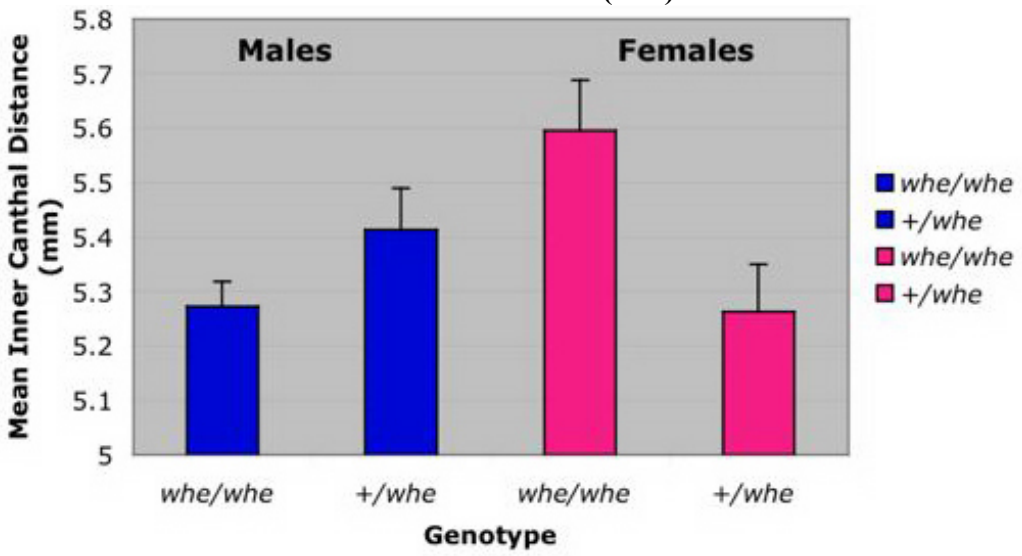
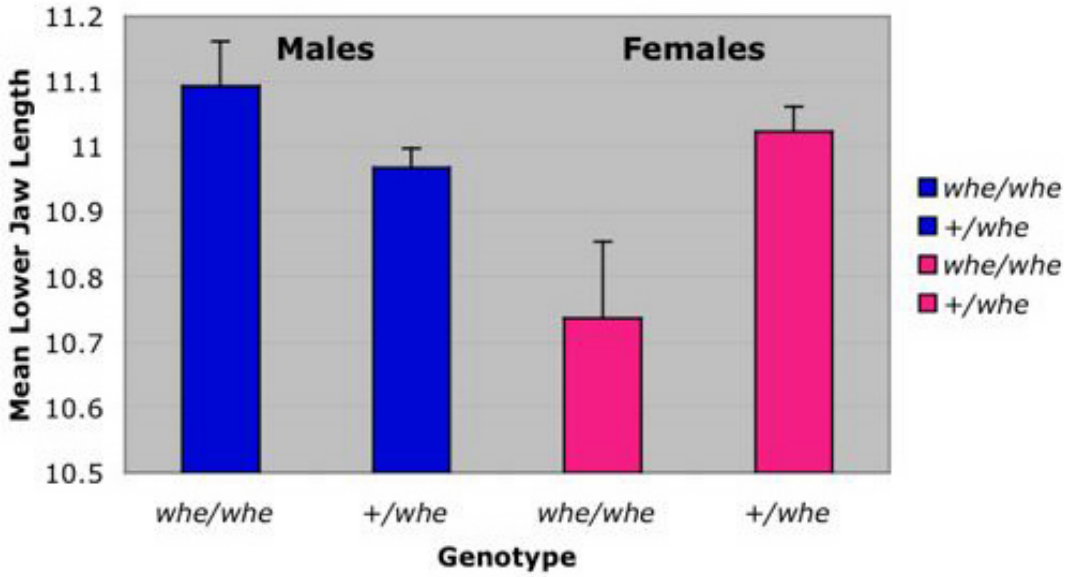


Figure 2: Mean Lower Jaw Length of Male and Female

***whe/whe* and Control Mice (n=6).**



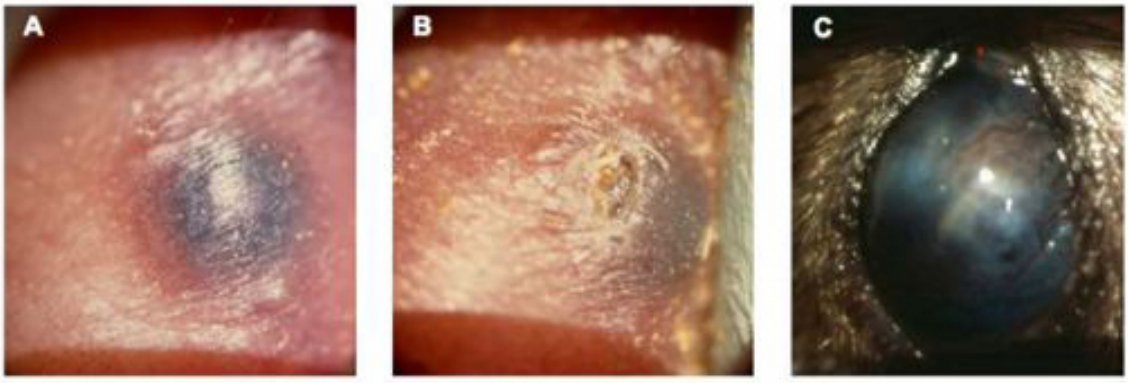
C. Hearing Tests

Hearing was assessed by ABR threshold analysis (Zheng et al. 1999) using two mutants and two controls of each gender at three months of age. The ABR results showed that both mutant and control mice have normal hearing.

D. Eye Examination

Eight mutants at four weeks of age were examined by a slit lamp and indirect ophthalmoscope. Results showed that the mice do not have a cataract. In all the mutants, the cornea has a white vascular area. Eye histology shows a vascular cornea and attachment of the cornea to the iris (synechia). The retina and lens appear normal in heterozygotes and in most homozygotes. One out of eight homozygotes showed an outer nuclear layer with rosettes and degeneration.

Figure 3: The eye appearance of *whe* mutant mice. (A) The normal control shows a closed eye at 2 days of age. (B) An open eye is shown in a *whe/whe* mouse at 2 days of age. (C) A cornea opacity and a white vascular area are shown in a *whe/whe* mouse at 5 months of age.



E. Pathology
One male mutant was perfused at nineteen weeks of age via cardiac infusion of Bouin's fixative following admission of anesthesia. Testicular atrophy was observed and osteoarthritis was noted in both hips of a female mutant perfused at fifty-four weeks of age.

Discussion
In normal human development, the eyes close during the eighth week of gestation and reopen at the seventh month of gestation (Juriloff, 2000). During normal development in the mouse, the eyelids grow, flatten across the eye and fuse with each other between days 14 and 16 of gestation and open approximately 14 days after birth (Harris, 1982 and Juriloff, 2000). When the eyelids do not fuse properly in the mouse, the eyes are open at birth and cause corneal opacity or degenerative changes of the eye when mice reach adulthood (Juriloff, 2000).

In the *whe* mutation reported here, the eyelid is either completely or partially open when neonates are two days old. Consequently, an infection of the eye develops and a characteristic white spot is present on one or both eyes. Further investigation of the mutant eye shows an atypical growth of blood vessels on the cornea. Histology shows cornea inflammation and iris and corneal attachment. This new mutation is a valuable model of abnormal eye development, even though the causative gene has not been identified.

Acknowledgements
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Mouse Genome Database (MGD) Mouse Genome Informatics Project, The Jackson Laboratory, Bar Harbor, Maine. World Wide Web (informatics.jax.org)

Zheng QY, Johnson KR, Erway LC (1999). *Assessment of hearing in 80 inbred strains of mice by ABR threshold analyses.* Hear Res 130, 94-107.

[1]Protocols
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.

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