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The Petit Rat (*pet/pet*), a New Semilethal Mutant Dwarf Rat with Thymic and Testicular Anomalies

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The petit rat (*pet/pet*) is a recently discovered semilethal mutant dwarf. The neonatal *pet/pet* rats had a low body weight and small thymus and testis. During the first 3 d after birth, 50% of the male and 80% of the female *pet/pet* pups were lost or found dead. Surviving *pet/pet* rats showed marked retardation of postnatal growth, and their body weights were 41% (female rats) and 32% (male rats) of those of normal rats at the adult stage. The *pet/pet* rats exhibited proportional dwarfism, and their longitudinal bones were shorter than those of controls without skeletal malformations. Most organs of male *pet/pet* rats, especially the thymus, testis, adipose tissue surrounding the kidney, and accessory sex organs, weighed markedly less at 140 d of age than did those of their normal counterparts. The thymus of *pet/pet* rats was small with abnormal thymic follicles. Testes from *pet/pet* rats exhibited 2 patterns of abnormal histology. Spermatogenesis was present in testes that were only slightly anomalous, but the seminiferous tubules were reduced in diameter. In severely affected testes, most of the seminiferous tubules showed degeneration, and interstitial tissue was increased. Plasma growth hormone concentrations did not differ between *pet/pet* and normal male rats. The dwarf phenotype of *pet/pet* rats was inherited as an autosomal recessive trait. These results indicate that the *pet/pet* rat has a semilethal growth-hormone-independent dwarf phenotype that is accompanied by thymic and testicular anomalies and low birth weight.

Abbreviations: GH, growth hormone; PET, petit; TBS, Tris-HCl buffered saline.

Many spontaneously mutant rat strains that display unusual phenotypes have been established as models of human disease to study the etiologies and pathologies of various diseases and to develop novel treatments and drugs. Recent progress in genome sequencing projects has made it possible to identify the genes responsible for the mutations defined by various phenotypes of interest, and phenotype-driven approaches have been used to discover the functions of various mammalian genes.[13] From a closed colony of Wistar–Imamichi rats, we have established several spontaneous mutant rat strains, including those showing osteochondrodysplasia,[23] male hypogonadism with renal hypoplasia,[25,31] hydronephrosis,[9,34] and lethal dwarfism with epilepsy[26,32]. These lines have been characterized phenotypically to evaluate their usefulness as disease models and to elucidate candidate genes responsible for the mutant phenotypes.[23-32]

We noted rats showing severe dwarfism in the 16th generation of +/+ coisogenic strain of the HDH hypogonadism strain, which is a normal (*hgn*-free) inbred strain derived from the HGN strain.[29] We succeeded in fixing the mutation by sister–brother mating of the carriers and have continued inbreeding to establish a new dwarf rat inbred strain named the petit (PET) rat.[4] We have characterized the phenotype of *pet/pet* rats to evaluate the novelty and utility of this strain as a disease model. This report is the first description of phenotypic characterization of *pet/pet* rats, including postnatal growth, mortality, organ weight, results of histologic examinations, and genetic mode of inheritance.

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Materials and Methods

Animals. Phenotypically normal (+/+ or +/*pet*) and *pet/pet* rats were collected from 1023 littermates of 152 litters obtained by crossing proven carriers in the 2nd to 14th generations of PET rats. Rats were maintained under conditions of controlled photoperiod (14 h light, 10 h dark), temperature (24 ± 1 °C), and relative humidity (50% ± 10%). Rats were fed a commercial chow (CR-LPF Pellets, Oriental Yeast, Tokyo, Japan) and given water ad libitum.[26-30,32] Rats were free from common pathogens for rats (Sendai virus, sialodacryoadenitis virus, Tyzzer disease virus, *Mycoplasma pulmonis*, *Bordetella bronchiseptica*, *Corynebacterium kutscheri*, *Pasteurella pneumotropica*, and *Streptococcus pneumoniae*). The animal procedures and experimental protocols followed the guidelines of the Animal Care and Use Committee of the Nippon Veterinary and Life Science University.

Growth. Newborn rats showing reduced body weight were identified as *pet/pet* rats, whereas the remaining rats were phenotypically normal. The resulting 8 female and 58 male *pet/pet* rats and 20 female and 117 male normal littermates were weighed at 1, 3, 7, 12, 18, and 21 d of age, and 6 female and 29 male *pet/pet* rats and 19 female and 52 male normal littermates were weighed once a week after 21 d of age.

Survival rates. A total of 119 female and 135 male *pet/pet* rats and 395 female and 374 male normal rats were used for comparison of survival rates from 0 to 98 d of age. The percentage survival rate on each day was calculated by dividing the number of surviving pups by the total number of neonates born.

Determination of the genetic mode of inheritance. The rats used in hereditary analysis were derived from the second through seventh generations of the PET strain. The incidence of *pet/pet* rats

was analyzed by χ^2 test to confirm the hypothesis that this mutation was inherited as a single autosomal recessive trait.[23-26,31]

Skeletal examination. To examine the skeletal systems, whole-body radiography of 3 *pet/pet* and 3 normal male rats at 91 d of age was performed by using a LaTheta scanner (LCT-100; Aloka, Tokyo, Japan).[26]

Anatomic and histologic examinations. To determine whether *pet/pet* rats had anatomically apparent defects at birth, 4 *pet/pet* and 6 normal neonates were euthanized by ether overdose and necropsied. To examine internal organs in surviving *pet/pet* rats, 8 *pet/pet* and 8 normal male rats at 140 d of age were euthanized by ether overdose and necropsied. The preputial gland, testis, epididymis, deferent duct, gland of the deferent duct, coagulating gland, seminal vesicle, prostate gland, bladder, penis, Cowper gland, bulbocavernosus muscle, levator ani muscle, adrenal gland, spleen, pancreas, adipose tissue surrounding the kidney, submandibular gland, sublingual gland, lateral lacrimal gland, liver, thymus, thyroid gland, heart, lung, brain, pituitary gland, eye, and gastrocnemius muscle of *pet/pet* and normal rats were isolated and weighed by using a digital balance.[24-27,30,31] Because the HDH strain was isolated from the HGN strain, which had been derived from the hydronephrosis (HNK) strain,[9,29,31,34] some of the rats in the PET strain had hydronephrosis. Therefore, we did not compare the renal weights between *pet/pet* and normal males. After measurement of organ weights, organs were fixed in 10% formalin in 0.1 M sodium hydrogen phosphate buffer, embedded in paraffin, and sectioned at a thickness of 5 µm. The sections were deparaffinized, hydrated, and stained with hematoxylin and eosin. The stained sections were examined with a light microscope (BX50, Olympus, Tokyo, Japan), and histologic photographs were obtained using a charge-coupled digital camera system (Penguin 600CL, Pixcera, Osaka, Japan).[26-30]

Plasma growth hormone (GH) concentration. Seven *pet/pet* and 6 normal males at 35 d of age were used to examine plasma growth hormone (GH) concentrations. Blood samples were collected from the vena cava with heparinized plastic syringes under light ether anesthesia. Plasma samples were obtained after centrifugation and stored at –20 °C until assayed for GH. The plasma concentrations of GH in *pet/pet* and normal male rats were measured using a rat growth hormone enzyme immunoassay kit (SPI-BIO, Massy, France).

Immunohistochemical detection of GH in the pituitary gland. The pituitary glands were removed from 3 *pet/pet* and 3 normal male rats at 35 d of age and washed with 0.9% saline, and paraffin sections were prepared as described earlier. The pituitary sections were soaked in 0.01 M Tris-buffered saline (TBS; pH 7.5), then soaked in methanol containing 3% hydrogen peroxide for 15 min to inactivate internal peroxidases, and soaked in TBS. The sections were incubated with antirat GH rabbit polyclonal antibody (1:1000; Biogenesis, Poole, UK) overnight at room temperature, rinsed with TBS, and then incubated with peroxidase-conjugated antiIgG polymer [Histofine Simple Stain Rat Max-Po (Multi); Nichirei, Tokyo, Japan]. The sections subsequently were incubated with 3,3′-diaminobenzidine tetrahydrochloride solution (Histofine Simple Stain DAB solution, Nichirei) for 5 min, and the reaction was stopped by soaking in distilled water. The sections were counterstained with hematoxylin.[26,28,29] For negative controls, the primary antibodies were replaced with normal rabbit serum.[26]

Statistical analysis. Body weights, organ weights, and GH concentrations are expressed as mean ± SD (SD), and differences between normal and *pet/pet* rats were assessed by using the unpaired Student *t* test (Excel X for Mac, Microsoft Corp. Redmond, WA).[26]

Results

Body growth. Newborn *pet/pet* rats were obviously smaller than their normal littermates (Figure 1 A). In addition, newborn *pet/pet* rats had extremely small thymuses (Figure 1 B), the weight (mean ± SD) of which was about 38% of that of a phenotypically normal thymus (*pet/pet*, 4.00 ± 0.12 mg; normal, 10.31 ± 1.17 mg). In addition, the testis of *pet/pet* rats weighed about 50% that of normal testis (*pet/pet*: 2.80 ± 0.56 mg; normal, 5.38 ± 0.90 mg). No other gross anomaly was detected in *pet/pet* rats. Although *pet/pet* and normal neonates varied slightly in their weights depending on the number of littermates, the body weights of *pet/pet* female and male rats were always 65% to 75% of those of normal littermates. Therefore we could easily differentiate between dwarf (*pet/pet*)

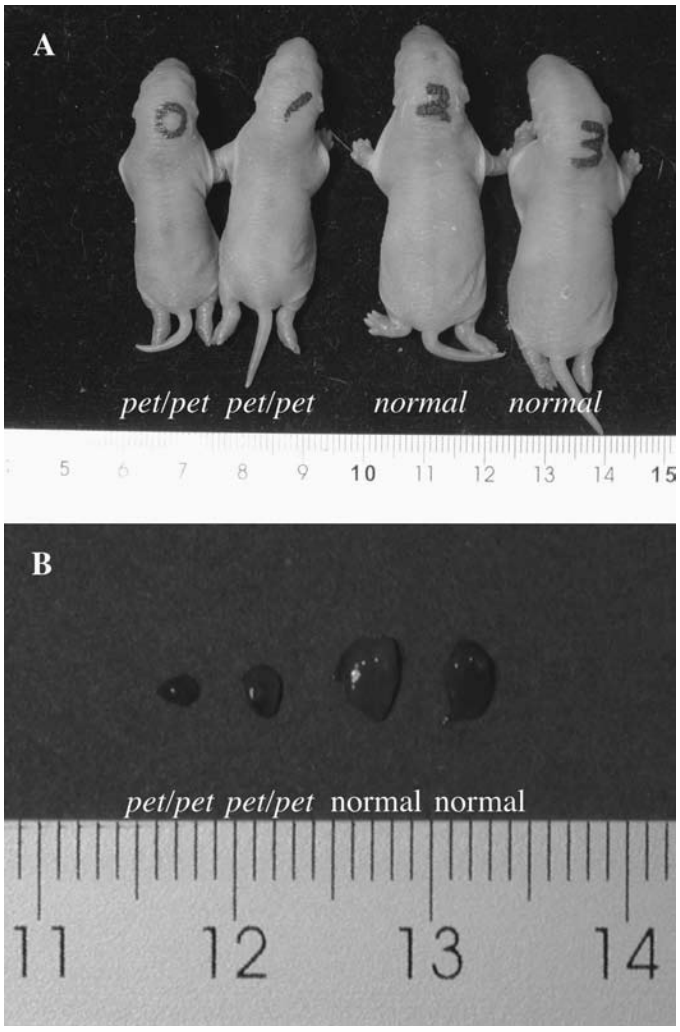


Figure 1. (A) Gross appearance and (B) thymus of newborn *pet/pet* and normal rats. The *pet/pet* rats were apparently smaller than their normal littermates. The *pet/pet* thymuses were extremely small in size compared with normal thymuses.

rats and normal littermates at birth. Overall, the body weights of *pet/pet* neonates were significantly less than those of normal littermates (female rats: *pet/pet*, 4.1 ± 0.4 g; normal, 6.1 ± 0.3 g; male rats: *pet/pet*, 4.7 ± 0.3 g; normal, 6.3 ± 0.5 g; $P < 0.05$ for both comparisons). The *pet/pet* rats also showed defective postnatal growth. Body weights were significantly ($P < 0.05$) lower in *pet/pet* than normal rats on all days examined. At 98 d after birth, the body weight of *pet/pet* female rats (112.1 ± 23.1 g) was about 41% that of their normal counterparts (298.5 ± 23.1 g), and the body weight of *pet/pet* male rats (142.2 ± 23.4 g) was about 32% that of their normal counterparts (443.2 ± 60.7 g; Figure 2). The dwarf phenotype of *pet/pet* rats was more apparent at the adult stage as compared with that at birth. The dwarfism in *pet/pet* rats was proportional (Figure 3 A); the ratio of body length to tail length was comparable between *pet/pet* (1.41 ± 0.04) and normal (1.41 ± 0.03) rats, and there were no skeletal anomalies in *pet/pet* rats except for longitudinal bone shortening (Figure 3 B).

Survival rates. Although *pet/pet* rats usually were born alive, most of the female rats and half of the male rats died or were lost the next day. Because we could discern the presence of milk in the stomach externally, we considered that the *pet/pet* neonates could suckle. Survival rates of *pet/pet* rats were 3% for female and 32% for male rats at 98 d of age, whereas survival rates of phenotypically normal rats were 83% for female and 87% for male rats at the same age (Figure 4).

Genetic mode of inheritance. The numbers of *pet/pet* and phenotypically normal rats were recorded at birth. Thereafter, all rats genotyped as *pet/pet* died early or showed severe growth retardation, indicating the accuracy of phenotyping at birth. The actual incidences of *pet/pet* female, male, and both female and male rats did not deviate significantly from the hypothesis that this mutation was inherited as a single autosomal recessive trait (Table 1).

Organ weights. Because of their high mortality rate, we could not collect a sufficient number of *pet/pet* female rats for statistical analysis of organ weights. We measured the absolute weights and calculated the relative weights [absolute weight (mg) × 100%/ brain weight (mg)] of major organs in *pet/pet* and normal male rats at 140 d of age. The absolute weights of all organs were significantly lower in *pet/pet* than in normal male rats. The relative weights of the thymus, adipose tissue surrounding kidney, testis,

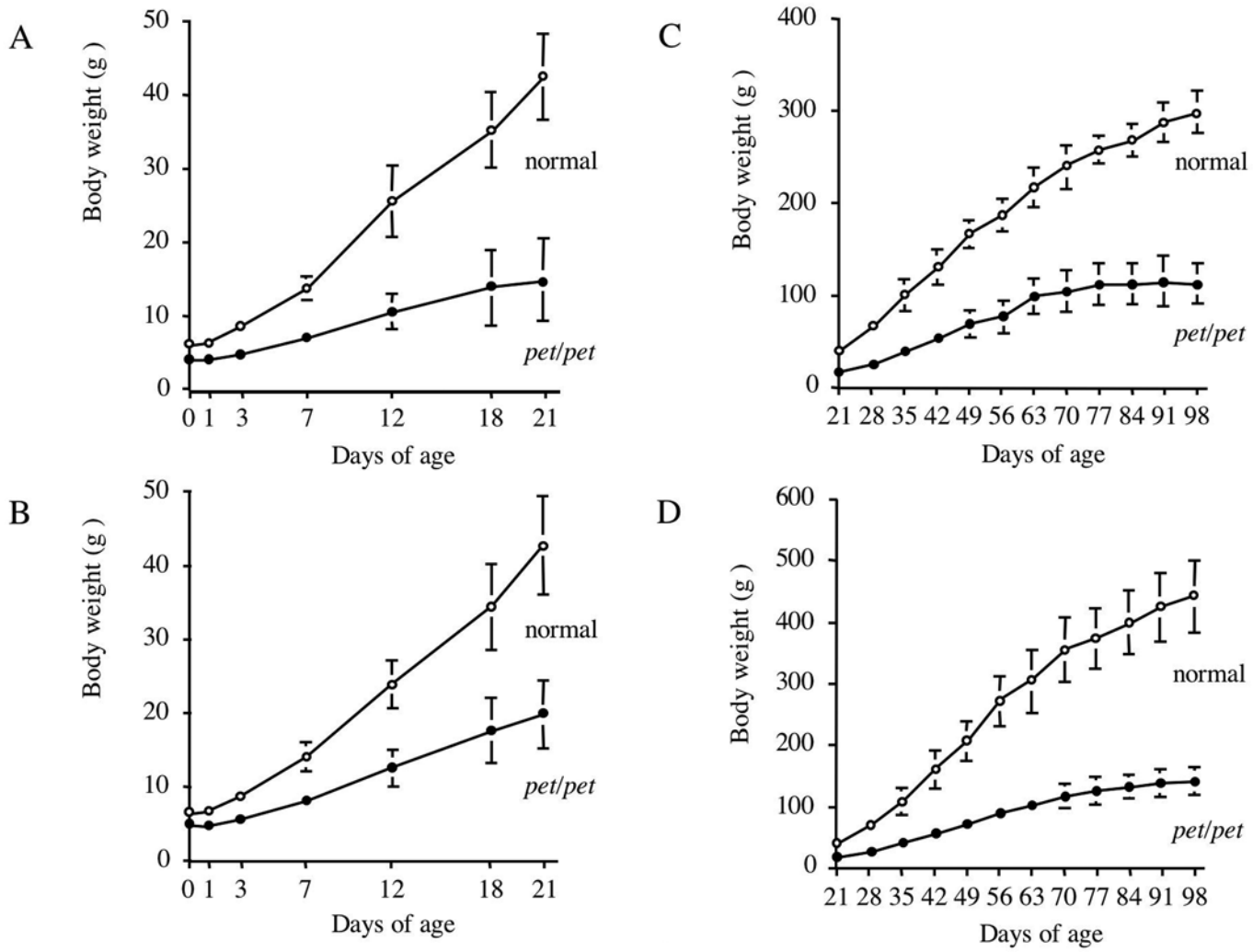
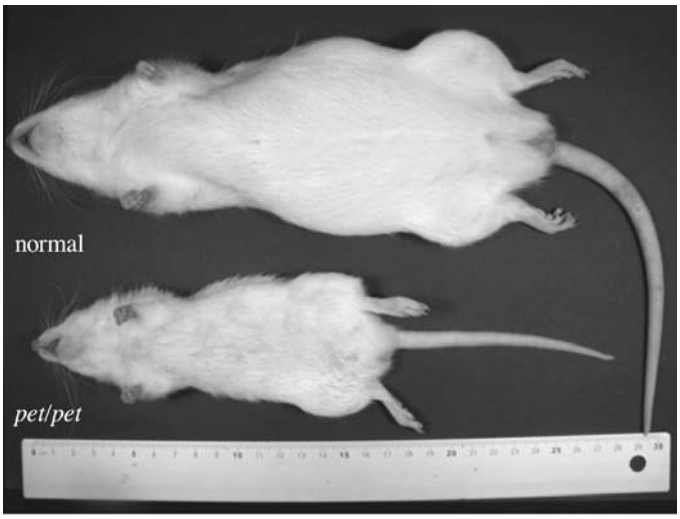


Figure 2. Changes in body weights of *pet/pet* and normal (A, C) female rats and (B, D) male rats. (A, B) During the nursing period. (C, D) After weaning. The body weights of *pet/pet* rats were significantly ($P < 0.05$) smaller than those of normal rats on all days examined.

Table 1. Fitness to the hypothesis of a single autosomal recessive trait as the genetic mode of transmission

	Observed		Expected			
	pet/pet	normal	*pet/pet*	normal	χ^2	*P*
Female rats	66	217	70.75	204.75	0.429	>0.50
Male rats	84	269	88.25	264.75	0.272	>0.50
Total	150	486	159	477	0.678	>0.25

and accessory sex organs including epididymis, gland of deferent duct, seminal vesicle, coagulating gland, prostate glands, and Cowper gland were much smaller in *pet/pet* than in normal male rats ($P < 0.0001$). The relative weights of the deferent duct, penis, bulbocavernosus, levator ani muscle, spleen, pancreas, submandibular gland, liver, heart, and gastrocnemius muscle were significantly ($P < 0.05$) lower in *pet/pet* than in normal males (Table 2).



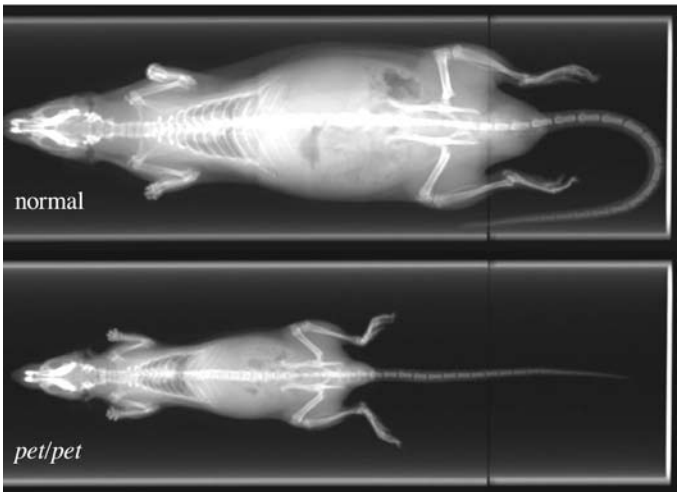


Figure 3. (A) External features of *pet/pet* and normal male rats at 91 d of age. The dwarfism of *pet/pet* rat was proportional and more severe than that at birth. (B) Radiographs of the same rats. There were no skeletal anomalies in *pet/pet* rats except for longitudinal bone shortening.

Histologic examinations. All of the organs of *pet/pet* and normal male rats collected at 140 d of age underwent histologic examination. Consistent with the lower absolute weights in *pet/pet* male rats, the cross-sectional areas of their organs were smaller than those of normal male rats. We could not detect any apparent histologic changes in most organs of *pet/pet* male rats, except for reduced organ cellularity and slight cellular infiltration into the interstitial tissue of kidney and gastrocnemius muscle (data not shown). However, the *pet/pet* testis and thymus, which showed remarkably decreased relative weights, had marked pathologic changes. The *pet/pet* testis exhibited 2 patterns of pathologic change: mild and severe. In testes with mild pathologic change, spermatogenesis appeared normal, but the diameters of seminiferous tubules were about 26% smaller than those of normal littermates. In severely affected testes, most seminiferous tubules had degenerated, and spermatogenesis was present only rarely. In the degenerated tubules, most of the germ cells were lost; Sertoli cells were present but had many vacuoles in the cytoplasm. The area of interstitial tissue and the number of cells in that area were increased (Figure 5). The different degrees of testicular anomaly were detected in different sides of the testis in the same rats. The *pet/pet* thymus was very small, with increased connective tissue.

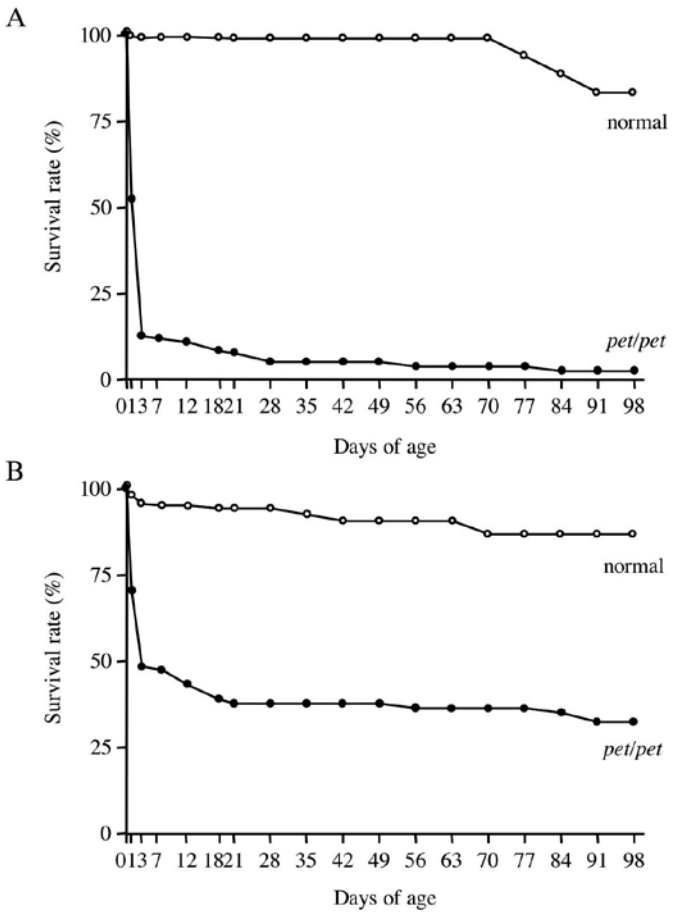


Figure 4. Survival rates from birth to adult stage in *pet/pet* and normal rats. Survival rate on each day examined was represented as the percentage of the number of surviving animals to the total number of newborn *pet/pet* and normal rats. (A) 10% of female and (B) 50% of male *pet/pet* rats were alive at 3 d of age, and the survival rates of *pet/pet* rats were (A) 3% for female rats and (B) 32% for male rats at 98 d of age.

Table 2. Body weights (g), body and tail lengths (cm), and organ weights (mg) of *pet/pet* and normal male rats at 140 d of age

	Absolute		Relative[a]	
	pet/pet	normal	*pet/pet*	normal
Body weight	153.8 ± 19.8[b]	515.3 ± 50.9	not done	not done
Body length	16.9 ± 0.6[b]	25.5 ± 0.8	not done	not done
Tail length	12.1 ± 0.5[b]	18.1 ± 0.5	not done	not done
Preputial gland	62.4 ± 12.5[b]	117.0 ± 24.4	5.6 ± 1.0	5.7 ± 1.1
Testes	644.1 ± 220.8[b]	3004.0 ± 425.3	59.1 ± 20.3[c]	149.8 ± 21.8
Epididymus	233.8 ± 94.2[b]	1112.1 ± 60.9	21.0 ± 7.7[c]	55.5 ± 4.1
Gland of deferent duct	10.8 ± 3.1[b]	44.6 ± 4.2	0.9 ± 0.2[c]	2.2 ± 0.1
Deferent duct	88.3 ± 22.6[b]	248.6 ± 16.8	7.8 ± 2.0[b]	12.3 ± 0.6
Coagulating gland	52.6 ± 7.9[b]	200.8 ± 28.8	4.8 ± 0.7[c]	10.0 ± 1.6
Seminal vesicle	273.8 ± 67.7[b]	1700.4 ± 142.2	25.1 ± 5.7[c]	84.7 ± 6.8
Prostate gland (ventral)	173.7 ± 31.1[b]	502.7 ± 94.4	15.7 ± 2.8[c]	24.9 ± 4.2
Prostate gland (dorsal)	108.1 ± 17.3[b]	377.6 ± 81.8	9.8 ± 1.5[c]	18.7 ± 4.1
Bladder	86.0 ± 16.8[b]	152.8 ± 16.4	7.7 ± 1.3	7.5 ± 0.6
Penis	168.2 ± 18.1[b]	353.7 ± 19.9	15.2 ± 1.1[b]	17.5 ± 1.1
Cowper gland	25.3 ± 6.1[b]	95.9 ± 13.0	2.3 ± 0.5[c]	4.7 ± 0.6
Bulbocavernosus	313.1 ± 86.0[b]	929.5 ± 85.8	28.3 ± 7.0[b]	46.1 ± 4.5
Muscle levator ani	108.7 ± 22.8[b]	281.7 ± 18.6	9.8 ± 1.7[b]	14.0 ± 1.2
Adrenal gland	25.9 ± 4.6[b]	42.5 ± 5.6	2.3 ± 0.3	2.1 ± 0.2
Spleen	445.0 ± 74.8[b]	986.8 ± 118.5	40.2 ± 5.9[b]	48.9 ± 5.3
Pancreas	485.5 ± 80.8[b]	1355.1 ± 214.1	43.9 ± 6.2[b]	67.5 ± 12.4
Submandibular gland	250.4 ± 26.4[b]	638.9 ± 37.2	23.0 ± 1.5[b]	31.8 ± 1.8
Sublingual gland	55.7 ± 10.1[b]	103.0 ± 7.8	5.1 ± 0.7	5.1 ± 3.3
Lateral lacrimal gland	131.4 ± 7.6[b]	208.6 ± 7.0	12.1 ± 0.7	10.3 ± 2.5
Liver	7012.5 ± 1657.7[b]	20791.3 ± 5187.1	634.7 ± 136.8[b]	1032.7 ± 253.2
Thymus	75.3 ± 43.7[c]	463.8 ± 69.7	6.7 ± 3.7[c]	23.0 ± 3.3
Thyloid gland	15.4 ± 4.4[b]	28.7 ± 4.0	1.3 ± 0.3	1.4 ± 0.2
Heart	715.7 ± 107.9[b]	1456.6 ± 91.8	64.8 ± 8.3[b]	72.3 ± 4.9
Lung	1006.7 ± 143.9[b]	1763.7 ± 321.8	91.5 ± 13.8	87.5 ± 15.3
Brain	1102.4 ± 56.0[c]	2014.7 ± 71.4		
Pituitary gland	4.7 ± 1.4[b]	10.5 ± 1.6	0.04 ± 0.01	0.05 ± 0.01
Gastrocnemius muscle	914.2 ± 126.2[b]	2553.6 ± 286.9	82.8 ± 9.5[b]	126.7 ± 12.8
Eye	180.4 ± 8.1[b]	292.6 ± 12.9	16.6 ± 1.0	14.5 ± 0.7
Adipose tissue surrounding kidney	582.0 ± 197.3[c]	8245.6 ± 510.4	53.3 ± 17.1[c]	411.1 ± 25.2

Data are presented as mean ± SD.
[a]Absolute weight (mg) x 100%/ brain weight (mg)
[b]Significantly ($P < 0.05$) smaller in *pet/pet* than in normal rats.
[c]Significantly ($P < 0.0001$) smaller in *pet/pet* than in normal rats.

In addition, abnormal thymic follicles were seen in the subcapsular region of the *pet/pet* thymus (Figure 6).

Detection of GH production in the pituitary gland and plasma GH concentration. The anterior pituitary glands in *pet/pet* and normal male rats contained similar numbers of GH-positive cells (Figure 7 A). Plasma GH concentrations were comparable between *pet/pet* and normal male rats (Figure 7 B).

Discussion

In the present study, we found that *pet/pet* rats showed severe dwarfism, a high mortality rate, and testicular and thymic anomalies. The dwarfism of *pet/pet* rats was characterized by not only postnatal severe growth retardation but also markedly decreased weight at birth. Because the birth weight of *pet/pet* rats was significantly less than that of normal rats, the growth retardation of *pet/pet* rats may begin during the embryonic period. In utero growth retardation has been reported to occur in several knockout mice with severe growth retadation;[10-12] these models also show a considerable rate of prenatal death.[10,12] However, the lack of significant deviations in the segregation ratio indicates that *pet/pet* rats are born alive. Mice deficient in the pleomorphic adenoma gene 1 show a similar postnatal dwarf phenotype associated with low birth weight and reduced fertility,[11] but they lack the postnatal lethality and thymic anomaly of *pet/pet* rats.

In addition to the PET strain, we have established 2 other mutant rat strains showing growth retardation. The *lde/lde* rats of

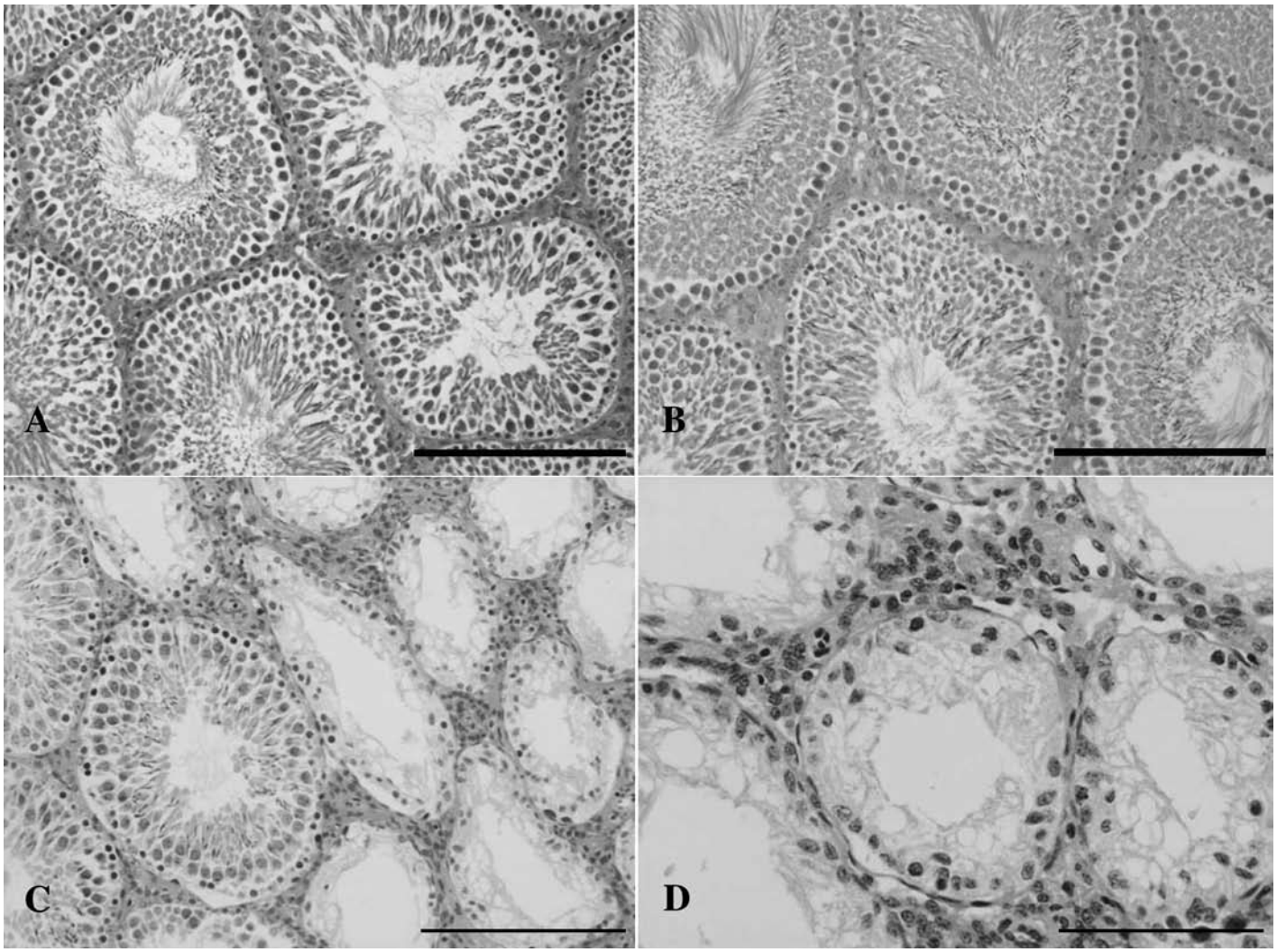


Figure 5. Histology of (A, C, D) *pet/pet* and (B) normal testes at 140 d of age. (A, B) In the slightly affected testis of *pet/pet* rats (A), the diameters of seminiferous tubules were smaller than those of normal male rats (B). (C, D) In the severely affected testis of *pet/pet* rats, most of the seminiferous tubules were degenerated, and spermatogenesis was found in only a few seminiferous tubules (C). Most germ cells at various developmental stages were degenerated, and indistinguishable condensed nuclei remained in the affected tubules. Sertoli cells had many cytoplasmic vacuoles, and interstitial tissue was increased (D). Bar, 100 µm (A, B, C), 50 µm (D).

the LDE strain show a severe dwarf phenotype with premature lethality.[26] Their growth retardations appears after 3 d of age, and they have a high incidence of epileptic seizures.[26,32] The *hgn/hgn* rats of the HGN strain show body growth retardation beginning in the embryonic period,[30] whereas their postnatal growth shows less retardation than that of *pet/pet* and *lde/lde* rats.[24,27] In addition, *hgn/hgn* rats show renal hypoplasia and testicular dysplasia.[24,25,27-31] Although mutant rats carrying *hgn*, *lde*, and *pet* have been identified in our facility, both phenotypic analysis and linkage mapping experiments clearly excluded the possibility that these defects were caused by the same mutation.[4,25,29,32]

Several dwarf strains resulting from GH deficiencies have been reported.[2,14,16,22,33] In contrast to these strains, *pet/pet* rats had a normal plasma GH concentration at 35 d of age and a similar number of GH-positive cells in the anterior pituitary gland as normal controls. These results indicate that the growth retardation in *pet/pet* rats is not caused by GH deficiency. Although mice deficient in insulin-like growth factor I show growth retardation from birth,[17] plasma levels of this hormone and expression of the GH receptor may be normal in *pet/pet* rats because they did not show any abnormal elevations in plasma GH levels.[38] Consistent with the observed proportional dwarfism, absolute weights of all organs examined were significantly smaller in *pet/pet* than in normal male rats. In addition, relative weights of most organs were somewhat decreased in *pet/pet* male rats, but those of the reproductive organs and thymus markedly reduced. These findings indicate that the growth of these organs was affected to a greater extent than overall body growth in *pet/pet* males, and this effect may be caused directly or indirectly by mutation of the *pet* gene.

The *pet/pet* rats were born alive, but most died or were lost between 1 and 3 d after birth. In particular, *pet/pet* female rats had a dramatically decreased birth weight and an extremely high mortality rate. Because those *pet/pet* rats that survived until 3 d after birth typically remained alive to the adult stage, the high mortality of *pet/pet* rats during the early postnatal period may be related to the severely low birth weight. Small size at birth may be associ-

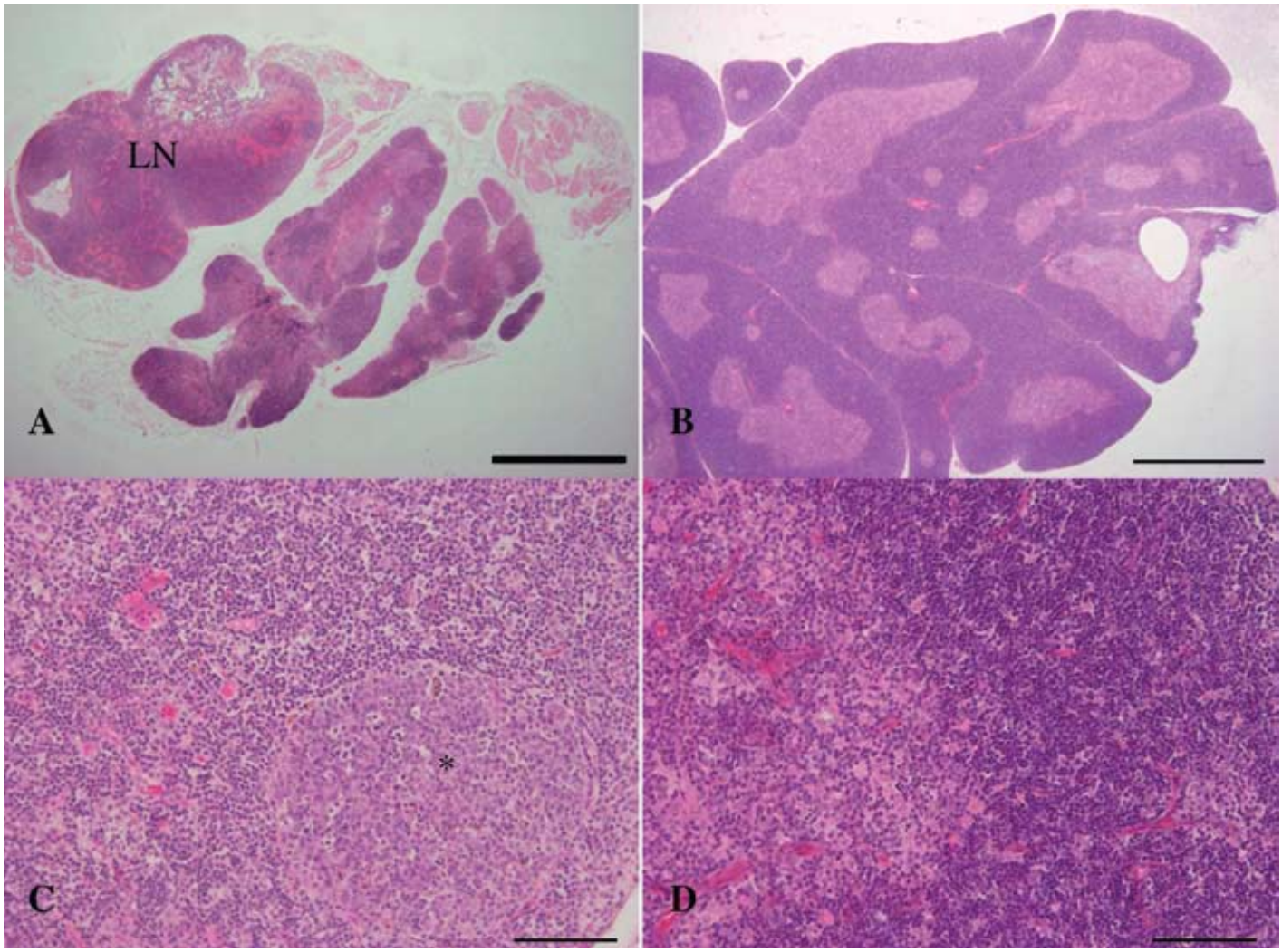


Figure 6. Histology of (A, C) *pet/pet* and (B, D) normal male thymus at 140 d of age. (A, B) The *pet/pet* thymus was grossly smaller than that of normal controls. The much smaller size of the *pet/pet* thymus was readily apparent when compared with the section of adjacent lymph node. (C, D) The *pet/pet* thymus contained abnormal thymic follicles (*) in the subcapsular region. Bar, 1 mm (A, B), 100 µm (C, D).

ated with an increased risk of infant early death[35] and metabolic diseases in adult life.[20] Because the growth retardation in most rat dwarf mutant strains becomes apparent during the nursing period or after weaning,[2,5,14,33] *pet/pet* rats may be a unique genetic model for severe dwarfism with low birth weight.

The testes of *pet/pet* neonates were small, and male *pet/pet* rats exhibited 2 grades of testicular anomaly at 140 d of age. Slightly anomalous testes showed normal spermatogenesis but reduced seminiferous tubule diameter. Although some cases of human subfertility may be associated with low birth weight, the pathologic mechanism governing the association remains to be determined.[7,8,36] Therefore, it may be useful to examine reproductive performance in *pet/pet* males. In severely affected *pet/pet* testes, spermatogenesis was rare, and degenerated seminiferous tubules were numerous. Although the cause of the phenotypic variation in the *pet/pet* testis at 140 d of age is unknown, it is unlikely to be due to genetic background variation because the different types of testicular defect were still present in rats of the 14th generation of the PET strain. In general, consequent phenotypes are epigenetically modified with advancing age. Because different degrees of testicular anomaly were detected on different sides of the testis in the same rat, the testicular variations at 140 d of age may be related to degenerative processes of the affected testis with advancing age. In addition to the degenerated seminiferous tubules, severely abnormal testes contained increase interstitial tissue. Leydig cell hyperplasia is associated with testicular dysfunction and alteration with aging.[6,37] Considering the significantly smaller relative weight of accessory sex organs, it may be useful to confirm the presence of Leydig cells in the interstitial tissue and to determine the testosterone production in the *pet/pet* testis.

In addition to testicular anomalies, *pet/pet* rats also showed severely small thymus. Growth retardation with involution of the thymus and spleen occurs in mice null for latent TGFβ binding protein 3.[3] In that model, the thymus defect is considered to be caused by elevated serum corticosterone levels. The small thymus in *pet/pet* rats likely is due to another mechanism, however, because we found that *pet/pet* rats have a small thymus at birth, which may be congenitally hypoplastic or due to premature involution. In addition, thymic lymph follicles were present in the capsular and subcapsular regions of the *pet/pet* thymus. Adult

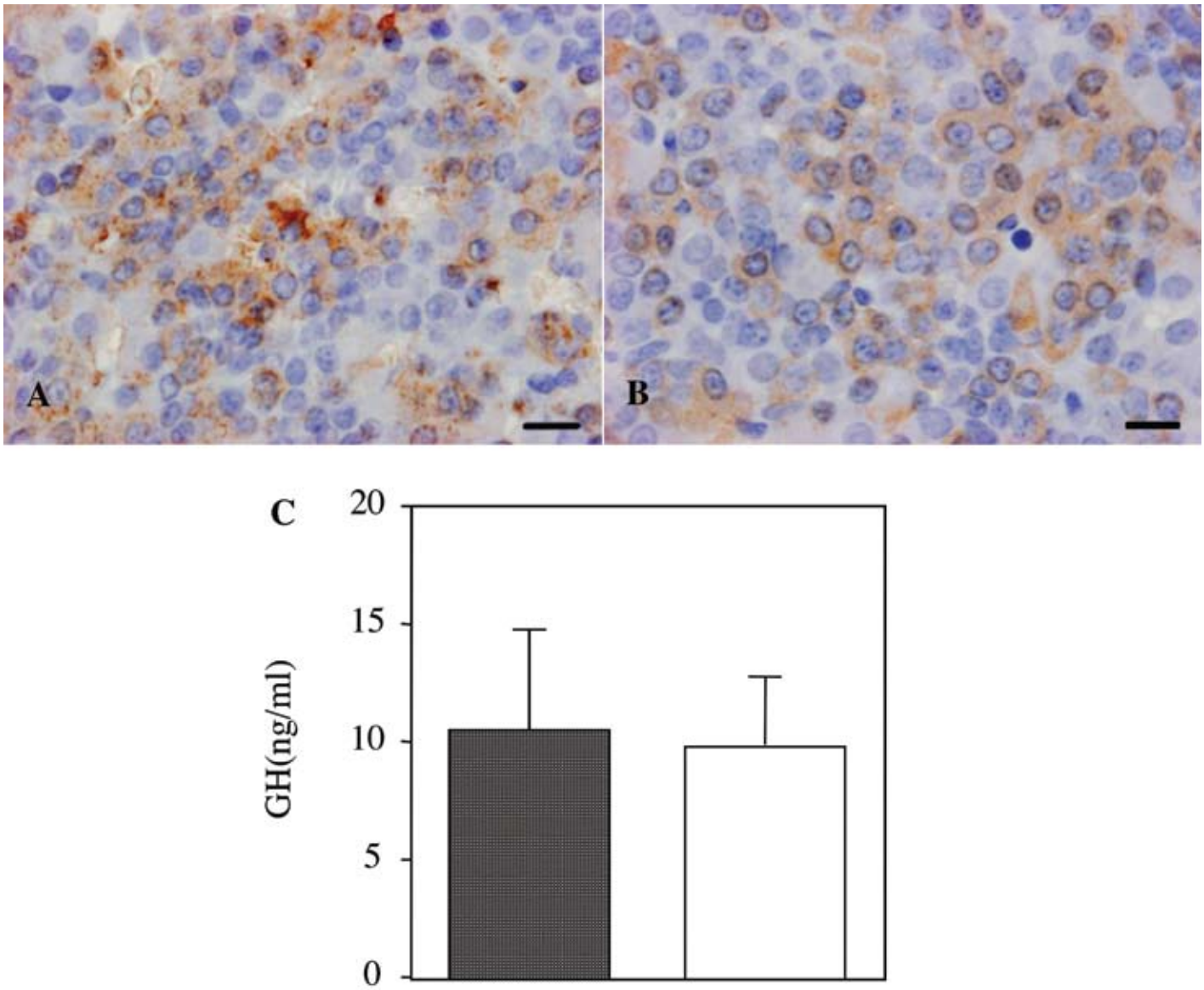


Figure 7. Immunostaining of GH in the anterior pituitary gland of (A) *pet/pet* and (B) normal male rats, and (C) plasma GH concentration at 35 d of age. (A, B) Numbers of GH-positive cells were similar between *pet/pet* and normal male rats. Bar, 50 µm. (C) Mean plasma GH levels were comparable between *pet/pet* (gray column) and normal (white column) males. Vertical bars, SD.

Wistar rats show rare thymic lymph follicles.[15] In humans, thymic follicles occur with some cases of autoimmune disease, such as myasthenia gravis, systemic lupus erythematosus, polymyositis, and nephropathy.[1,18,19,21] On histologic examination, we found a slightly increased cellular infiltration into the interstitial tissue of the kidney and gastrocnemius muscle in *pet/pet* rats (data not shown). The possibility of secondary autoimmune disease in *pet/pet* rats will be addressed in future studies.

In conclusion, we have characterized *pet/pet* rats, which show postnatal semilethality, severe dwarfism from birth, an extremely small thymus, and testicular anomalies, all of which were closely associated with the newly found autosomal recessive mutation. We named the gene responsible for the phenotype described here *pet*, for 'petit'.

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