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Phenotypic Characterization of Spontaneously Mutated Rats Showing Lethal Dwarfism and Epilepsy

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We have characterized the phenotype of spontaneously mutated rats, found during experimental inbreeding in a closed colony of Wistar Imamichi rats. Mutant rats showed severe dwarfism, short lifespan (early postnatal lethality), and high incidence of epileptic seizures. Mutant rats showed growth retardation after 3 d of age, and at 21 d their weight was about 56% that of normal rats. Most mutant rats died without reaching maturity, and 95% of the mutant rats had an ataxic gait. About 34% of the dwarf rats experienced epileptic seizures, most of which started as 'wild running' convulsions, progressing to generalized tonic–clonic convulsions. At age 28 d, the relative weight of the testes was significantly lower, and the relative weight of the brain was significantly higher, in mutant than in normal rats. Histologically, increased apoptotic germ cells, lack of spermatocytes, and immature Leydig cells were found in the mutant testes, and extracellular vacuoles of various sizes were present in the hippocampus and amygdala of the mutant brain. Mutant rats had significantly increased concentrations of plasma urea nitrogen, creatinine, and inorganic phosphate, as well as decreased concentrations of plasma growth hormone. Hereditary analysis showed that the defects were inherited as a single recessive trait. We have named the hypothetically mutated gene as *lde* (lethal dwarfism with epilepsy).

Abbreviations: GH, growth hormone; BUN, blood urea nitrogen; CRE, creatinine; GLU, glucose; TG, triglycerides; IP, inorganic phosphate; CPK, creatine phosphokinase; ALP, alkaline phosphatase; GPT, glutamic–pyruvic transaminase; GOT, glutamic–oxaloacetic transaminase; WR, wild-running

Many mutant rat strains have been used to study the etiologies and treatments of various human diseases, including hypertension, obesity, diabetes, epilepsy, infertility, and dwarfism. Recent progress in the Human Genome Project has made it possible to identify the mutated gene defined by the phenotype of interest. Spontaneously mutated animals are useful not only as models of human diseases but for the identification of functionally important genes. Our laboratory has established several spontaneously mutated rat inbred strains, including those for osteochondrodysplasia,[20] male hypogonadism (HGN),[21] hydronephrosis,[30] and dwarfism with thymic hypoplasia.[6] All of these strains have originated from a closed colony of Wistar Imamichi rats. As an example, we have used *hgn/hgn* rats of the HGN strain to assay renal function[23,24] and gonad development,[25] and we have identified the gene responsible for *hgn*.[26]

During the process of experimental rat inbreeding in a closed colony of Wistar Imamichi rats, we identified several abnormal immature rats showing severe growth retardation and high excitability. These dwarf rats also experienced epileptic seizures and died without reaching maturity. The mutant phenotype seemed to be transmitted as an autosomal recessive trait. We named this mutated allele *lde* (lethal dwarfism with epilepsy) and started sister–brother inbreeding to establish an inbred LDE strain. Several rat models of dwarfism and epilepsy have been used to understand these pathologic conditions and to identify novel therapeutic methods.[19,33] Phenotypic characterization of mutant rats is important for establishing the value of each strain as a disease model. We have characterized the phenotype of *lde/lde* rats by studying their postnatal growth, life span, and organ weight. We also performed histologic examinations of various tissues, as well as observing their epileptic seizure, assaying their blood chemistry, and confirming the genetic inheritance of their mutation.

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Materials and Methods

Animals. The rats used in this study were mutant (*lde/lde*) and phenotypically normal (+/+ or +/*lde*) littermates derived from 254 litters of the third to sixth generation of the LDE inbred strain. Rats were bred and fed under the same conditions as described previously[25] and kept in a clean conventional animal room under controlled light (light:dark, 14:10). Experimental procedures and care of animals were in accordance with the guidelines of the Animal Care and Use Committee of Nippon Veterinary and Life Science University.[26]

Life span and body weight. The pups were weighed at 3, 7, 12, 18, and 21 d of age and once weekly after weaning. Rats showing severe growth retardation were regarded as mutant rats, with genotype (mutant or phenotypically normal littermates) usually determined by comparing body weights at 21 d of age. In total, 45 female and 44 male mutant and 144 female and 122 male normal rats were used for comparisons of body growth and lifespan. Skeletal X-rays were taken using an X-ray CT system for experimental animals (LaTheta LCT-100, Aloka, Tokyo, Japan).

Observations of epileptic seizures and gait anomaly. Spontaneous incidents of epileptic seizure were examined in 56 male and 65 female mutant rats. Each rat was observed for more than 6 h each day until they died, and the day on which a seizure was first observed was recorded. Each such seizure was observed until it ended. The incidence of gait anomaly was examined in 14 female and 12 male normal and 19 female and 20 male mutant rats at 21 d of age.

Organ weight and histologic examination. We euthanized 8 normal male and 10 normal female and 15 mutant male and 20 mutant female rats at 28 d of age by ether overdose and weighed their major organs on an electric balance. Harvested organs were fixed in 4% buffered formalin for 1 h, except that pituitary glands and testes were fixed in Bouin solution overnight. Sagittal and coronal semithin pieces of fixed brain were serially sectioned at 3 to 5 mm before embedding. All fixed tissues and organs were embedded in paraffin (Paraffin pellet, Wako, Oosaka, Japan) and serially sectioned at 3 µm as described.[22-26] Sections were deparaffinized in xylene, hydrated in graded alcohol, and immersed in water, after which they were stained with hematoxylin and eosin and examined under a light microscope. Images were obtained using a digital camera system attached to the microscope.[23-26]

Determination of the mode of inheritance. Mutant pups were detected by low body weight at weaning in 83 of the 254 litters examined. Because some rats with low body weights in 50 of the litters died before weaning, we used the remaining 33 litters, in which all low weight pups detected at birth survived until 21 d of age, for the determination of mode of inheritance. The incidence of mutant rats was analyzed by χ^2 test to confirm that the mutation was transmitted as an autosomal recessive trait.[20,21]

Immunohistochemical detection of growth hormone (GH) in pituitary gland. After pituitary sections were immersed in 0.01 M phosphate-buffered saline (pH 7.5), they were immersed in methanol containing 3% periodic acid for 15 min to inactivate internal peroxidases and soaked in phosphate-buffered saline. The sections were incubated overnight with antibody against rat GH (1:12800 dilution; rabbit polyclonal, Biogenesis, Poole, UK), rinsed in phosphate-buffered saline, and incubated with a labeled secondary antibody (Histofine Simple Stain Rat PO Multi, Nichirei, Tokyo, Japan) to detect the primary antibody. The slides subsequently were incubated with 3,3′-diaminobenzidine tetrahydrochloride and counterstained with hematoxylin.[25,26]

Blood examination and urinalysis. Blood samples were collected from the vena cava with a heparinized plastic syringe under light ether anesthesia. Plasma samples were obtained and stored at –20 °C until assayed. Plasma samples from 4 normal and 5 mutant 28-d-old rats of each sex were used for blood chemistry analysis. Plasma concentrations of urea nitrogen (BUN), creatinine (CRE), glucose (GLU), triglyceride (TG), Ca^{2+}, inorganic phosphate (IP), creatine phosphokinase (CPK), alkaline phosphatase (ALP), glutamic–pyruvic transaminase (GPT), glutamic–oxaloacetic transaminase (GOT), Na^+, K^+, and Cl^- were measured automatically (Dri-Chem 3500V, Fujifilm Medical Company, Tokyo, Japan). Blood cell counts and dipstick urinalyses (Ames Multistix, Miles-Sankyo, Tokyo, Japan) were performed for 3 normal and 3 mutant males at 56 d of age as previously reported.[22]

Plasma GH hormone assay. Plasma concentrations of GH were measured in 7 normal males, 8 normal females, 8 mutant males, and 8 mutant females at 28 d of age by enzyme-linked immunofluroescent assay (Amersham Bioscience UK, Buckinghamshire, England).

Statistical analysis. Body weights, organ weights, blood chemistry assays, and GH concentrations were reported as means and standard deviations, and differences between normal and mutant rats were evaluated by using unpaired Student t tests ($P < 0.05$) (Excel X for Mac, Microsoft Corp, Redmond, WA). Linear regression analysis was performed using Prism 4 (GraphPad Software, San Diego, CA) to examine body growth with advancing age and to compare slopes between normal and mutant rats.[23]

Results

Growth and survival. At 21 d of age, the body weights of mutant rats were less than those of normal rats in both male (21.7 ± 4.0 g versus 38.5 ± 7.0 g, respectively) and female (21.4 ± 5.7 g versus 36.2 ± 8.3 g, respectively) rats. At this age, the body weight of mutant rats were about 56% of that of normal littermates (male: 56.0% ± 7.0% [range, 36.9% to 76.7%]; female, 56.4% ± 11.1% [range, 30.6% to 79.0%]). Therefore, all rats could be categorized as phenotypically normal (+/+ or +/*lde*) or mutant (*lde*/*lde*) rats by comparing body weights; moreover, because all the rats had been numbered on their ears, we found that average body weights were significantly ($P < 0.05$) lower in mutant than in normal rats beginning at 3 d of age (mutant male, 7.84 ± 0.91 g; normal male, 8.20 ± 1.02 g; mutant female, 7.28 ± 1.20 g; normal female, 7.95 ± 1.13). Linear regression analyses of data obtained during the nursing and postweaning periods showed significant ($P < 0.05$) correlations between body growth and age in mutant and in normal rats of both genders during both periods (Figure 1), whereas the regression slopes were significantly ($P < 0.023$) lower in mutant than in normal rats during both periods in both genders. The dwarfism of the mutant rats was of the proportional type (Figure 2 A), and there were no gross anomalies in their skeletons (Figure 2 B). After categorization by body weight at 21 d of age, the number of living mutant rats was recorded. All male mutant rats died before 77 d of age, and mutant females died before 84 d of age (Figure 3). During these periods, most of the phenotypically normal rats grew normally and survived (mortality rate, 1.5%).

Observation of epilepsy. We detected epileptic seizures in 33.8% (22 of 65) of mutant male rats and 33.9% (19 of 56) of mutant female rats, with seizures first detected between 16 to 63 d of age. Three patterns of epileptic seizure were observed in mutant rats. In the most typical pattern (type I), the seizures started with 'wild running' (WR) convulsions, during which the rats suddenly ran and jumped aggressively in their cages; the rats then fell and experienced generalized tonic–clonic convulsions, and after the convulsions, the rats remained lying down without movement for a few minutes. In the second seizure pattern (type II), rats experienced only WR but not generalized seizures. In the third pattern (type III), the rats alternately repeated WR-like and clonic convulsions of forelimbs and head. In addition, 95% of the mutant rats but none of the normal rats had ataxic gait.

Organ weights. We assayed the absolute and relative weights (absolute weight [mg] × 100/body weight [g]) of major organs in both genders at 28 d of age to determine the influence of the mutated gene on the growth and function of body organs. Consistent with the low body weights of mutant rats, the absolute weights of all organs (except brain in male rats) were significantly ($P < 0.05$) lower in mutant than in normal rats. The relative weights of testes and spleen in male rats and thymus in both genders were significantly ($P < 0.01$) lower in mutant than in normal rats, whereas the relative weights of adrenal and pituitary glands in female rats

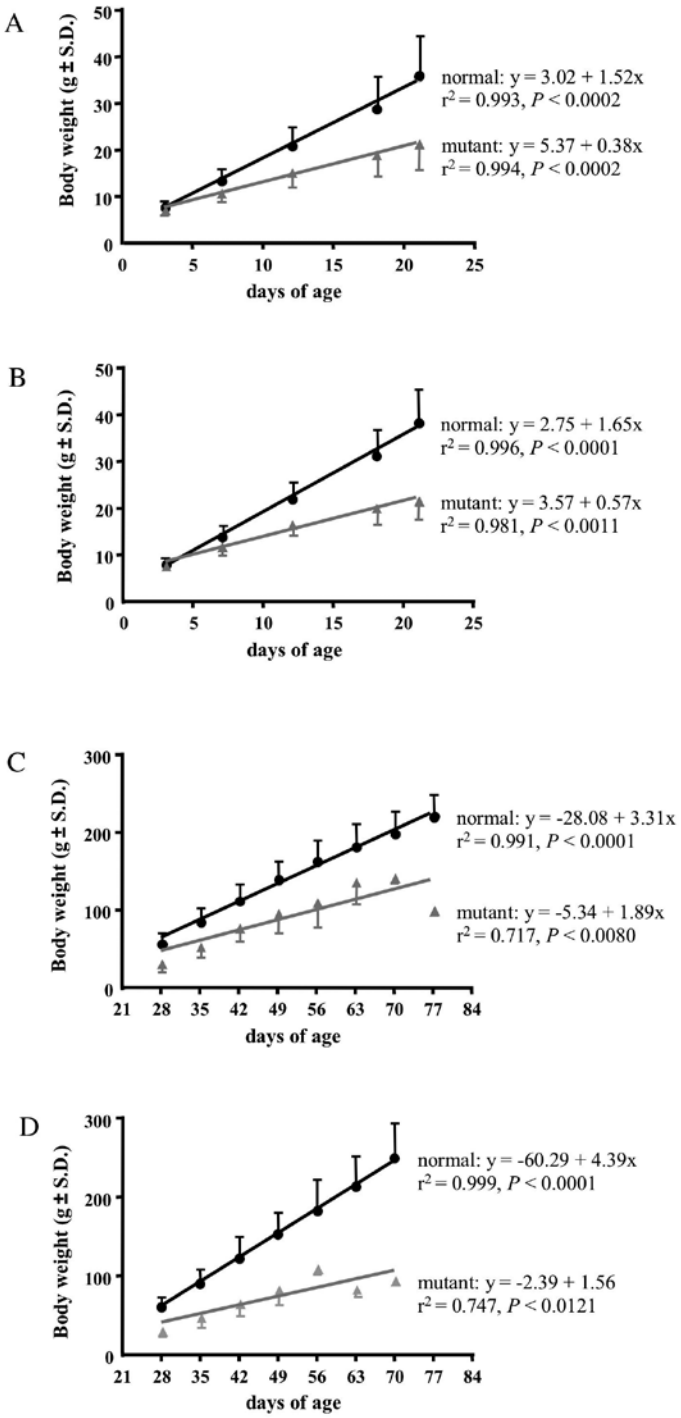

Figure 1. Growth of normal and mutant female (A and C) and male (B and D) rats during nursing (A and B) and after weaning (C and D). Growth and age were significantly correlated ($P < 0.05$) in normal and mutant rats of both genders. At all ages examined, the body weight was significantly ($P < 0.05$) less in mutant than in normal rats (data not shown). Regression slopes were significantly smaller in mutant than in normal rats ($P < 0.0001$ for male rats during both periods and for female rats 21 d of age and younger; $P = 0.0228$ for female rats older than 21 d).

and kidney, lungs, and brain in both genders were significantly ($P < 0.01$) higher in mutant than in normal rats (Table 1).

Blood examination and urinalysis. The concentrations of electrolytes (Ca^{2+}, Na^{+}, K^{+}, and Cl^{-}), aberration enzymes (CPK, ALP, GPT, GOT), GLU, and TG were comparable in the normal and mutant rats, whereas the levels of BUN, CRE, and female IP were significantly ($P < 0.05$) higher in mutant than in normal rats (Table 2). The number of red blood cells was comparable between normal ($6.91 \pm 0.28 \times 10^6/\mu l$) and mutant ($7.08 \pm 0.15 \times 10^6/\mu l$) male rats. Protein excretion to urine also was within the normal range (≤30 mg/dl) in the mutant rats.

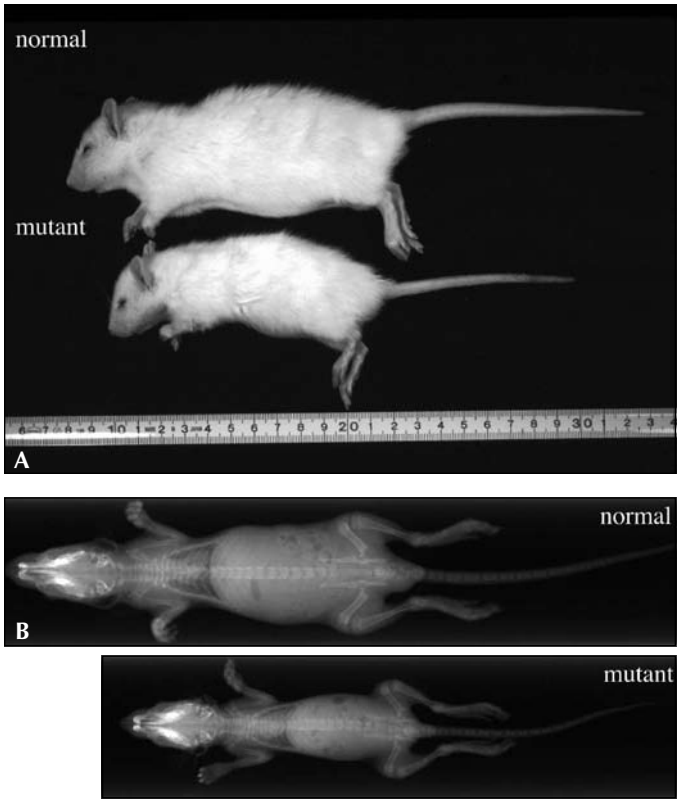

Figure 2. (A) External features of normal and mutant rats at 28 d of age. Mutant rats have small body size, with proportional dwarfism. (B) X-ray photograph of skeletal system.

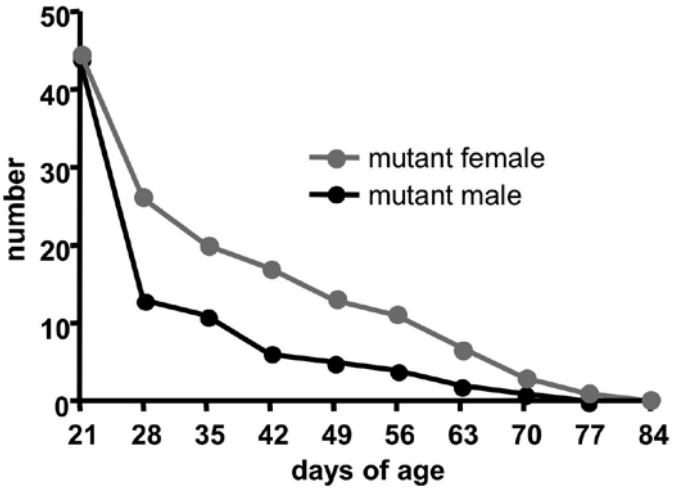

Figure 3. Survival of mutant rats. After 21 d of age, the number of surviving mutant rats gradually decreased. All mutant male rats died by 77 d of age, and all mutant female rats by 84 d of age.

Table 1. Organ weights in 28-d-old rats

	Absolute (mg)		Relative[a]	
	Normal	Mutant	Normal	Mutant
Female rats				
Ovary	13.0 ± 2.9	6.9 ± 2.8[c]	20.5 ± 5.3	21.6 ± 7.3
Uterus	67.2 ± 31.1	37.4± 22.6[c]	107.1± 55.5	122.5 ± 77.8
Adrenal g.	7.3 ± 2.4	4.8 ± 1.5[b]	11.7 ± 4.1	15.4 ± 4.0[d]
Spleen	154.4 ± 31.3	71.3 ± 34.6[c]	244.3 ± 61.4	217.9 ± 83.1
Pancreas	366.5 ± 104.1	205.1 ± 84.3[c]	568.7 ± 127.9	636.1 ± 163.6
Kidney	346.7 ± 53.9	227.1 ± 59.1[c]	540.0 ± 42.0	728.7 ± 116.5[e]
Liver	2890.0 ± 406.2	1595.6 ± 606.3[c]	4541.3 ± 612.3	4968.5 ± 1390.7
Thymus	217.2 ± 30.2	77.1 ± 51.1[c]	343.9 ± 68.0	231.8 ± 148.4[c]
Heart	301.3 ± 23.9	160.3 ± 56.8[c]	477.1 ± 74.0	505.3 ± 127.2
Lung	447.4 ± 37.1	267.5 ± 71.1[c]	705.5 ± 90.0	861.2 ± 160.5[e]
Brain	1426.4 ± 45.6	1338.3 ± 107.2[c]	2254.2 ± 274.7	4542.0 ± 1375.3[e]
Pituitary	2.9 ± 1.0	1.9 ± 0.6[b]	4.5 ± 1.7	6.2 ± 1.6[e]
Male rats				
Testis	162.2 ± 35.2	42.2 ± 13.4[c]	288.1 ± 40.6	159.4 ± 334[c]
Adrenal	7.6 ± 2.0	3.2 ± 1.4[c]	13.5 ± 2.5	11.8 ± 4.2
Spleen	146.9 ± 30.4	49.7 ± 24.7[c]	262.2 ± 39.8	176.9 ± 70.6[c]
Pancreas	285.1 ± 86.2	141.2 ± 75.8[c]	512.9± 153.9	498.2 ± 197.7
Kidney	310.0 ± 37.6	192.4 ± 45.1[c]	560.3± 95.9	734.6 ± 111.2[e]
Liver	2488.6 ± 497.7	1298.4 ± 561.1[c]	4453.8 ± 707.8	4667.8 ± 1335.0
Thymus	164.8 ± 29.6	61.3 ± 31.7[c]	292.6 ± 24.9	215.9 ± 64.6[c]
Heart	275.0 ± 18.7	131.1 ± 39.5[c]	496.6 ± 60.8	487.1 ± 65.4
Lung	391.4 ± 30.7	235.9 ± 51.8[c]	705.5 ± 75.7	903.5 ± 136.1[e]
Brain	1402.4 ± 98.6	1302.2 ± 144.4	2527.7 ± 253.9	5174.0 ± 1380.5[e]
Pituitary	2.5 ± 0.7	1.4 ± 0.4[c]	44 ± 0.9	5.4 ± 1.4

Values are presented as mean ± 1 standard deviation (n = 10 normal female, 20 mutant female, 8 normal male, or 15 mutant male rats).
[a]absolute weight (mg) × 100/body weight (g).
[b]Significantly ($P < 0.05$) less than value for normal rats.
[c]Significantly ($P < 0.01$) less than value for normal rats.
[d]Significantly ($P < 0.05$) greater than value for normal rats.
[e]Significantly ($P < 0.01$) greater than value for normal rats.

Table 2. Plasma concentrations of biochemical markers in 28-d-old rats

	Female rats		Male rats	
	Normal	Mutant	Normal	Mutant
BUN (mg/ml)	12.6 ± 4.3	40.3 ± 3.7[c]	10.1 ± 2.7	35.6 ± 12.8[d]
CRE (mg/ml)	0.48 ± 0.05	0.64 ± 0.11[d]	0.45 ± 0.06	0.58 ± 0.04[d]
GLU (mg/ml)	169.0 ± 26.7	145.4 ± 26.5	155.0 ± 30.1	157.4 ± 38.9
TG (mg/ml)	60.8 ± 12.3	74.2 ± 24.3	76.0 ± 15.8	108.2 ± 124.5
Ca^{2+} (mg/ml)	9.5 ± 1.0	8.9 ± 0.6	8.9 ± 0.7	8.8 ± 0.6
IP (mg/ml)	9.5 ± 0.6	10.6 ± 0.6[c]	9.73 ± 0.8	10.3 ± 1.2
CPK (U/I)	37.5 ± 26.1	316.8 ± 131.7	253.8 ± 60.1	365.2 ± 157.9[b]
ALP (U/I)	792.3 ± 408.4	1427.6 ± 619.5[a]	965.8 ± 444.2	1304.2 ± 393.7
GPT (U/I)	37.5 ± 26.1	32.4 ± 17.4	30.3 ± 8.5	110.0 ± 91.8
GOT (U/I)	nd	nd	89.3 ± 11.5[a]	282.5 ± 177.9[b]
Na^{+} (mEq/l)	nd	nd	141.0 ± 0.8	141.2 ± 2.6
K^{+} (mEq/l)	nd	nd	4.1 ± 0.5	4.4 ± 0.5
Cl^{-} (mEq/l)	nd	nd	98.5 ± 2.1	101.2 ± 4.1

nd, not determined.
Values are shown as mean ± 1 standard deviation. n = 4 normal or 5 mutant rats, except for [a]n = 3 normal rats and [b]n = 4 mutant rats.
[c]Significantly ($P < 0.05$) greater than value for normal rats.
[d]Significantly ($P < 0.01$) greater than value for normal rats.

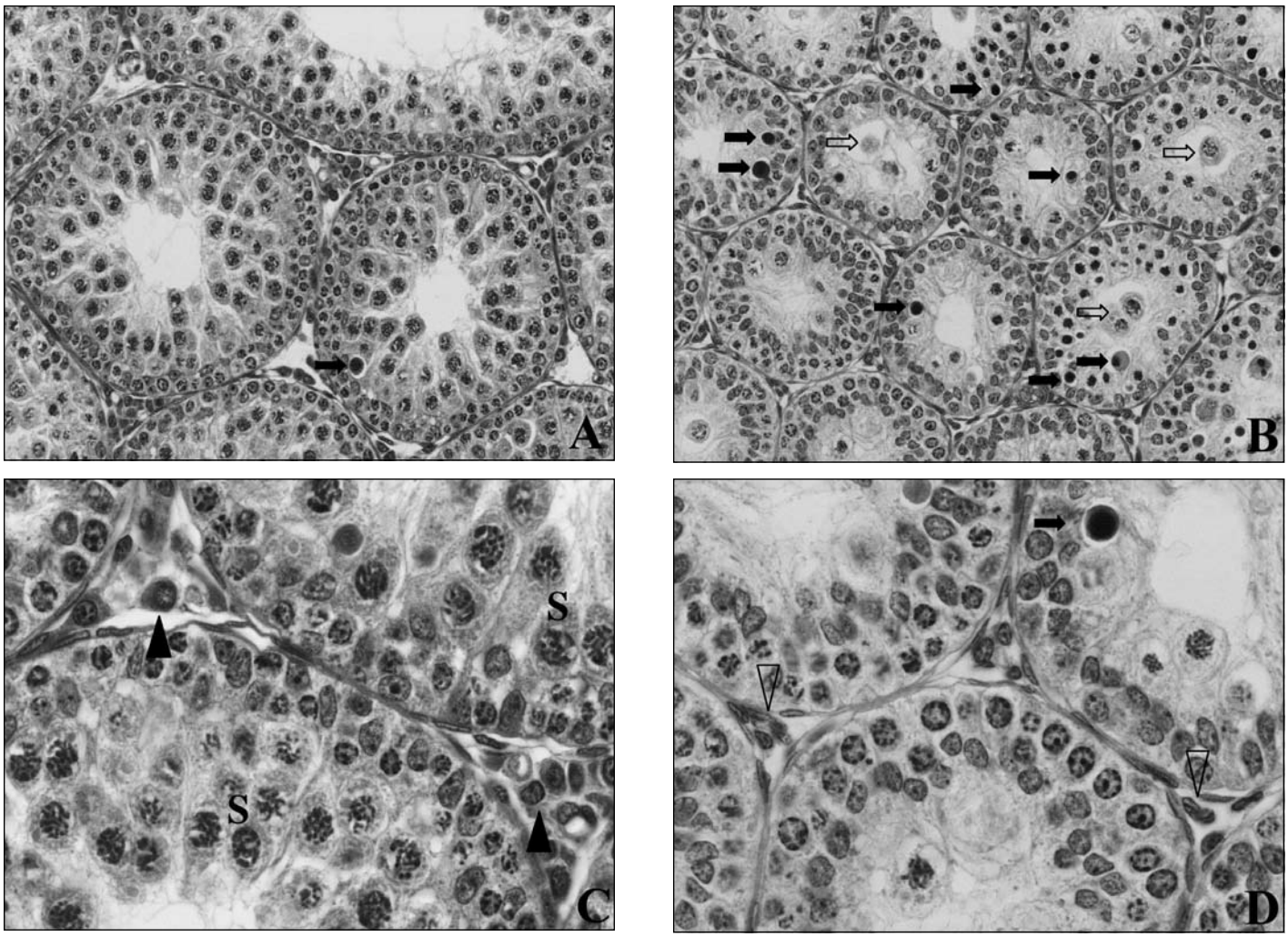

Figure 4. Testicular histology of normal (A, C) and mutant (B, D) testes at 28 d of age at middle (A, B; magnification, ×270) and high (C, D; magnification, ×670) magnification. A, B) The diameters of seminiferous tubular sections were narrower in mutant than in normal testes. Many apoptotic germ cells were detected in mutant testes (dark arrows), along with deciduous germ cells, which fell into the lumen from the basal compartment of the tubules (clear arrows). (C, D) In seminiferous tubules of normal testes, spermatocytes (S) entering meiosis were found in the luminal area, whereas spermatocytes were present only rarely in the seminiferous tubules of mutant rats. In interstitial tissue, round Leydig cells (dark arrowheads) were plentiful in normal testes, but most Leydig cells in mutant testes were spindle-like shaped (clear arrowheads). Hematoxylin and eosin staining.

Histologic examinations. Histologic sections from all of the organs obtained at necropsy at 28 d of age were examined. Except for the smaller area of the histologic sections, due to the smaller size of the organs, we did not detect any obvious pathologic alterations in most tissues of the mutant rats. Mutant testes, however, showed dramatic pathologic alterations. Whereas normal testes contained well-developed seminiferous tubules and round, mature Leydig cells, the testes of mutant males showed immature seminiferous tubules with decreased diameter, increased number of apoptotic germ cells, and the presences of deciduous germ cells in the center of the tubules and spindle-shaped mesenchymal Leydig-like cells in the interstitium. In addition, although the luminal area of normal seminiferous tubules contained many spermatocytes, no normal spermatocytes were present in the tubules of mutant rats (Figure 4). Although external appearance of brain was apparently normal in mutant rats at 28 d of age, the hippocampus showed obvious pathologic changes on histopathology (Figure 5 A, B). In mutant rats, the CA1 region of the hippocampus had many extracellular vacuoles (Figure 5 C, D), which were localized around pyramidal cells and varied in size (Figure 5 E, F), and similar vacuoles were found in the amygdaloid body (Figure 5 C, D, G, H). In contrast no vacuoles were found in the hippocampus and amygdaloid body of normal rats. Other portions of brain in mutant rats lacked obvious alteration at a light-microscopic level. Further, although mutant rats had elevated levels of BUN and CRE, we could not find any pathologic alteration in their kidneys.

Pituitary GH production and plasma GH concentration. Although the anterior pituitary lobes of both normal and mutant rats contained many GH-positive cells, there was less cytoplasm in the cells from mutant rats (Figure 6 A, B). Plasma GH levels were slightly lower in mutant than in normal rats of both genders, but the differences were not statistically significant (Figure 6 C).

Mode of inheritance. To confirm the mode of inheritance, we recorded the incidence of mutant rats in each litter. The actual incidence of dwarfism in male, female, and total rats did not deviate

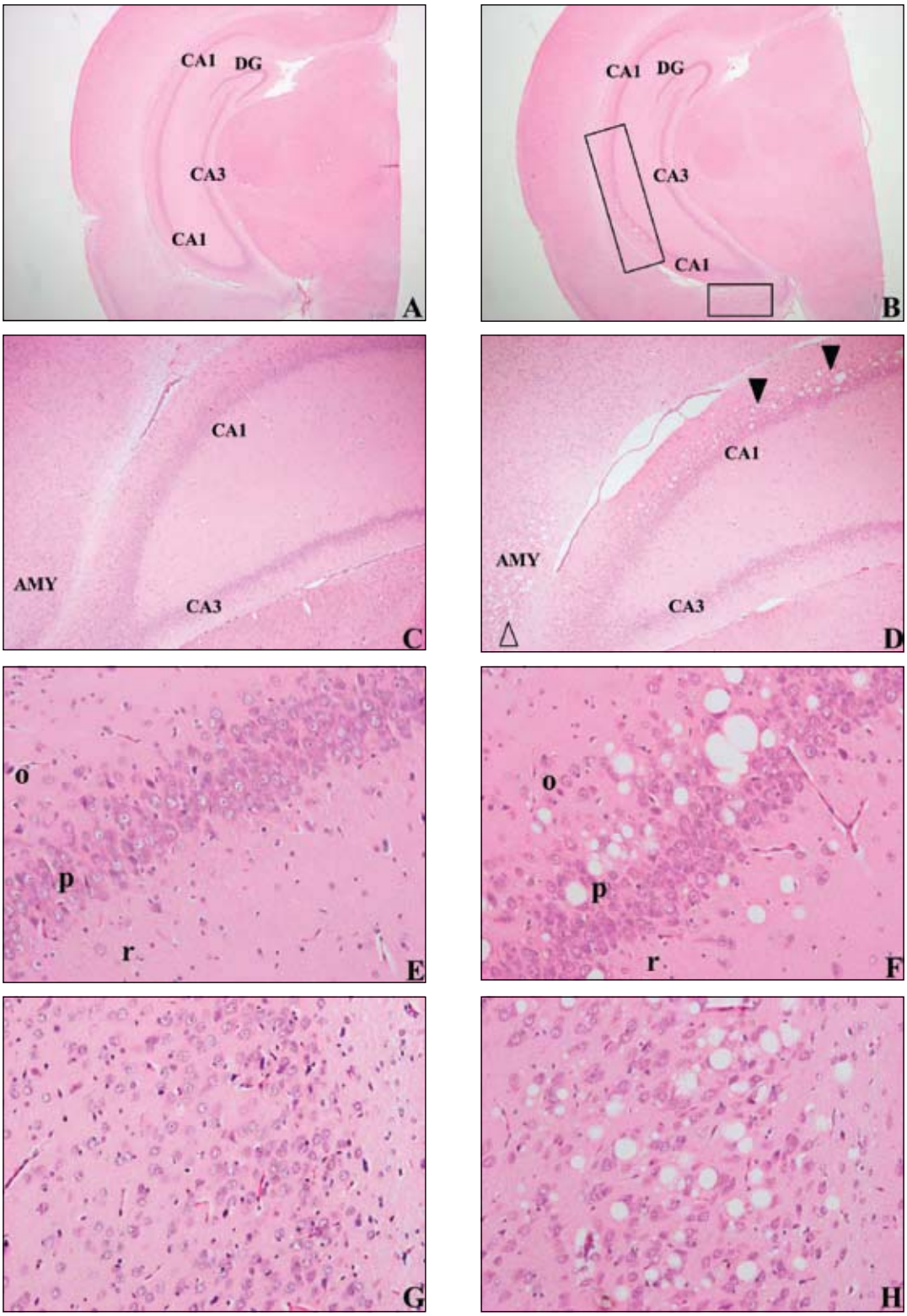

Figure 5. Coronal sections of hemicerebrum containing the hippocampus in normal (A, C, E, G) and mutant (B, D, F, H) male rats at 28 d of age at low (A, B; magnification, ×8), middle (C, D; magnification, ×27), and high (E, F, G, H; magnification, ×135) magnifications. (A, B) Basic architectures are comparable between normal and mutant hippocampuses, but clusters of vacuoles were present in mutant rats (open squares). (C, E, G) No vacuoles were detected in the hippocampus and amygdaloid body of normal rats. (D) In mutant rats, however, many extracellular vacuoles were found in the CA1 region of the lateral hippocampus (dark arrowheads) and in the amygdaloid body (clear arrowhead). (F) Many vacuoles with various sizes were present around and (H) in the amygdaloid body. DG, dentate gyrus; AMY, amygdaloid body; o, oriens layer; p, pyramidal layer; r, radiant layer. Hematoxylin and eosin staining.

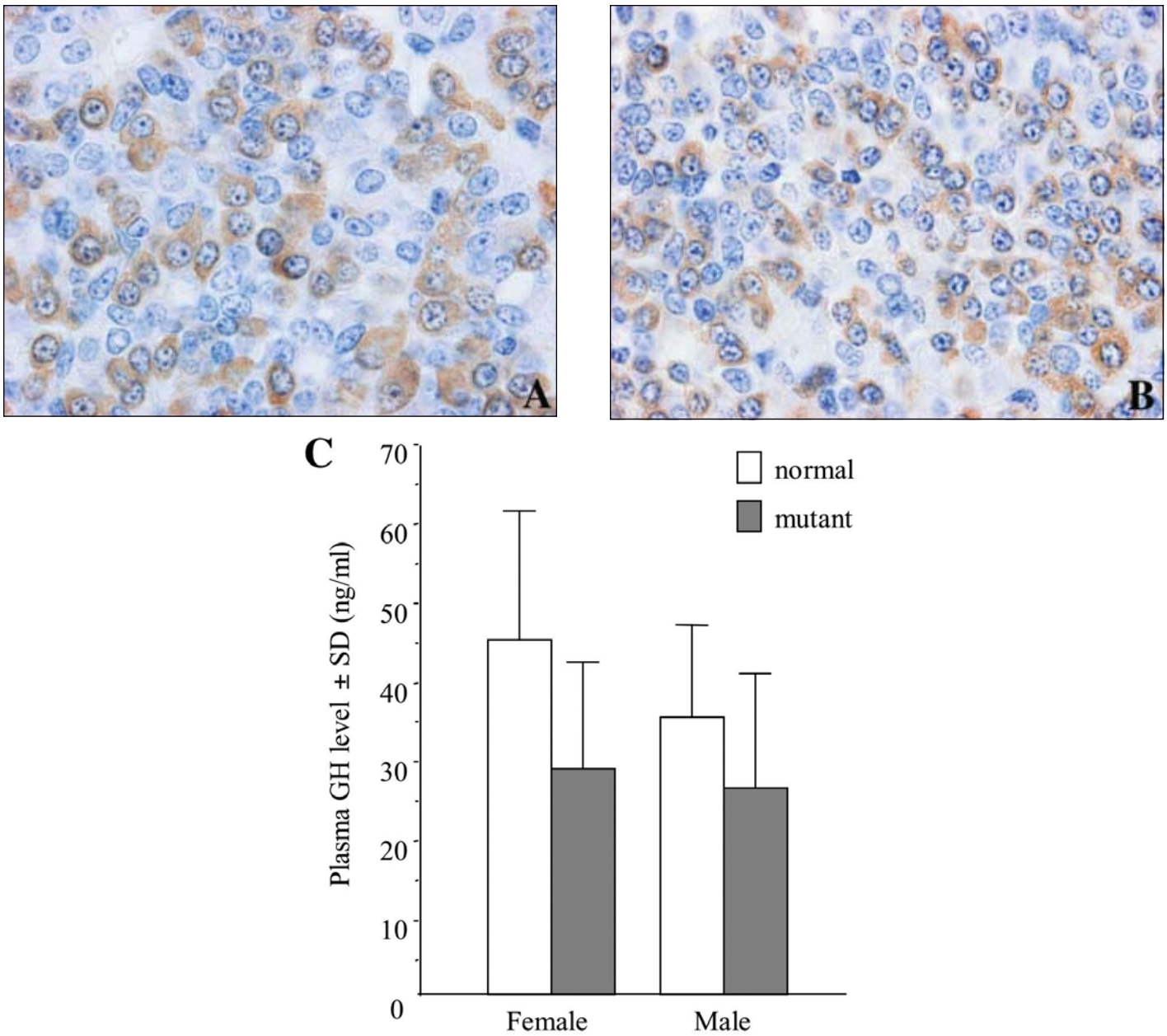

Figure 6. Immunostaining of GH in the anterior lobe of the pituitary gland in (A) normal and (B) mutant male rats (magnification, ×670) and (C) plasma GH concentration in 28 d old rats, determined by enzyme-linked immunosorbent assay. A, B) Similar numbers of GH-positive cells (stained brown) were present in normal and mutant pituitaries, but the cytoplasm of cells containing GH seemed to be slightly smaller in mutant than in normal male rats. (C) Plasma GH levels (ng/ml) were slightly lower in mutant than in normal rats of both sexes, but the differences were not statistically significant. Bars, 1 standard deviation.

significantly from the hypothesis that the defect was transmitted by a single autosomal recessive trait (Table 3).

Discussion

We have phenotypically categorized mutant *lde/lde* rats by comparing their body weights at 21 d of age with those of their normal littermates. We found that the mutant rats had lower body weights during both the nursing period and after weaning. Regression analysis showed that body growth retardation in mutant rats became gradually more apparent with advancing age, with surviving female mutants weighing about 50% and surviving male mutants about 40% that of normal respective adult rats. Several other mutant strains of dwarf rats have been described, in which growth retardation appears after (*dw/dw*[5] and *mri/mri*[7]) and before (*dr/dr*[29] and *rdw/rdw*[15]) weaning. All of these previously developed mutant strains are fertile and do not show the epilepsy or lethality displayed by *lde/lde* rats, suggesting that *lde* is a novel spontaneous mutation associated with dwarfism in rats.

Epileptic seizures occurred in 34% of the mutant rats identified by dwarfism but not in the remaining 66% of dwarf rats. None of the normal rats experienced epileptic seizures, suggesting that these dwarf rats have a congenital defect that causes spontaneous

Table 3. Fitness to the hypothesis for the genetic mode of transmission[a]

	Observed		Expected			
	Normal	Mutant	Normal	Mutant	χ^2	P
Total rats	228	83	233.3	77.8	0.47	>0.50
Female rats	126	41	125.3	41.8	0.018	>0.90
Male rats	102	42	108	36	1.33	>0.25

[a]The hypothesis was a single autosomal recessive trait.

epileptic seizures. A limitation in our ability to detect spontaneous seizures and the early death of the mutant rats before seizure detection may reduce the apparent incidence of seizures in these rats. In preliminary experiments, we found that an audio sound at 15 KHz (88 dB) induced convulsions in 90% of mutant dwarf rats and 0% of normal rats.[28] The WR convulsions and generalized tonic–clonic convulsions that occurred in *lde/lde* rats have also been described in NER,[17,18] IER,[1,32] tremor,[16] and WER[31] rats, but no incidences of dwarfism and lethality have been reported in these other strains. The other important characteristic of epilepsy in our mutant dwarf rats was that seizures began during the early postnatal period, making *lde/lde* rats a potential animal model for pediatric epileptic disease. In addition, most *lde/lde* rats showed ataxic gait, in contrast with the ataxia and male sterility (AMS) mouse,[10] we did not detect any marked pathologic changes in the cerebella of *lde/lde* rats. Although the cause of postnatal death in *lde/lde* rats is unknown, repeated seizures, small body size, and ataxia may be disadvantageous for their survival.

External features of our mutant rats indicate that their dwarfism is a proportional type, with the weights of most organs being less than 65% of that in normal rats at 28 d of age. However, the absolute weight of mutant brain was greater than 90% that of normal brain, and the relative weight of mutant brain was about twice that of normal brain. Similar disproportional brain growth has been reported to occur in *dr/dr*[29] and *rdw/rdw*[15] rats. Because the GH gene is mutated in *dr/dr* rats[29] and because the level of plasma GH is extremely low in *rdw/rdw* rats,[15] postnatal rapid growth in the size of brain likely is independent of growth hormone. Although we expected that GH deficiency would be involved in the dwarfism of *lde* rats, we detected GH-producing cells in the anterior lobe of their pituitary glands, and our preliminary immunoblotting experiments showed that the GH was of normal molecular weight.[27] Moreover, although the plasma GH level was slightly lower in mutant than in normal rats, the difference was not significant, suggesting that the severe type of dwarfism observed in *lde/lde* rats cannot be explained solely by low levels of plasma GH. GH receptor-knockout mice, an animal model of Laron syndrome,[4] show dwarfism, but their plasma GH level is elevated due to the lack of negative feedback for GH secretion caused by a reduced level of plasma insulin-like growth factor I.[35] Because *lde/lde* rats did not show elevated levels of plasma GH, they might have normal expression and function of GH receptor and insulin like growth factor I.

Consistent with the marked reduction of both absolute and relative testicular weight (26% and 55% of normal, respectively), seminiferous tubules containing spermatocytes were observed only rarely in mutant testes, and Leydig cells were more immature. These observations indicate that the responsible gene for *lde* was involved in postnatal testicular development and that the *lde* gene might have a direct or indirect influence on the differentiation and function of these testicular cells. It is unclear, however, whether the testicular defect is present in the testis itself or in the hypothalamus–pituitary axis. Examination of postnatal testicular pathogenesis and plasma gonadotropin levels might help determine whether the dwarfism and hypogonadism in mutant rats are caused by pleiotropic effects of *lde* or secondary effects of defective pituitary hormones.

In *lde/lde* brain, characteristic vacuoles were detected in the CA1 region of the hippocampus and in the amygdaloid body. Anomalies in the temporal lobe involving the hippocampus are believed to be related to the incidence of epilepsy, and organic lesions such as hippocampal sclerosis and microdysgenesis have been detected in some cases of temporal lobe epilepsy.[2,14] In epileptic models of rats, neuronal microdysgenesis in the CA1 of hippocampal formation reportedly is involved in epileptogenesis in IER rats.[32] Although extracellular vacuole formations (spongy degeneration) are distributed widely in the central nervous systems of tremor[16] and SER[13] rats, the abnormal activation in hippocampal neurons is considered to be associated with the occurrence of epileptic seizures in these rats.[34] In addition, neurons in the amygdala play an important role in the occurrence of limbic epilepsy induced by audiogenic kindling in Wistar audiogenic rats.[9] Therefore, although similar vacuoles in hippocampus and amygdala have not been reported in human epileptic disease and other animal models of epilepsy, the vacuole formation in these areas of *lde/lde* brains may be associated with the incidence of epilepsy in these rats. These findings indicate that *lde/lde* rats may be a potential animal model for studying temporal lobe epilepsy. Endocrine disorders and reproductive problems have been reported in patients with epileptic disease,[8,12] and the limbic system containing hippocampus is closely associated with the hypothalamus, which is important for the development and regulation of gonads.[11] Therefore, the pathologic alterations in the testes and hippocampus of mutant rats may be related, due to functional alteration in the hypothalamus.

We found that the plasma concentrations of BUN and CRE were higher in mutant than in normal rats. Although these are clinical markers of renal insufficiency, our histologic examination did not reveal any marked pathologic alterations in mutant kidneys. Furthermore, urostick tests and blood examinations did not identify any manifestations of proteinuria and anemia. Therefore, if renal excretive function is reduced in the mutant rats, the degree of dysfunction may not be severe. Alternatively, the increases in plasma BUN and CRE may be related to the increased production of these compounds. The spontaneously epileptic rat (SER) strain has elevated levels of BUN, combined with growth retardation and motile epileptic seizures.[3] Because *lde/lde* rats similarly show severe growth defects and motile epileptic seizures, the production of urea-nitrogen and creatinine may be increased due to hypercatabolism and muscle disruption. Although renal clearance tests may eliminate the possibility of renal insufficiency, the severe dwarfism and lethality in these animals make it difficult to

collect sufficient amounts of urine from the mutant rats.

In conclusion, we have phenotypically characterized a strain of spontaneously mutated rats that arose during experimental inbreeding of a Wistar Imamichi closed colony. We found that the mutant rats showed severe dwarfism, short lifespan (early postnatal death), high incidence of epileptic seizures, and histologic anomalies in testes and hippocampus. Hereditary analysis indicates that these traits were inherited as a single recessive trait. We have named the hypothetical mutated gene *lde* (lethal dwarfism with epilepsy).

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