

Inherited Ateliotic Dwarfism in Mice

Characteristics of the mutation, little, on Chromosome 6

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RECENTLY, attention has been focused on inherited diseases affecting growth in human beings¹. Several of these disorders, including familial pan-hypopituitarism, isolated growth hormone deficiencies type I and type II, and Laron-type dwarfism, have in common the improper functioning of the anterior pituitary gland. Advances in the therapeutic treatment of individuals with these disorders have been possible through increased understanding of pituitary physiology, development of diagnostic tools for early detection of such disorders, and the availability of human growth hormone. Further progress in the treatment of human pituitary growth disorders now depends on acquiring additional information that is difficult or impossible to obtain from human beings. This knowledge may be accessible by studying similar or possibly identical inherited diseases in laboratory mammals.

We have been fortunate to find in the laboratory mouse a recessive, autosomally inherited growth defect that appears to resemble a condition similar to the human disorder, isolated growth hormone deficiency type I. This paper describes the mode of inheritance and chromosomal location of the new mutation, designated little (gene symbol *lit*), and its effects on growth and on pituitary prolactin and growth hormone.

Material and Methods

One small female and two small male mice were noted in the C57BL/6J strain in the Production Depart-

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ment of the Jackson Laboratory. To determine the mode of inheritance of the abnormality, affected C57BL/6J individuals were mated to unrelated C57BL/6J mice, to mice of the C3H/HeJ strain, and to mice of the ABP stock. In each case, the F₁'s were intercrossed to produce F₂'s. In addition, matings were made between affected C57BL/6J mice and normal sibs. Linkage tests were conducted using mice of various linkage testing stocks obtained from the Mouse Mutant Stocks Center of the Jackson Laboratory.

Studies of body weight and bone dimensions were conducted to monitor growth of mutant and normal mice. In the first type of experiment, all new-borns in a number of litters, produced from matings of C57BL/6J *lit/lit* females to C57BL/6J *lit/+* males, were toe-clipped for future identification and weighed every 7 days until 36 days of age. Like-sex individuals were isolated together at weaning (21 to 28 days of age). In addition, an identical growth experiment was conducted involving mice born from matings of C57BL/6J-*lit/lit* females to (C57BL/6J × ABP)F₁-*lit/+* males.

In the second type of growth experiment, bone dimensions and body weights were determined on 30-, 50-, and 90-day-old like-sex pairs of *lit/lit* and *lit/+* sibs from matings of C57BL/6J-*lit/lit* females by C57BL/6J-*lit/+* males. The skeletons of each pair were prepared simultaneously as recommended by Green², with minor modifications. Individual bones were removed from the skeleton, boiled in water to facilitate removal of muscle, dried, and measured using a sliding micrometer. Long bone measurements represent diaphyseal lengths since the epiphyses are removed with the potassium hydroxide treatment. Skull lengths were measured along the mid-line from the anterior end of the nasal bones caudally to the most posterior projection of the occipital bone. Skull widths were measured from side to side just posterior of the zygomatic arch—squamosal bone junction. Scapula length represents the longest overall dimension, whereas the width is the widest region of the blade near the medial border.

Growth hormone (GH) and prolactin (PRL) content in whole pituitaries of C57BL/6J-*lit/lit* females and males, and *lit/+* female mice were determined from acrylamide gels as follows: fresh pituitaries were homogenized in a 10 percent sucrose solution prepared in 0.05M sodium phosphate buffer (pH 7.4). The homogenate was adjusted to a concentration of 6 mg/ml. One hundred μ l aliquots of the homogenates were layered onto 10 percent

acrylamide resolving gels⁵. Electrophoresis was performed in the Buchler Acrylamide Disc Electrophoresis System for 1½ hours, 2.5 ma/gel. The gels were stained in Amido Schwartz for 15 minutes, and destained in 7 percent acetic acid overnight. To facilitate identification of the albumin band in the pituitaries, electrophoresis was also performed on mouse plasma using 2µl of plasma per gel tube. Purified mouse PRL and GH were run simultaneously for reference purposes.

All mice used in these studies were fed a diet of Old Guilford 96W consisting of 22.5 percent protein, 7.5 percent fat, and 0.6 percent vitamin supplementation. Food and water were available ad libitum. Special care was taken when weaning litters including *lit/lit* individuals to place extra-long spouts on the water bottles and pellets of food on the cage floor to insure that there would be no retardation of growth because of inability to obtain sufficient quantities of food and water. These caging conditions were observed until all *lit/lit* individuals had grown sufficiently to easily reach the standard water spout and food hopper.

Results and Discussion

Inheritance

The mode of inheritance of the small phenotype was determined by mating the abnormal mice to unrelated C57BL/6J mice and intercrossing the F₁'s. Since none of the F₁ young was small, whereas approximately 25 percent of the F₂ offspring were small (line 4, Table I), the small body size appeared to be due to an autosomal recessive mutation. Other crosses involving C57BL/6J little and normal individuals were also made (lines 1-3, Table I). In addition, F₁ mice, produced by mating C57BL/6J little females to either C3H/HeJ or ABP males, were intercrossed to produce F₂'s (lines 5 and 6, Table I). All of the results are consistent with the hypothesis that the little phenotype is produced by a

single recessive autosomal mutation with full penetrance. The mutation was named little, gene symbol *lit*.

Several crosses were made to determine the linkage of *lit*. One of these crosses involved mating a C57BL/6J-*lit/lit* female to an ABP male and intercrossing the F₁'s to produce F₂'s. ABP is a highly inbred stock of mice homozygous for 5 recessive genes not carried by C57BL/6J, including the waved-1 (*wa-1*) locus. In the repulsion intercross, we noted that among 208 F₂ offspring, none was *wa-1/wa-1 lit/lit* (111 + +, 54 *wa-1* +, 43 + *lit*, 0 *wa-1 lit*), indicating that *lit* was linked to the Chromosome 6 gene *wa-1*. To confirm this, we mated a C57BL/6J-*lit/lit* female to a *Hd Mi^{wh}/+* + male and mated the F₁ males carrying both dominant mutations back to C57BL/6J-*lit/lit* females (*Hd*, hypodactyly; *Mi^{wh}*, white). Offspring were classified as *lit/lit* if they displayed both small body size and small anterior pituitary. The results, given in Table II, show that the order of these three loci on Chromosome 6, with percent recombination ± its standard error is *Hd*-3.3 ± 0.8-*lit*-14.0 ± 1.1-*Mi^{wh}*.

Reproductive characteristics

Early in the course of these studies it was noted that *lit/lit* C57BL/6J or hybrid females lost their first litters because of failure of milk production. Second and subsequent litters are raised. In addition, only 50 per cent of *lit/lit* C57BL/6J males sired first litters. Of those that sired a first litter, only 50 percent sired second litters. Third litters were rare. The reproductive performance of *lit/lit* hybrid males is unknown. A more detailed analysis of the reproductive characteristics of the little mouse is warranted.

Growth parameters

The failure of *lit/lit* C57BL/6J mice to maintain normal growth was apparent by weaning. A study of body

Table I. Segregation of little (*lit*) mice

Mating		Offspring		Total	Observed little %	Expected little %
♀	♂	<i>lit</i>	+			
<i>lit/lit</i> C57BL/6J	<i>lit/lit</i> C57BL/6J	44	0	44	100.0	100.0
<i>lit/lit</i> C57BL/6J	<i>lit/</i> + C57BL/6J	274	290	564	48.6	50.0
<i>lit/</i> + C57BL/6J	<i>lit/lit</i> C57BL/6J	110	137	247	44.5	50.0
<i>lit/</i> + C57BL/6J	<i>lit/</i> + C57BL/6J	159	547	706	22.5	25.0
<i>lit/</i> + (C57BL/6J × C3H/HeJ)F ₁	<i>lit/</i> + (C57BL/6J × C3H/HeJ)F ₁	102	298	400	25.5	25.0
<i>lit/</i> + (C57BL/6J × ABP)F ₁	<i>lit/</i> + (C57BL/6J × ABP)F ₁	43	165	208	20.7	25.0

Table II. Offspring of cross of *Hd* + *Mi^{wh}/+* + *lit* + male by + *lit* +/+ *lit* + female

Chromosome from ♂ parent								Total
+ <i>lit</i> +	<i>Hd</i> + <i>Mi^{wh}</i>	<i>Hd</i> ++	+ <i>lit</i> <i>Mi^{wh}</i>	<i>Hd</i> <i>lit</i> +	++ <i>Mi^{wh}</i>	<i>Hd</i> <i>lit</i> <i>Mi^{wh}</i>	+++	
182	194	29	31	7	5	3	0	451

weight was conducted to determine when in the neonatal period the genetic defect influenced general body growth. Body weight measurements were taken from mice of the C57BL/6J strain and offspring of the (C57BL/6J × ABP)-F₁ × C57BL/6J cross during the interval of 1 to 36 days of age. Results are given in Table III. Mean body weights of *lit/lit* and *lit/+* individuals were compared separately by sex for each time period with *t* tests. Significant differences in weights were first noted between C57BL/6J *lit/lit* and *lit/+* females at day 15 ($t = 2.43$, $df = 67$, $P < 0.025$), between C57BL/6J *lit/lit* and *lit/+* males at day 15 ($t = 4.01$, $df = 65$, $P < 0.001$), between hybrid *lit/lit* and *lit/+* females at day 15 ($t = 2.25$, $df = 22$, $P < 0.05$), and between hybrid *lit/lit* and *lit/+* males at day 22 ($t = 5.09$, $df = 33$, $P < 0.001$). These results indicate that the *lit* mutation is already operative before weaning. It should be noted that, although at birth the mean weights of mutant and normal were not significantly different, there was a tendency for mutants to weigh less. It is possible that the effect of the *lit* gene has already occurred by birth.

Human dwarfism is considered to be of the ateliotic type, if, by visual inspection, the adult body proportions appear normal⁸. When careful measurements are made, however, the adult ateliotic dwarf shows child-like proportions of large head, long torso, and shortened limbs. Since little mice appeared to have an ateliotic dwarfism, measurements were made to determine if this, in fact, was the case. The length and width of representative samples of endochondral bone (radius, humerus, tibia, femur) and intramembrane bone (scapula, skull) were determined on 30-, 50-, and 90-day-old C57BL/6J *lit/lit* and *lit/+* like-sexed littermates. These data along with the body weights of 18 to 19 pairs of little and normal littermates weighed sequentially at 30, 50, and 90 days are presented in Table IV. Since we found that long bone widths were more subjective than lengths with respect to where measurements were to be taken and less precise because of the small absolute size, we have presented only the width data from scapulae and skulls (Table IV).

Paired *t* tests were performed on bone measurements from mutant and normal mice of the same age and sex. As may be seen in Table IV, bone lengths of *lit/lit* mice

in all age groups were significantly smaller than *lit/+* controls. Scapula width was significantly smaller in *lit/lit* mice compared to controls across the three age groups. The modest amount of skull width data available indicated *lit/lit* male skulls were significantly smaller at 30 days, but not different at 90 days, from *lit/+* controls.

When the average bone measurements of *lit/lit* mice are considered as a percent of *lit/+* control values, there is a remarkable tendency for *lit/lit* bone dimensions to be approximately 80 percent of normal. There is also a slight tendency for the bones of *lit/lit* individuals to be relatively smaller at both 50 and 90 days of age than at 30 days of age. Although the data on skull dimensions are too incomplete to clearly discern trends in relative measurements, it does appear that of the bone types, the skull is most normal in size.

Finally, it should be noted in Table IV that the relative body weights of both male and female little mice generally remain just under 60 percent of *lit/+* controls throughout the experimental period examined.

We conclude from these data that the limbs, scapula, and skull length are proportionately reduced in size at 30 days and after, that the relative difference between bones of little and normal littermates increases between 30 and 50 days of age as *lit/+* mice experience accelerated growth associated with puberty, that the skulls of *lit/lit* males are closest to normal size of all skeletal features considered, and that body weight of little mice is approximately 50 to 60 percent of normal after 4 weeks of age. These features are indicative of ateliotic dwarfism.

Pituitary growth hormone and prolactin

Autopsies of little mice revealed that the anterior pituitary gland was very small, implying a possible defect in the functioning of this gland. Evaluation of mouse pituitary GH and PRL for changes under various physiological conditions has been accomplished using techniques of bioassay⁶, acrylamide gel electrophoresis^{3,5}, and radioimmunoassay^{9,10}. We chose acrylamide disc electrophoresis as the preferred method since both PRL and GH can be evaluated simultaneously.

Table III. Body weights (g) of *lit/lit* and *lit/+* mice

Sex	Genotype	Age in days					
		1	8	15	22	29	36
C57BL/6J							
♀♀	<i>lit/lit</i>	1.38 ± 0.02 (34)*	3.44 ± 0.07 (34)	5.43 ± 0.13 (34)	6.05 ± 0.14 (34)	7.61 ± 0.16 (32)	8.58 ± 0.16 (30)
	<i>lit/+</i>	1.43 ± 0.03 (35)	3.76 ± 0.09 (35)	5.89 ± 0.13 (35)	7.72 ± 0.16 (35)	11.45 ± 0.23 (34)	14.54 ± 0.26 (32)
♂♂	<i>lit/lit</i>	1.38 ± 0.04 (25)	3.40 ± 0.09 (25)	5.12 ± 0.09 (25)	5.90 ± 0.15 (25)	7.46 ± 0.18 (24)	8.87 ± 0.21 (23)
	<i>lit/+</i>	1.42 ± 0.02 (42)	3.71 ± 0.09 (42)	5.82 ± 0.12 (42)	7.73 ± 0.16 (42)	12.19 ± 0.26 (40)	15.70 ± 0.29 (39)
Hybrid							
♀♀	<i>lit/lit</i>	1.55 ± 0.03 (17)	3.30 ± 0.08 (17)	5.73 ± 0.16 (17)	6.74 ± 0.17 (17)	8.01 ± 0.12 (17)	9.21 ± 0.15 (17)
	<i>lit/+</i>	1.60 ± 0.05 (7)	3.64 ± 0.21 (7)	6.41 ± 0.27 (7)	9.06 ± 0.38 (7)	13.51 ± 0.35 (7)	17.43 ± 0.34 (7)
♂♂	<i>lit/lit</i>	1.46 ± 0.05 (14)	3.21 ± 0.13 (14)	5.54 ± 0.19 (14)	6.61 ± 0.24 (14)	7.93 ± 0.20 (14)	9.59 ± 0.25 (14)
	<i>lit/+</i>	1.57 ± 0.03 (21)	3.43 ± 0.11 (21)	5.96 ± 0.18 (21)	8.57 ± 0.27 (21)	14.02 ± 0.43 (21)	18.48 ± 0.36 (21)

* Number in parentheses is number of individuals

Table IV. Selected bone dimensions (mm) and body weight (g) of *lit/lit* and *lit/+* mice 30, 50, and 90 days of age*

Age (days)	30			50			90						
	<i>lit/lit</i>	<i>lit/+</i>	No. pairs	<i>lit/lit</i>	<i>lit/+</i>	No. pairs	<i>lit/lit</i>	<i>lit/+</i>	No. pairs				
Endochondral bone													
Radius length	♀	7.74 ± 0.12	8.73 ± 0.07	±0.10	7	8.28 ± 0.09	9.83 ± 0.13	±0.12	6	8.68 ± 0.18	10.38 ± 0.14	±0.18	5
	♂	7.62 ± 0.13	8.72 ± 0.27	±0.27	6	7.95 ± 0.36	10.12 ± 0.05	±0.31	4	8.78 ± 0.09	10.72 ± 0.15	±0.12	6
Humerus length	♀	7.59 ± 0.12	9.13 ± 0.07	±0.07	7	7.63 ± 0.08	10.03 ± 0.22	±0.21	6	8.82 ± 0.32	11.28 ± 0.25	±0.27	5
	♂	7.68 ± 0.13	9.35 ± 0.18	±0.22	6	8.00 ± 0.30	10.82 ± 0.26	±0.37	4	9.00 ± 0.14	11.93 ± 0.07	±0.17	6
Femur length	♀	8.30 ± 0.13	10.07 ± 0.16	±0.15	7	9.73 ± 0.24	13.10 ± 0.34	±0.26	6	10.52 ± 0.37	13.90 ± 0.21	±0.35	5
	♂	8.63 ± 0.38	10.38 ± 0.24	±0.34	6	9.70 ± 0.11	13.05 ± 0.33	±0.34	4	10.50 ± 0.08	14.15 ± 0.10	±0.13	6
Tibia length	♀	11.59 ± 0.17	13.24 ± 0.15	±0.18	7	12.67 ± 0.22	15.65 ± 0.14	±0.31	6	13.68 ± 0.12	16.68 ± 0.15	±0.22	5
	♂	11.50 ± 0.28	13.57 ± 0.31	±0.29	6	13.38 ± 0.30	15.73 ± 0.18	±0.18	4	13.47 ± 0.28	16.52 ± 0.31	±0.54	6
Intramembranous bone													
Scapula													
length	♀	7.36 ± 0.13	8.74 ± 0.19	±0.19	7	7.68 ± 0.15	10.10 ± 0.16	±0.18	6	8.60 ± 0.23	10.98 ± 0.22	±0.31	5
	♂	7.35 ± 0.08	8.78 ± 0.16	±0.13	6	7.80 ± 0.14	10.43 ± 0.05	±0.17	4	8.58 ± 0.06	11.35 ± 0.14	±0.16	6
width	♀	4.33 ± 0.06	5.63 ± 0.21	±0.19	7	4.78 ± 0.09	6.90 ± 0.10	±0.07	6	5.44 ± 0.21	7.24 ± 0.07	±0.19	5
	♂	4.28 ± 0.07	5.68 ± 0.01	±0.12	6	4.80 ± 0.18	7.10 ± 0.12	±0.18	4	5.37 ± 0.06	7.52 ± 0.12	±0.11	6
Skull													
length	♂	18.25 ± 0.30	20.23 ± 0.19	±0.28	6	—	—	—	—	19.32 ± 0.26	22.67 ± 0.43	±0.61	6
	♂	9.63 ± 0.05	10.45 ± 0.14	±0.17	6	—	—	—	—	9.82 ± 0.19	10.58 ± 0.30	±0.31	6
Body weights	♀	7.73 ± 0.16	13.08 ± 0.29	—	19	10.37 ± 0.25	17.99 ± 0.33	—	19	13.55 ± 0.44	21.74 ± 0.68	—	19
	♂	7.62 ± 0.15	14.01 ± 0.42	—	18	11.13 ± 0.23	21.77 ± 0.25	—	18	16.84 ± 0.37	27.04 ± 0.38	—	18

* All bone measurements, except 90 day skull width, of *lit/lit* mice of either sex were significantly different at the 0.01 level, or less, from the comparable bone dimensions of *lit/+* mice by paired *t* tests
 † SE_{*i*} = standard error of mean difference

A photograph of gels containing electrophoresed proteins from pituitary homogenates of *lit/+* females, *lit/lit* females, *lit/lit* males, and purified mouse PRL and GH are presented in Figure 1.

It is immediately apparent that both GH and PRL are markedly deficient in *lit/lit* females and *lit/lit* males. In fact, the concentration of PRL is so low that the band is barely visible in freshly destained gels. Radioimmunoassay data, to be reported in detail elsewhere¹ have confirmed these findings.

The gels containing purified GH or PRL have, in addition to the band designated as GH or PRL, additional faster migrating bands. These bands are also evident in gels prepared from fresh pituitary homogenates of *lit/+* females. These proteins may be degeneration products of GH or PRL. It should also be noted that pituitary homogenates from either *lit/lit* females or *lit/lit* males contain one or more bands located between PRL and the buffer front that are barely observable in the *lit/+* female. Cheever, et al.³ have also observed similar bands in gels prepared from pituitary homogenates of Snell's dwarf (*dw/dw*) mice¹¹. As in the case with little mice, most of the pituitary gland of dwarf mice is composed of the posterior part. Therefore, it is most likely that these bands are of posterior pituitary gland origin.

The little mouse as animal model for isolated growth hormone deficiency type 1

We believe that there are a number of parallelisms to be drawn between the mouse dwarfism caused by the *lit* mutation and human dwarfism caused by isolated GH deficiency type 1. Both disorders are inherited as fully recessive traits. In both instances, the onset of growth retardation is first detectable in infancy. There is a proportionate reduction in bone size (80 percent of normal) and body weight (60 percent of normal) in the mutant mouse, and a similar but more variable retardation of bone age in relation to chronological age in young human dwarfs. There is an excellent growth response to GH therapy in the little mouse² and the human dwarf⁶. Little females show delayed sexual maturation but reach full fertility. Puberty in human female dwarfs is also delayed, yet fertility is normal⁷. Little males have delayed sexual maturation and a marked degree of infertility. Human male dwarfs also have delayed sexual maturation; fertility data have not been published.

Summary

A new autosomal recessive mutation in the mouse, little (*lit*), has been shown to be located on Chromosome 5. The mutation in the homozygous state causes ateliotic dwarfism that is first detected at 15 days of age by decreased body weight. Long bone lengths are significantly reduced. Skull width, however, is not affected. Female little mice are fully fertile; they may lose their first litters. Although most of the little males sire one or two litters, they rarely sire a third litter. Analysis of pituitary extracts electrophoresed on acrylamide gels reveal a significant reduction of the two anterior pituitary hormones, GH and PRL, in both male and female little mice. Because the little mouse shares a number of

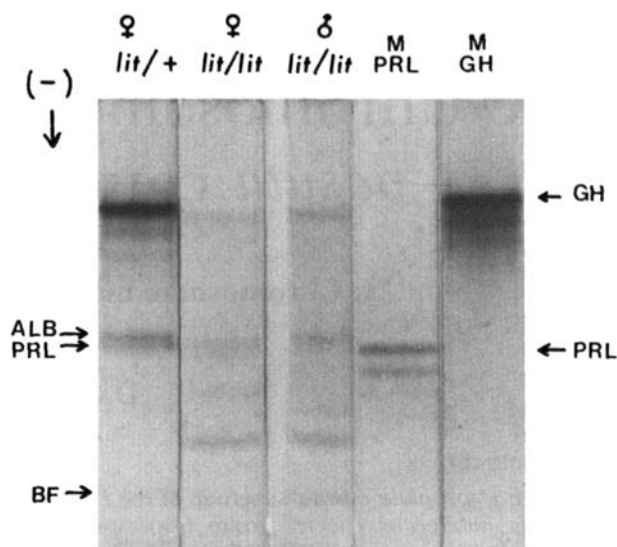


FIGURE 1—Homogenates of whole pituitary glands and purified mouse PRL and GH in 12 percent acrylamide gels. In order, left to right: *lit/+* ♀, *lit/lit* ♀, *lit/lit* ♂, PRL, and GH. The bands corresponding to the buffer front (BF), PRL, albumin (ALB), and GH are identified.

similarities with the human ateliotic dwarfism, isolated growth hormones deficiency type 1, it may be a useful animal model for this inherited human growth disorder.

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