

Long bone abnormality (*lbab*): a new mouse mutation on Chromosome 1 causing small size and skeletal abnormalities

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Mutation Symbol: *lbab*

Mutation Name: long bone abnormality

Strain of origin: PL/J

Current strain name: B6.PL-*Nppclbab*/GrsrJ

Stock #004521 (jaxmice.jax.org)

Phenotype category: size, skeletal, limbs

Abstract

We report here a new autosomal recessive mutation that arose spontaneously in the PL/J strain at The Jackson Laboratory, which we have named "long bone abnormality" (*lbab*). The *lbab* mutation, when homozygous, causes overall smaller body size with proportionate dwarfing of all organs with the possible exception of the testes. We mapped the *lbab* mutation to Chr 1, between the markers *DIMit9* and *DIMit488*. Mouse mutants demonstrating small body size and skeletal defects are important models for a variety of human abnormalities such as dwarfism. An OMIM search of dwarfism produced 246 entries. Mutant mouse models are valuable tools for the study of the pathology contributing to these abnormalities and can aid in the molecular identification of responsible genes. *Bwtq1* (body weight QTL1) and *Col6a3* (procollagen, type VI, alpha 3) are located in this region of Chr 1.

Origin and Description

The *lbab* mutation was found at The Jackson laboratory in 1996 by Arcel Dullas in a production colony (room-annex 12). The mutation arose on the PL/J strain (stock # 000680) at generation F171.

Homozygous mutants are recognizable by 10 days of postnatal development by an overall smaller body size. The mutants exhibit proportionate dwarfing of all organs with the possible exception of the male reproductive tract. One-month-old males have exceptionally small testes, however mature homozygous males do breed and have normal testis size by palpation. The mutants have proportionately shorter tails. Brain size is conserved. Mutants exhibit shorter long bones, and knee joints are "scrambled" in some mutants. Abnormalities are seen in the femoral and tibial growth plates of some mutants. The mutation is being transferred to a C57BL/6J background (now at N6) because it would not breed on PL/J. Growth plates appear less affected on the C57BL/6J background than on the original PL/J background. The mutation is currently maintained by progeny testing and outcrossed to C57/B6J when a mutant is available.

Phenotypic Characterization

The eyes of 4 mutants and 2 controls were examined with an ophthalmoscope and all showed normal eyes with the exception of one mutant, which had *rdl* retained from the PL/J strain, and not likely due to the mutation.

Allometric comparisons made with 2 homozygous male mutants and two male control animals showed an overall smaller body size with proportionate dwarfing of all organs except the reproductive tract, in which the testes were exceptionally small. X-rays of a homozygous 5-week-old female and a litter mate control showed that the mutant has much shorter long bones than the control. The mutant has well shaped vertebra and no kyphosis at this age. Two mutants observed at 9 weeks of age look like the long bones have stopped growing (progressive dwarfing) in that the proportionality of the ratio of long bones to spine gets greater at this later age.

Histological techniques. Tissues for histopathological examination were prepared from both *lbab/lbab* and control animals. Tissues were removed from seventeen animals deeply anesthetized with tribromoethanol (Avertin) and fixed by intracardiac perfusion of Bouin's solution following a flush of saline. After demineralization in Bouin's, multiple cross sections of all portions of the spinal cord and brain were prepared. Cross and longitudinal sections of lumbar muscles, fore and hindlimb muscles and samples of each of the somatic organs (liver, spleen, pancreas, stomach, small intestine, colon, cecum, lungs, thymus and heart) were also prepared. For light microscopy sections of all tissues were stained with hematoxylin and eosin (H&E). Brain and spinal cord sections were also stained with luxol-fast blue-cresylecht violet (LFB-CV) and Bodian's stain. Specimens from 2 *lbab/lbab* and 2 *lbab/?* mice were cleared and stained with alizarin red S and alcian blue to demonstrate bone and cartilage (Green, M.C., 1952). Nine of the seventeen mutants examined had growth plate abnormalities, while eight had normal appearing growth plates. Of those with abnormalities, some animals had disorganization of both the femoral and tibial growth plates, while others had problems only in the femoral plates. Testis biopsy done on a homozygous mutant showed that this male has normal sperm heads and many sperm tails.

Genetic Analysis

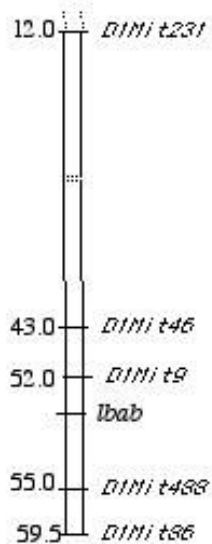
lbab is inherited as a recessive mutation as shown by traditional intercross analysis. Tests for allelism were done and found to be negative with the following mutants: Brachymorphic (*bm*) 0/42 progeny born, achondroplasia (*cn*) 0/61 progeny born, osteochondrodystrophy (*ocd*) 0/19 (4 unclassifiable) progeny born, and small (*sml*) 0/59 progeny born.

For linkage analysis a CAST/Ei male was mated to a homozygous (PL/J-*lbab* x C57Bl/6J) female. F1 hybrids from this initial cross were then intercrossed to produce F2 progeny. The F2 progeny were scored visually for phenotype and spleens and tail tips from 96 *lbab/lbab* animals were collected and stored at -70C for subsequent DNA typing to map the mutation. DNA was extracted from the frozen tail tips by a standard hot sodium and Tris (HotSHOT) procedure (Truett, et al., 2000). Polymerase chain reactions

with MIT primer pairs (MapPairs, Research Genetics, Huntsville Ala.) were carried out in 10 ul total volume reactions containing 20 ng genomic DNA, by previously described methods (Ward-Bailey et al. 1996).

Mutation segregation ruled out Chromosome X linkage. Autosomal linkage was analyzed with PCR markers using a pooled DNA approach (Taylor et al. 1994). A genome sweep of F2 progeny from the CAST intercross was begun by typing a pooled sample of DNA (made from 27 of the *lbab/lbab* F2 animals) for segregating MIT microsatellite markers. Linkage of *lbab* was first detected on Chr 1 with *DIMit231* and *DIMit9* using the pooled sample. DNA samples were then typed for the individual 96 animals (192 meioses) with these two markers and three additional Chr 1 markers. Recombination estimates with standard errors and best gene order are centromere - *DIMit231* - 18.5+/-6.54 - *DIMit46* - 3.36+/-1.34 - *DIMit9* - 2.09+/-1.03 - ***lbab*** - 4.74+/-1.53 - *DIMit488* - 2.8+/-2.74 - *DIMit86*. Gene order and recombination frequencies were calculated with the Map Manager computer program (Manley 1993). The map position for *lbab* on Chr 1 is 53.5 cM, according to MGD placement. The complete chromosome (Chr1) linkage data for 96 *lbab/lbab* mice have been deposited in the Mouse Genome Database.

Chromosome 1



Skeletal and somatic growth parameters of *lbab* mice on the original PL/J background

A: Phenotype		B: Allometric Comparisons		
	<i>lbab/lbab</i> as % normal		<i>lbab/lbab</i>	normal
body weight	59%	tail lg/total lg	36%	41%
tail length	56%	femur lg/total lg	80%	85%
back length	65%	body fat/total b wt	14.5%	14%
total length	61%	BMC/total lg	11%	14%
femur length	63%	BMC/total body wt	10%	12%
BMC - bone mineral content	57%	BMD- bone mineral density (g/cm ²)	0.033	0.035

Parameters of growth: *lbab* (n=7-16) vs. normal (n=4-12) age-matched controls; males and females, 1-4 months of age, gender and ages combined. The data presented in the table above demonstrate that *lbab* mice are ateliotic dwarfs.

A: Skeletal measurements in *lbab/lbab* mice are consistently approximately 60% of the same measurements in normal littermates. In addition, body weight is proportionately reduced.

B: Furthermore allometric comparisons indicate that *lbab* mice are normally proportioned compared to normal littermates, and have only slightly reduced BMD by PIXImus. Growth curves for developmental time points from 1 to 4 months when mice reach skeletal maturity are similar to normal littermates of both sexes for body weight and measurements of skeletal growth including tail, back, and femur lengths. Homozygous *lbab/lbab* mice are smaller and of shorter stature at birth and remain so in spite of relatively normal rates of growth. Because group sizes were small, no gender comparisons were made.

Histological sections of *lbab/lbab* long bones:

Figure 1:

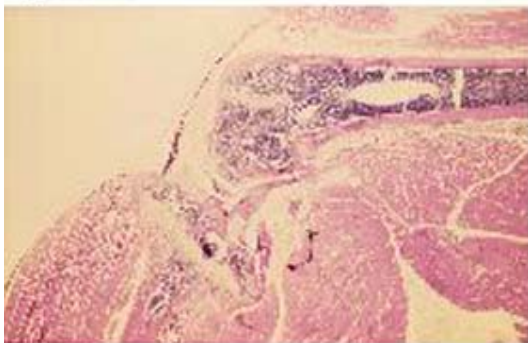
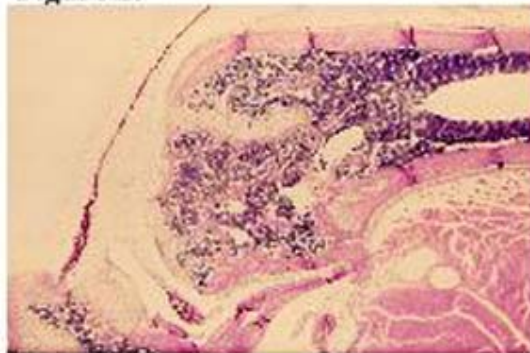


Figure 2:



The knee of a 1-month-old *lbab/lbab* mouse stained with H&E is shown at 2.5X

magnification. The growth plate of the tibia (figure 1) appears relatively normal; however the growth plate of the femur is not (figure 2).

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Addendum

The *lbab* mutation was found to be an A to G transversion in exon 2 of the natriuretic peptide type C gene, which substitutes an argenine for a glycine in a conserved domain. See Jiao et al., *BMC Genet* 2007, 8:16.