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Original

Impaired Development of Somatotropes, Lactotropes and Thyrotropes in Growth-Retarded (*grt*) Mice

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Abstract: Congenitally primary hypothyroid growth-retarded (*grt*) mice exhibit a characteristic growth pause followed by delayed onset of pubertal growth. We characterized the developmental pattern of somatotropes, lactotropes and thyrotropes in the anterior pituitary, as well as plasma levels of their secretory hormones, in *grt* mice. Compared with normal mice, the weight of *grt* pituitary gland was similar at 8 weeks of age but significantly heavier after 12 weeks of age. Compared with normal mice, there were significantly fewer somatotropes in the *grt* pituitary until 8 weeks of age, but the number gradually increased up to 48 weeks. The number of lactotropes in *grt* mice was consistently lower than that in normal mice from 2 through 48 weeks, whereas the number of thyrotropes in the *grt* pituitary was consistently higher than in the normal pituitary. Thyrotropes in the *grt* pituitary exhibited hypertrophy and hyperplasia with less intensive thyroid-stimulating hormone (TSH) immunoreactivity than normal thyrotropes. In normal mice, the sum of the relative proportions of these cells plateaued at 8 weeks, where it remained up to 48 weeks of age. In *grt* mice, these proportions almost reached normal levels at 12 weeks of age but gradually declined after 24 weeks. Plasma growth hormone concentrations did not differ between *grt* and normal mice until 24 weeks of age. Compared with normal mice, *grt* mice exhibited significantly lower plasma prolactin and thyroxine levels but higher TSH levels. These findings indicate that development of somatotropes, lactotropes and thyrotropes in *grt* mice is impaired, being followed by altered hormone secretion. (J Toxicol Pathol 2009; **22**: 187–194)

Key words: *grt* mouse, anterior pituitary, somatotrope, lactotrope, thyrotrope

Introduction

The anterior pituitary gland synthesizes and releases several types of tropic hormones that are classified as five major hormones, namely, growth hormone (GH), prolactin (PRL), thyroid-stimulating hormone (TSH), adrenocorticotropic hormone (ACTH) and gonadotropic hormone (GTH). These hormones regulate thyroidal, adrenal and gonadal function in addition to lactation, bodily growth and somatic development[1].

Several spontaneous mutant dwarf animals have been used to gain insight into genes involved in dwarfism. Strains of mice that exhibit autosomally inherited dwarfism include Snell dwarf (*dw*)[2] and Ames dwarf (*df*)[3], both of which are forms of primary hypopituitarism. The *dw* and *df* mice fail to produce PRL, TSH and GH[4,5] and are useful animal models of panhypopituitarism. Hypothyroid mice (*hyt*)[6] and rats (*rdw*)[7] are known to be the forms of primary hypothyroidism that are associated with a congenital thyroid hormone deficiency. The thyroid of *hyt* mice lacks functional TSH receptors[8,9], with a relatively small somatotrope (GH cell) population in the pituitary[10] and a high plasma TSH concentration[11]. The pituitary of *rdw* rats is composed of reduced numbers of GH and PRL cells[7], exhibiting declined levels of GH and PRL mRNA and elevated levels of TSH mRNA. In association with these phenomena, plasma GH and PRL levels are low[12,13] and plasma TSH levels are high[13–15].

The growth-retarded (*grt*) mouse is a mutant with congenital primary hypothyroidism that shows a characteristic growth pause followed by delayed onset of pubertal growth[16]. Plasma concentrations of thyroxine (T_4) are significantly lower in *grt* mice compared with controls[16], whereas TSH concentrations are greatly elevated[17,18]. The unresponsive nature of TSH receptors to TSH is considered to be attributable to dysfunction of the *grt* thyroid gland[19].

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Sasaki *et al*. reported that a missense mutation of tyrosylprotein sulfotransferase 2 (*Tpst2*) is responsible for this phenotype and proposed that tyrosine sulfation of TSH receptors by TPST2 is crucial for TSH signaling and thyroid gland function[18]. Severe postnatal growth retardation and hormonal abnormalities are observed in *grt* mice[16]. In male *grt* mice, delayed testicular development is associated with reduced fertility[20]. Ovarian and uterine development is defective in female *grt* mice, which results in infertility[21,22]. The density of immunoreactive GH and PRL cells is lower in *grt* mice than in normal mice at 12–13 months of age[16]. However, the developmental pattern of these cells in the anterior pituitary of *grt* mice has not been described, nor has the endocrine status of these hormone levels in the plasma. Thus, we examined the developmental pattern of somatotropes, lactotropes (PRL cells) and thyrotropes (TSH cells), as well as developmental changes in plasma hormone concentrations, in normal and *grt* mice.

Materials and Methods

Animals

Phenotypically male normal (+/+ or *grt*/+) and growth-retarded (*grt/grt*) mice were obtained as described previously[19]. The mice were maintained under controlled temperature (23 ± 1°C), relative humidity (60 ± 5%) and lighting (08:00–20:00) conditions. Commercial laboratory chow (CRF-1, Charles River Japan, Inc., Kanagawa, Japan) and tap water were available *ad libitum*. All experimental procedures were performed in accordance with the "Institutional Guidelines for Animal Care and Use" of Saitama University (Saitama, Japan).

Hormones and antisera

Mouse PRL (AFP-6476C), mouse GH (AFP-10783B), rabbit anti-mouse PRL serum (AFP-131078) and monkey anti-rat GH serum (NIDDK-anti-rGH-S-5) were supplied by the Pituitary Hormone & Antisera Center, Harbor-UCLA Medical Center, Los Angeles, CA, USA. Goat anti-rabbit IgG serum (HAC-RBA2-05GTP91), goat anti-monkey IgG serum (HAC-MKA2-02GTP88), rabbit anti-rat GH (HAC-RT25-02RBP85), rabbit anti-rat PRL (HAC-RT26-02RBP85) and rabbit anti-rat TSH (HAC-RT29-01RBP86) serum were the gifts from the Institute for Molecular and Cellular Regulation, Gunma University, Maebashi, Japan.

Immunohistochemistry

To examine developmental changes in the three types of hormone-producing cells in the anterior pituitary, pituitary gland specimens were prepared from male *grt*/*grt* and phenotypically normal (*grt*/+ and +/+) mice at 2, 5, 8, 12, 24 and 48 weeks of age. All mice were sacrificed under anesthesia. The mice were divided into two groups for the following studies. Pituitaries from 8-, 12-, 24- and 48-weeks were carefully isolated, weighed and stored at –20°C to assay protein (not reported here). For histological studies, pituitaries with the median eminence attached were carefully removed from 2-, 5-, 8-, 12-, 24- and 48-week-old mice and fixed with acetic acid-free Bouin's fluid for at least 24 h to identify the exact orientation in histological sections. The pituitaries were dehydrated in graded ethanol and embedded in paraffin. Sagittal sections (4 μm thick) were cut and processed immunohistochemically by the peroxidase anti-peroxidase complex method using rabbit antiserum against rat GH, rat PRL and rat TSH. Deparaffinized sections were incubated in a solution of 3% H_2O_2 for 20 min to block endogenous peroxidase activity. After rinsing twice with 10 mM phosphate-buffered saline (PBS, pH 7.4), the sections were treated with diluted normal swine serum (1:20; Dako, Glostrup, Denmark) for 30 min at room temperature. The sections were rinsed with PBS and then incubated sequentially with anti-GH serum (1:4000), anti-PRL serum (1:4000) or anti-TSH serum (1:6000) overnight at 4°C, followed by swine anti-rabbit IgG (1:50; Dako) for 1 h at room temperature and then rabbit peroxidase anti-peroxidase complex (1:200, Dako) for 1 h at room temperature. The sections were rinsed in PBS, and the peroxidase activity was visualized with 0.1% diaminobenzidine (Wako Pure Chemical Industries, Osaka, Japan) in 0.05 M Tris-HCl (pH 7.4) containing 0.005% H_2O_2. After washing in distilled water, the sections were counterstained with hematoxylin.

Counting of immunoreactive cells

The pituitaries from 3 mice in each group were used. Each sample was sectioned sagittally through the pituitary, and then the approximately one-quarter (1/4), half (midsagittal position) (1/2) and three-quarters (3/4) positions were selected for analysis. The number of immunoreactive cells as well as the number of total pituitary cells on the three sections was counted under a light microscope. The ratio of the number of immunoreactive cells to the number of total pituitary cells was calculated and expressed as a percentage.

Hormone determinations

Blood samples were collected from 4–17 mice in each experimental group (except for normal mice at 48 weeks of age; n=2) between 14:00 and 18:00 h by orbital puncture. Plasma samples were obtained by centrifugation at 4°C and stored at –30°C for analysis. Radioimmunoassays for plasma GH and PRL were performed according to the method described previously[23]. Purified mouse GH and mouse PRL were used as antigens and reference standard, respectively. Plasma T_4 and TSH levels were assayed using radioimmunoassays for T_4 (Ortho-Clinical Diagnostics, Raritan, NJ, USA) and rat-TSH (GE Healthcare Bio-Sciences Corp., Piscataway, NJ, USA), respectively, according to the manufacturer's instructions.

Statistical analysis

Values were expressed as the mean ± SEM. The statistical significance ($P<0.05$) of differences was determined using Student's or Welch's *t*-test.

Results

Pituitary growth

The weights of male normal (1.42 ± 0.12 mg) and *grt* (1.53 ± 0.09 mg) pituitaries were comparable at 8 weeks of age. The pituitaries of male *grt* mice (1.58 ± 0.05 mg) were significantly heavier than those of normal mice (1.18 ± 0.03 mg) at 12 weeks of age and were approximately 5.4-fold heavier than those of normal mice at 48 weeks of age (5.92 ± 1.09 vs. 1.50 ± 0.05 mg, respectively; Fig. 1).

Immunohistochemistry of GH, PRL and TSH cells

Representative photomicrographs of GH, PRL and TSH cells in sagittal sections of anterior pituitary glands from 5-week-old male *grt* and normal mice are shown in Fig. 2. In the normal mouse pituitary, GH and PRL cells were uniformly widespread (Fig. 2, A and C). In *grt* mice, there were fewer GH and PRL cells than in normal mice (Fig. 2, B and D). The number of TSH cells was greater in *grt* mice than in normal mice (Fig. 2, E and F). TSH cells in the *grt* pituitary exhibited hypertrophy and hyperplasia, and their immunoreactivity for TSH was weak compared with that of the thyrotropes in the normal pituitary.

Developmental changes of GH, PRL and TSH cells in anterior-lobe cells GH cells

The number of GH cells was significantly lower in *grt* mice than in normal mice from 2 weeks of age (713 ± 23 and 1772 ± 119, respectively) to 8 weeks of age. However, the numbers of GH cells gradually increased with age in *grt* and normal mice from 12 weeks to 48 weeks of age (4638 ± 555 and 5371 ± 636, respectively; Fig. 3A).

PRL cells

At 2 weeks of age, the number of PRL cells was significantly lower in *grt* mice than in normal mice (3.7 ± 2.7 and 68 ± 51, respectively). Normal mice had a pubertal rise in the number of PRL cells at 8 weeks of age. However, the rise was considerably delayed in *grt* mice, and the number of PRL cells was significantly lower than in normal mice up to 48 weeks of age (1058 ± 153 and 2626 ± 522, respectively; Fig. 3B).

TSH cells

There were significantly more TSH cells in *grt* mice than in normal mice at 2 weeks of age (581 ± 38 and 299 ± 59, respectively). This increase of TSH cells in the *grt* pituitary remained significant up to 48 weeks of age (1490 ± 163 and 436 ± 11, respectively; Fig. 3C).

Total pituitary cells

There was no significant difference in the total number of anterior pituitary cells between *grt* and normal mice from 2 weeks of age (4508 ± 145 and 4845 ± 252, respectively) to 24 weeks of age. However, the value for *grt* mice became significantly higher than that for normal mice at 48 weeks of age (19095 ± 753 and 12405 ± 971, respectively; Fig. 3D).

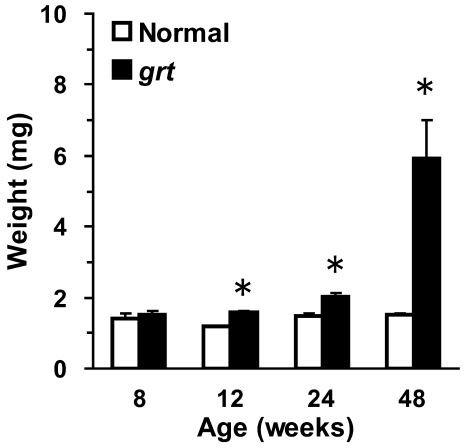

Fig. 1. Pituitary weight in normal and growth-retarded (*grt*) male mice. Values represent the mean ± SEM of 3 to 5 mice for each group. *Significantly different from age-matched normal mice (P<0.05).

Percentages of GH, PRL and TSH cells

The percentage of GH cells per section was significantly lower in *grt* mice than in normal mice at 2 weeks of age (15.9 ± 1.0% and 36.5 ± 0.7%, respectively) and then gradually increased up to 12 weeks of age. Thereafter, the percentage of GH cells in *grt* mice gradually decreased; the values were significantly lower in *grt* mice compared with normal mice at 24 and 48 weeks of age (Fig. 4). The percentage of PRL cells in *grt* and normal mice was comparable at 2 weeks of age (0.08 ± 0.06% and 1.5 ± 1.2%, respectively), but the percentage in *grt* mice was significantly lower than in normal mice from 5 through 48 weeks of age (Fig. 4). In contrast, the percentage of TSH cells was significantly greater in *grt* mice than in normal mice from 2 weeks (13.0 ± 1.2% and 6.2 ± 1.1%, respectively) through 48 weeks of age (7.8 ± 0.6% and 3.6 ± 0.4%, respectively; Fig. 4). In normal mice, the sum of the relative proportions of these cells plateaued at 8 weeks, where it remained up to 48 weeks. In *grt* mice, these proportions almost reached normal levels at 12 weeks of age but gradually declined after 24 weeks (Fig. 4).

Plasma hormone concentrations

In normal mice, plasma GH levels rose at 5 weeks of age and then peaked with a value of 26 ng/ml at 8 weeks of age. There was no pubertal rise in plasma GH in *grt* mice at this time, although small and transient rises of GH levels were observed after 5 weeks of age. Plasma GH levels did not differ significantly between *grt* and normal mice up to 24 weeks of age. At 48 weeks, however, GH levels in *grt* mice were significantly higher than in normal mice (Fig. 5A). Plasma PRL levels in normal mice had a transient pubertal peak at 5 weeks of age (63 ng/ml) and then gradually decreased to adult levels where they remained up to 48 weeks of age. In *grt* mice, PRL levels lacked a pubertal rise and were significantly lower than those of normal mice from 5 weeks through 48 weeks of age (31.1 ± 2.2 and 47.6 ± 2.9

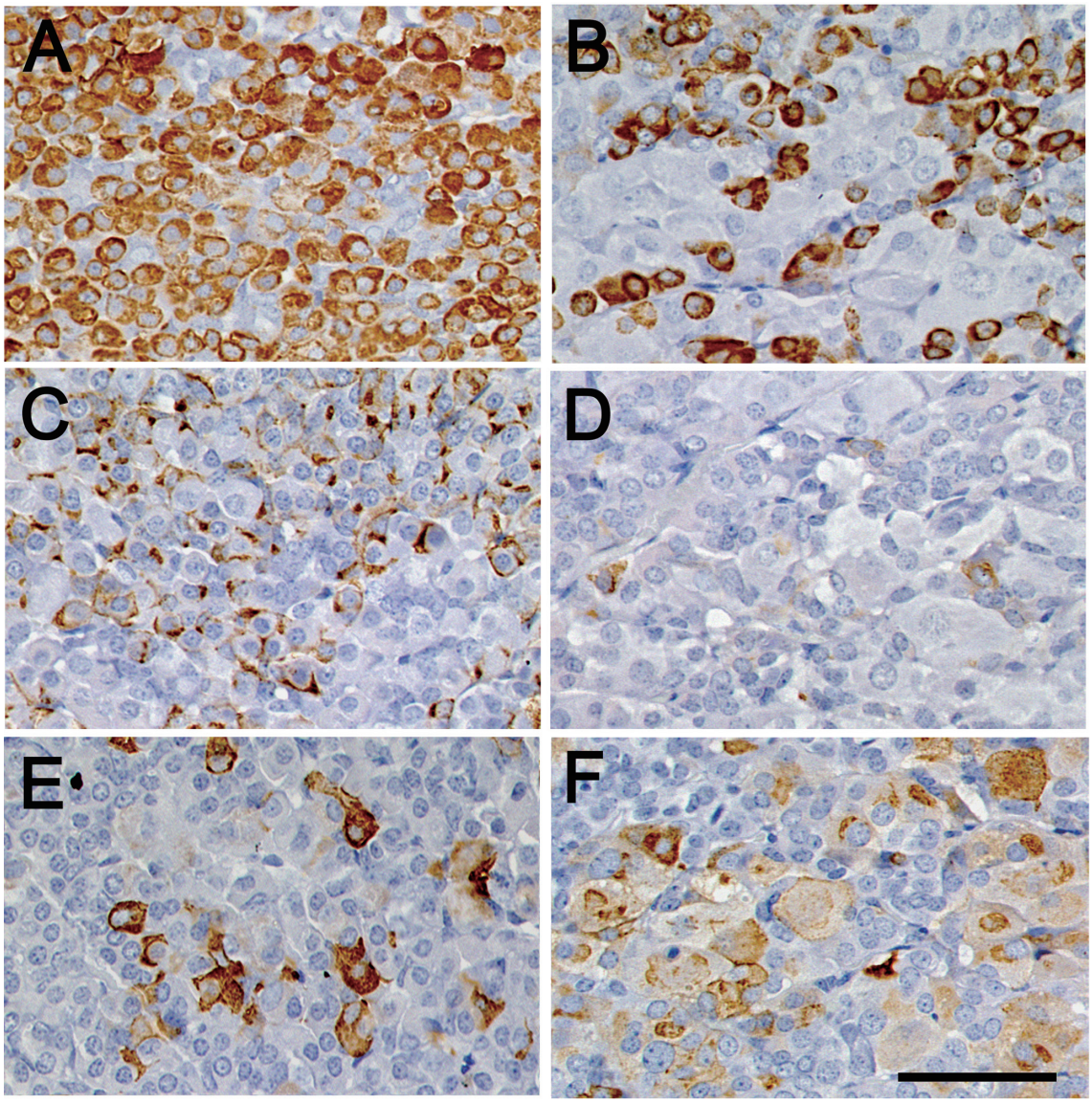

Fig. 2. Representative photomicrographs of anterior pituitary sections from 5-week-old normal (A, C, E) and *grt* (B, D, F) male mice. The sections were immunostained with antisera against rat growth hormone (GH) (A, B), prolactin (PRL) (C, D) and thyroid-stimulating hormone (TSH) (E, F) and counterstained with hematoxylin. Scale bar, 50 μm.

ng/ml, respectively; Fig. 5B). Plasma TSH levels in normal mice remained between 25 and 40 ng/ml at all ages examined. In *grt* mice, plasma TSH levels were dramatically higher than those in normal mice from 2 through 48 weeks of age (465.5 ± 107.7 and 36.3 ± 6.4 ng/ml, respectively; Fig. 5C), whereas plasma T_4 levels in *grt* mice were invariably lower than those in normal mice throughout the period from 2 to 48 weeks of age (13.8 ± 1.6 and 24.3 ± 0.7 ng/ml, respectively; Fig. 5D).

Discussion

The present study was conducted to evaluate the developmental pattern of GH, PRL and TSH cells, as well as circulating plasma hormone levels, in a congenital hypothyroid mouse, *grt*. The pituitary growth and developmental composition ratios of these cells were altered drastically in *grt* mice (Figs. 1–4). In contrast to GH and PRL cells, TSH cells in the *grt* mouse pituitary are increased in number, although their immunoreactivities were relatively weak, presumably because they were in a state of hypersecretion.

In rats treated with the reversible goitrogen propylthiouracil, the percentage of TSH cells in the pituitary increases and that of GH cells decreases[24,25]. In neonatally thyroidectomized rats, PRL mRNA levels, PRL contents in the pituitary and serum PRL concentrations are decreased[26]. Propylthiouracil treatment leads to a marked increase in circulating TSH[27]. These phenomena are in accordance with our results in *grt* mice, all coming from the deceased levels of plasma thyroid hormone.

The *grt* mice used in the present experiment, which have

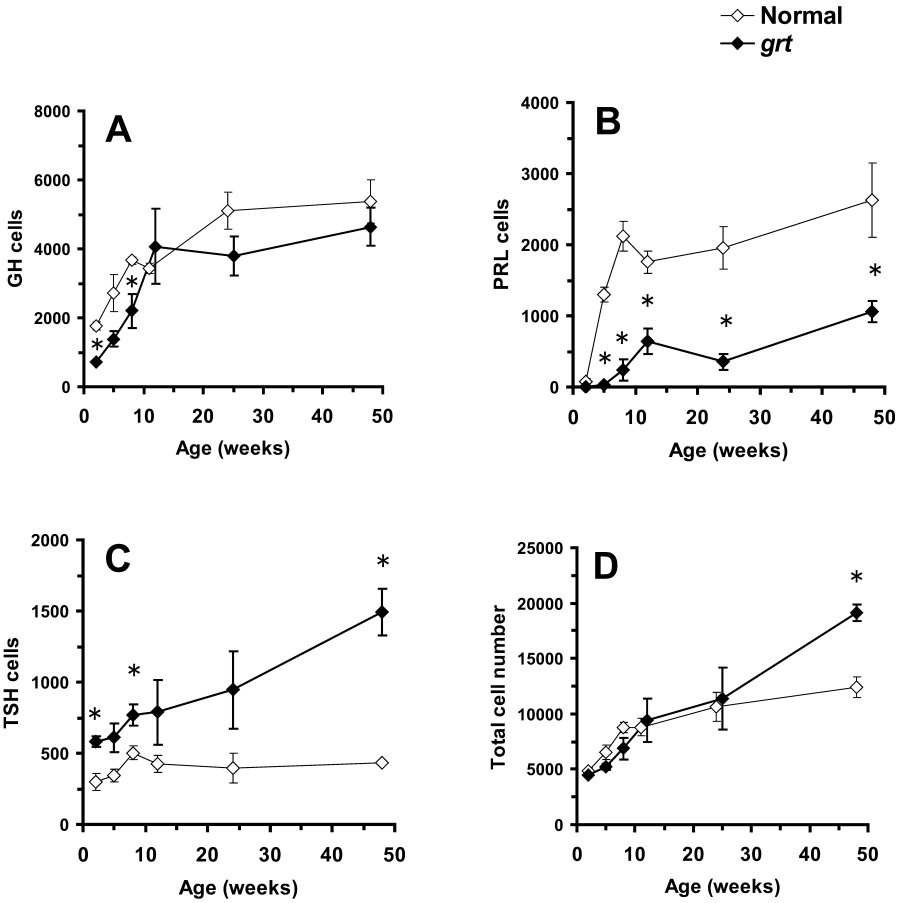

Fig. 3. Developmental changes in the numbers of GH- (A), PRL- (B), TSH-immunoreactive cells (C) and total pituitary cells (D) in normal and *grt* male mice. Each point represents the mean ± SEM of 3 mice. *Significantly different from age-matched normal mice ($P<0.05$).

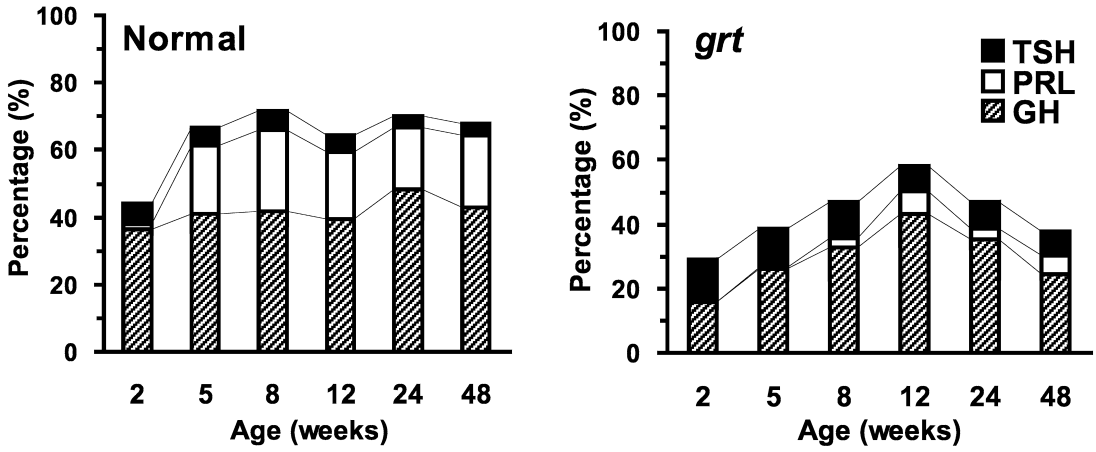

Fig. 4. Mean percentages of GH, PRL and TSH cells in the pituitaries of developing normal and *grt* male mice.

characteristics of congenital hypothyroidism, are derived from phenotypically normal DW/J mice by autosomal recessive inheritance. The growth pattern of *grt* mice differs from that of *dw* mice[16], which have been used as a rodent model of panhypopituitarism[2]. The *dw* phenotype is caused by a point mutation in the gene encoding pituitary transcription factor-1 (Pit-1), which is an essential factor for the development and proliferation of GH cells, PRL cells and TSH cells [28]. In the case of *dw* mice, the pituitary lacks GH, PRL and TSH cells, as demonstrated by immunocytochemically using both light microscopy[29] and electron microscopy[30]. In association with this, immunoassayable GH and PRL levels

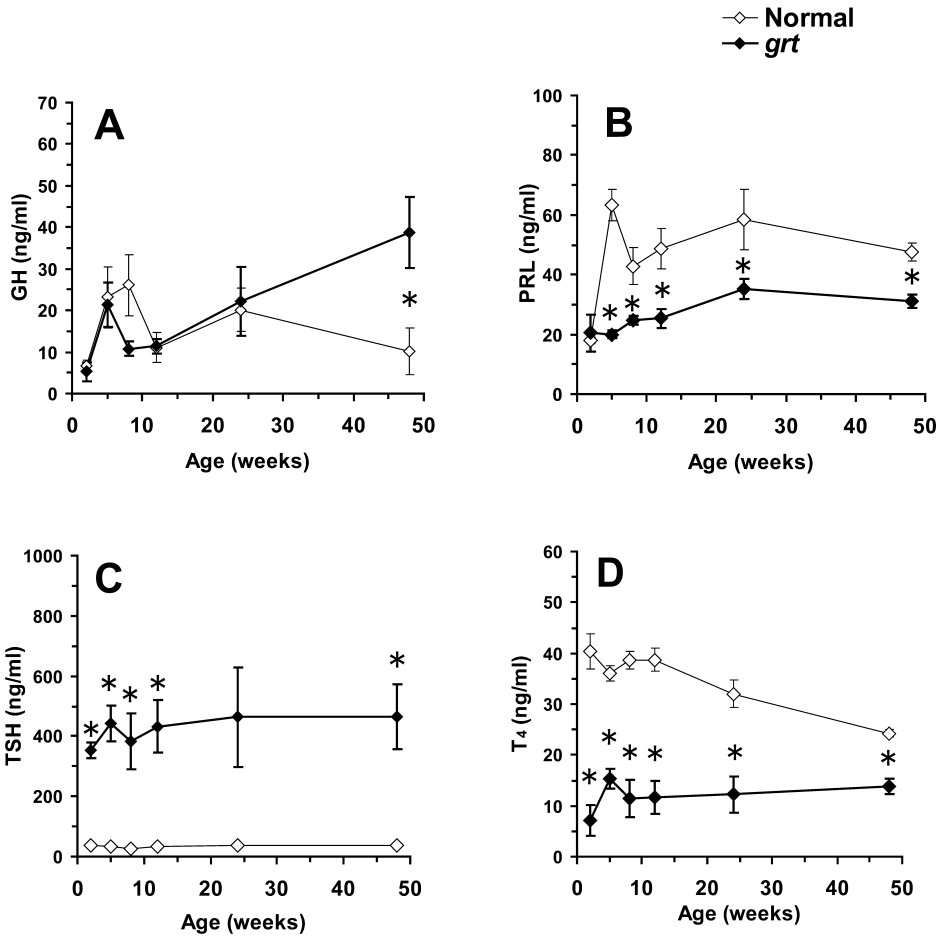

Fig. 5. Developmental changes in the plasma GH (A), PRL (B), TSH (C) and T_4 (D) levels in normal and *grt* male mice. Data points represent the mean ± SEM of 4 to 17 mice. *Significantly different from age-matched normal mice ($P<0.05$).

are extremely low[31]. Both *hyt* mice and *rdw* rats are also other dwarf rodent models with congenital primary hypothyroidism. There are fewer GH cells in *hyt* mice than in wild type[10]. In *rdw* rats, GH and PRL cells in the anterior lobe are fewer than in normal rats[7], and again both plasma and mRNA levels of GH and PRL expression in the pituitary are reduced, whereas plasma and mRNA levels of TSH are elevated[13–15].

In the present experiment, a delay of GH cell development was observed in *grt* mice during the first 8 weeks after birth (Fig. 3). According to Yoshida *et al.*[16], GH content in the *grt* pituitary gradually increases with age but remains below normal until about 1 year of age. A delay of body weight increase in *grt* mice compared with normal mice has also been noted. The difference in body weight between *grt* and normal mice becomes apparent by 3 weeks of age and most evident (about half of the value for normal mice) at about 1 month of age. However, *grt* mice gradually catch up with normal mice in terms of body weight, and no difference in weight is noted between *grt* and normal mice at about 1 year of age. Collectively the results from the present study and the study by Yoshida *et al.*[16] indicate the importance of pituitary GH in the rapid somatic growth and metabolic change at puberty. In contrast to the development changes in the *grt* pituitary cells, plasma GH levels did not differ between *grt* and normal mice except at 48 weeks (Fig. 5A). At present, it is difficult to explain the discrepancy between the number of pituitary GH cells and circulating GH levels at each developmental stage. GH is known to be released in a pulsatile manner, particularly in young animals[32], and this might be one of the reasons for the disaccord between the plasma GH levels and GH cell population.

PRL plays a pivotal role in reproduction and increases the number of luteinizing hormone receptors and responsiveness to luteinizing hormone in mouse testis[33]. Fertility is reduced in male *grt* mice and is associated with delayed testicular development[20]. In the present study, *grt* mice possessed fewer PRL cells and exhibited lower plasma PRL levels than normal mice (Figs. 2–5). Targeted disruption of the PRL gene in mice partially affects reproductive development and function; PRL deficiency reduces fertility along with delayed testicular development[34]. Thus, reduced fertility in *grt* mice at young ages, but not in subsequent stages, is considered to be due in part to insufficient PRL production.

TSH stimulates thyroid hormone synthesis and release from the thyroid gland and induces thyroidal folliculogenesis. TSH secretion is under the dual control by hypothalamic thyrotropin-releasing hormone (TRH) and thyroid hormones[35]. An experimental study on hypothyroid animal indicates that thyroid hormones not only regulate GH synthesis and secretion of GH but also affect differentiation and proliferation of GH cells[36]. The reduction in GH mRNA during the hypothyroidal state is accompanied by a decrease in serum GH and pituitary GH content[37,38]. In *grt* mice, the number of PRL cells was reduced and plasma PRL levels remained low as compared with normal mice (Figs. 2 and 3). A transgene ablation experiment revealed that most PRL cells are derived from GH cells [39,40]. Thus, the reduction of PRL cells in *grt* mice is likely to be a consequence of reduced numbers of GH cells.

It is known that TSH, PRL and GH cells are derived from a common undifferentiated pituitary transcription factor-1 (Pit-1) precursor cell[28]. Accordingly, another explanation for the underdevelopment of GH and PRL cells in *grt* mice may be that the overproduction of TSH cells from Pit-1 precursor cells reduces the potency of generation of GH and PRL cells from the precursor cells. A study using thyroid hormone-deficient mice showed that thyroid hormone replacement rescues the dwarfism, prevents and reverses the hypertrophy and hyperplasia of TSH cells and normalizes the population of PRL cells and GH cells[41]. These findings clearly demonstrate that thyroid hormone is critical for developing a normal component of PRL and GH cells.

The anterior pituitaries of *grt* mice contain various cells as observed in that of normal mice, namely, PRL cells, GH cells, TSH cells, ACTH cells, GTH cells and chromophobic cells[16]. According to Hibasami *et al*., chromophobic cells in *grt* anterior lobes show conspicuous proliferation, resulting in distinct hyperplasia. They concluded that the proliferation of these cells is secondary to the hypothyroidism and is a likely cause of the enlarged anterior lobes that are observed in *grt* mice[42]. In the present experiment, we also noticed that the *grt* pituitary became significantly heavier than the normal pituitary (Fig. 1), and the total number of anterior pituitary cells in *grt* mice was significantly greater than that in normal mice (Fig. 3D). Accordingly, the reduction in the percentages of GH, PRL and TSH cells as observed after 24 weeks of age is considered to be a consequence of increased numbers of chromophobic cells.

The gene responsible for the *grt* mutation is *Tpst2*, which plays a crucial role in tyrosine sulfation of TSH receptor[18]. Impaired tyrosine sulfation of the TSH receptor molecules may reduce responsiveness to TSH and eventually cause thyroidal hypofunction in *grt* mice. T_3 replacement therapy restores growth retardation[16] and serum TSH levels[17], indicating the importance of thyroid hormone for normalization of *grt* mice. The present study indicates that early-onset of growth retardation and the subsequent pubertal delay in *grt* mice might be associated with, at least in part, the impaired development of GH, PRL and TSH cells that accompanies primary congenital hypothyroidism.

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