Anterior pituitary function in Rathke's cleft cysts *versus* nonfunctioning pituitary adenomas

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Abstract. Although Rathke's cleft cysts (RCCs) are common sellar/parasellar lesions, studies examining pituitary function in patients with nonsurgical RCC are limited. This study aimed to clarify the importance of RCCs, including small nonsurgical ones, as a cause of hypopituitarism by determining the prevalence of pituitary hormone secretion impairment and its relationship to cyst/tumor size in patients with RCC and in those with nonfunctioning pituitary adenoma (NFA). We retrospectively investigated the basal levels of each anterior pituitary hormone, its responses in the stimulation test(s), and cyst/tumor size in patients with RCC ($n = 67$) and NFA ($n = 111$) who were consecutively admitted to our hospital for endocrinological evaluation. RCCs were much smaller than NFAs (median height, 12 vs. 26 mm). The prevalence of gonadotropin, PRL, and GH secretion impairment in RCC was lower in comparison to NFA (19% vs. 44%, 34% vs. 61%, and 24% vs. 46%, respectively), whereas the prevalence of TSH and ACTH secretion impairment was comparable (21–27% and 17–24%, respectively). A significant positive relationship between cyst/tumor size and number of impaired hormones was observed in both groups, but smaller cysts could cause hormone secretion impairment in RCC. Stimulation tests suggested that most hormone secretion impairment was attributable to the interrupted hypothalamic-pituitary axis in both groups. Therefore, RCC, even small ones, can cause pituitary dysfunction. Different mechanisms may underlie hypothalamic-pituitary interruption in RCC and NFA.

Key words: Rathke's cleft cyst, Nonfunctioning pituitary adenoma, Pituitary function, Hypopituitarism

PITUITARY TUMORS are the most common cause of hypopituitarism, accounting for more than half of all cases [1, 2]. Conversely, hypopituitarism is found in 10–46% of patients with pituitary incidentalomas [3]. Because hypopituitarism is associated with premature mortality [1, 4], it is necessary to properly diagnose and treat hypopituitarism in patients with pituitary tumors.

Hypopituitarism in pituitary tumors is thought to be caused by the compression of glandular tissue and/or vessels, which can interrupt the hypothalamic-pituitary axis and damage tissue directly or indirectly (*via* ischemia). The reported findings that hypopituitarism is more severe in larger pituitary adenomas [5, 6] and that increased intrasellar pressure (although not tumor size in this study) is related to pituitary dysfunction [7] seem to support this hypothesis. The preoperative prevalence of impaired secretion of GH, gonadotropins, TSH, and

ACTH is reported to be 36–97%, 43–57%, 19–25%, and 26–48%, respectively, among surgically-treated cases of nonfunctioning pituitary adenoma (NFA) [8–10].

Rathke's cleft cysts (RCCs) are common sellar/parasellar lesions that are reported in 11–33% of routine autopsy cases [11, 12] and which account for 12–28% of pituitary masses (excluding functioning adenomas) that are incidentally discovered by magnetic resonance (MR) imaging [13, 14]. Hypopituitarism in RCCs that needed surgical resection has been repeatedly reported in literature [15, 16]. However, studies examining pituitary function in patients with nonsurgical RCC are limited; one study reported that 31% of 62 patients presented with pituitary dysfunction [17] and another reported that 24% of 75 patients presented with dysfunction [18]. Because asymptomatic RCC is not a surgical indication, pituitary function of patients with small RCCs may not be completely evaluated. Moreover, hypopituitarism in RCCs was not related to cyst size in a case series of 37 patients [19].

This study aimed to clarify the importance of RCCs, including nonsurgical ones, as a cause of hypopituitarism. We retrospectively investigated the prevalence of

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the impaired secretion of individual anterior pituitary hormones and relationships between the pituitary dysfunction and cyst/tumor size in patients with RCCs in comparison to patients with NFAs. We also examined hormone responses in stimulation tests to clarify the characteristics of the impaired hormone secretion in RCCs and NFAs.

Materials and Methods

Subjects

Overall, 67 patients with RCC and 111 patients with NFA who were admitted to our hospital for an endocrinological evaluation between April 2005 and March 2017 were retrospectively examined (Table 1). No patients were surgically treated prior to the evaluation; 31 RCC and 105 NFA patients were histologically diagnosed after surgery, whereas the others were diagnosed

based on MR and computed tomography (CT) imaging. The diagnostic criteria for RCC on MR imaging were as follows: 1) a round, ovoid or pear-shaped nonenhancing mass, 2) showing neither a fluid-fluid level nor septation, and 3) when having an off-midline location on coronal imaging, presenting no T2-hypointense rim [20]. Calcified lesions on CT imaging were excluded [11]. All non-surgical RCC patients except two who dropped out of follow-up had MR imaging 1–7 times (median and mode 3) for two years after the evaluation, and the lesions remained fulfilling the criteria. Cystic lesions diagnosed as pituitary apoplexy of NFAs, which was defined as having a typical history (*e.g.*, acute-onset headache, blindness or ophthalmoplegia) and/or histological findings of hemorrhage or hemorrhagic infarction, did not meet the criteria.

The study was approved by the Clinical Research Ethics Committee of Kanazawa Medical University

Table 1 Clinical features and prevalence of anterior pituitary hormone impairments in patients with Rathke's cleft cysts and patients with nonfunctioning pituitary adenomas

	RCCs	NFAs	<i>p</i> -value
Female (F)/Male (M), <i>n</i> (%)	41 (61.2)/26 (38.8)	51 (45.9)/60 (54.1)	0.063*
Age, <i>y</i>	51.4 (20.7–77.8)	60.0 (25.9–86.6)	0.0002†
Reasons for being found; Headache/Visual disturbance/ Hypopituitarism/Incidentally/Others, <i>n</i>	30/7/10/16/4	27/32/5/45/2	0.0001#¶
Surgery, <i>n</i> (%)	31 (46.3)	105 (94.6)	<0.0001*
Cyst/tumor size			
Maximal height, mm	12 (6–34)	26 (12–47)	<0.0001†
Maximal width, mm	15 (6–26)	25 (11–50)	<0.0001†
Maximal depth, mm	10 (5–24)	20 (11–46)	<0.0001†
Anterior pituitary hormone impairments			
TSH, <i>n</i> (%)	13/63 (20.6)	30/111 (27.0)	0.36*
Basal TSH; Low/High/Normal, <i>n</i>	1/2/10	1/3/26	0.70#
Gonadotropin, <i>n</i> (%)	13/67 (19.4)	49/111 (44.1)	0.0011*
Amenorrheic F with low LH or FSH/Non-amenorrheic F with low estradiol/M with low testosterone, <i>n</i>	6/1/6	19/3/27	0.85#
PRL, <i>n</i> (%)	23/67 (34.3)	68/111 (61.3)	0.0006*
Hyper-/Hypoprolactinemia, <i>n</i>	20/3	64/4	0.36*
GH, <i>n</i> (%)	16/67 (23.9)	50/109 (45.9)	0.0039*
Reduced response to IHH, <i>n</i> (%)	14/54 (25.9)	37/68 (54.4)	0.0018*
Reduced response to GHRH, <i>n</i> (%)	9/63 (14.3)	30/78 (38.5)	0.0022*
Decreased IGF-I, <i>n</i> (%)	6/63 (9.5)	21/107 (19.6)	0.088*
ACTH, <i>n</i> (%)	11/65 (16.9)	27/111 (24.3)	0.34*
Reduced response to IHH, <i>n</i> (%)	8/52 (15.4)	14/68 (20.6)	0.63*
Reduced response to CRH, <i>n</i> (%)	8/59 (13.6)	24/76 (31.6)	0.016*
Decreased basal cortisol, <i>n</i> (%)	2/62 (3.2)	4/109 (3.7)	>0.99*

Data are presented as the number (%) or median (range). *, Fisher's exact test; †, Mann-Whitney *U*-test; #, chi-square test; ¶, excluding others. RCCs, Rathke's cleft cysts; NFAs, nonfunctioning pituitary adenomas; IHH, insulin-induced hypoglycemia.

(No. I103). An opt-out consent process was announced through the hospital's website and printed matter.

Endocrinological evaluation

Because assay kits for certain hormones were changed once or twice during the study period, each reference range at the time was used in the data analysis. For calculation, *e.g.*, averaging, earlier assay results were transformed to the corresponding latter assay values (or the earliest and last to the middle) according to their regression curves.

Impaired secretion of each hormone was defined according to the Endocrine Society Clinical Practice Guideline [21], with some modifications. Impaired TSH secretion was defined as decreased, normal, or mildly increased TSH with low free T4. Impaired gonadotropin secretion was defined as a decreased or normal level of either LH or FSH with low testosterone in men or low estradiol for the follicle period in premenopausal women; in amenorrheic or postmenopausal women, it was defined as low LH or FSH for the postmenopausal reference range. Impaired GH secretion was defined as a reduced GH response to insulin-induced hypoglycemia (IIH) (0.1 U/kg, in principle, IV). IIH with a glucose nadir >45 mg/dL was not used. When the IIH test was not performed or the data were invalid, a reduced response to GHRH (100 µg, IV) was substituted. Reduced responses to both tests were defined as a peak GH level of ≤3.0 ng/mL. When these stimulation test data were not available, an insulin-like growth factor-I (IGF-I) level of <2 standard deviations (SD) in a normal Japanese population [22] was substituted. Impaired ACTH secretion was defined as a reduced cortisol response to either IIH or CRH (100 µg, IV) with a peak level of <18 µg/dL (measured by Elecsys® cortisol [Roche Diagnostics] or its equivalent values of other assay kits). When these tests were not validly performed, a basal cortisol level of <3.0 µg/dL at 6–9 AM was substituted. TRH (500 µg, IV) and GnRH (100 µg, IV) tests were also performed. In addition to cortisol, ACTH levels were measured during the IIH and CRH tests. Clinically overt diabetes insipidus based on subjective symptoms and urinary specific gravity was found in only two RCC patients and one NFA patient, so that evaluation of neurohypophyseal function was excluded.

Pituitary gland images

Plain and gadolinium-enhanced MR images of the pituitary gland were obtained in all patients. All sagittal and coronal images were reevaluated, and the maximal height, width, and depth of the cyst/tumor were measured through all slices.

Statistical analysis

Data are presented as the median and range or the percentiles for cyst/tumor size. Hormone concentrations (except for cortisol) are presented as the mean and SD after logarithmic transformation, because those values were approximated with log normal distribution. The area under the curve (AUC) was calculated using a trapezoidal method (with the lower limit of measurement as the baseline). The statistical significance of differences was examined using Fisher's exact test or chi-squared test for prevalence, the Mann-Whitney *U* test (and unpaired *t*-test when homogeneity of variance was not denied by an *F* test) for cyst/tumor size, Spearman's rank correlation coefficient for correlations, and a repeated measures analysis of variance (ANOVA) for hormone responses. Regarding the AUC of the hormone response, the Mann-Whitney *U* test (and unpaired *t*-test) was (were) used for comparisons between two groups and an ANOVA followed by Tukey's *post-hoc* test was used for comparisons among three groups. *P* values of <0.05 were considered to indicate statistical significance. The analyses were performed using StatView for Windows, version 5.0 (SAS Institute, Cary, NC).

Results

Cyst/tumor size

The median cyst/tumor size of RCCs was smaller in comparison to NFAs in maximum height, width, and depth (Table 1).

Prevalence of impaired anterior pituitary hormone secretion

The number and percentage of cases with impaired secretion of each hormone among examined cases are shown, along with the numbers of included cases with different statuses or the criteria for impairment that were met (Table 1).

There was no significant difference in the prevalence of impaired TSH secretion between the patients with RCC and those with NFA; four RCC cases were excluded because of preexisting thyroid disease. Basal TSH levels were decreased in 3–8% and mildly increased (up to 6.84 mIU/L) in 10–15% of cases with impaired TSH secretion. The prevalence of impaired gonadotropin secretion in RCC patients was lower than that in NFA patients. Impaired PRL secretion was defined as hyperprolactinemia or hypoprolactinemia (see Discussion), the prevalence of which was lower in RCC patients (approximately 90% showed hyperprolactinemia). The overall prevalence of impaired GH secretion was also lower in RCCs, whereas no significant difference was found in the prevalence of impaired ACTH secretion; two NFA

cases lacked GH data, and two RCC cases were excluded from the evaluation of ACTH due to preexisting steroid usage. Although impaired secretion of GH and ACTH was primarily defined according to the IHH test, valid tests were performed in 81% and 61% of the RCC and NFA cases, respectively. The substituted GHRH test detected impaired GH secretion less frequently than the IHH test, whereas the CRH test detected impaired ACTH secretion equivalently or more frequently. In the cases where both the IHH test and the GHRH or CRH test were performed, 49% of patients with reduced GH responses to IHH showed reduced responses to GHRH and 86% of patients with reduced cortisol responses to IHH showed reduced responses to CRH (refer to *Hormone responses to stimulation tests*). Decreased IGF-I and basal cortisol levels were much less frequently observed.

Comparison of cyst/tumor size between patients with and without hormone secretion impairment

The cyst/tumor size (represented by the sum of three dimensions) was compared between patients with and without impaired secretion of each hormone in the RCC and NFA groups (Fig. 1). It was significantly greater in patients with impaired TSH, gonadotropin and GH secretion (and PRL, when height was compared within NFAs; $p = 0.016$, Mann-Whitney U test) in comparison to patients without the impairment of these hormones in both the RCC and NFA groups, whereas a significant difference according to ACTH impairment was only found in RCCs. Examining the data distribution, it was evident that small cysts of a size that rarely led to impaired hormone secretion in NFAs could cause impairment in the RCC group; the 75th percentile of the cyst/tumor size in the RCC cases with impairment was between the 10th and 25th percentiles in NFA cases with impairment as to all the hormones.

Number of impaired pituitary hormones

The relationships between the cyst/tumor size (represented by the sum of three dimensions in Fig. 2; the separate dimensions in Supplementary Fig. 1) and the number of impaired hormones were examined. Significant positive correlations were observed in every parameter of cyst/tumor size in both groups. However, in comparison to tumors of the NFA group, smaller cysts tended to cause more hormone secretion impairment in the RCC group. NFA cases with pituitary apoplexy did not seem to have any specific tendencies.

Hormone responses to stimulation tests

The hormone responses in the stimulation tests are presented in Figs. 3–5 and Supplementary Fig. 2. The response curves in cases with impaired hormone secre-

Fig. 1 Comparison of cyst/tumor size (height + width + depth) between patients with and without impaired secretion of each anterior pituitary hormone (as to PRL, with hyper-, hypo-, and normo-prolactinemia) in the Rathke's cleft cyst (RCC) and nonfunctioning adenoma (NFA) groups. The box contains values between the 25th and 75th percentiles (central line, median), and vertical lines represent the 10th and 90th percentiles. †, Mann-Whitney U test; ‡, unpaired t -test. For statistical analysis of PRL secretion impairment, cases of hyper- and hypoprolactinemia are mingled.

tion were compared between the RCC and NFA groups, while the curve in cases without impairment (both groups were merged) was presented for convenience. The difference between cases with and without impairment in each group was analyzed as AUCs.

The responses of TSH to TRH in cases with impaired TSH secretion, which did not decrease compared with responses in cases without impairment, were similar in the RCC and NFA groups (Fig. 3). Fifty percent and 67%

Fig. 2 Correlations between the numbers of impaired hormones in individual cases and their cyst/tumor sizes (represented by the sum of three dimensions) in patients with RCC and those with NFA (cases with pituitary apoplexy are presented separately). *, Spearman's rank correlation coefficient; for convenience, regression lines (solid for RCCs, dotted for NFAs) are drawn.

Fig. 3 TSH responses to TRH in patients with and without impaired TSH secretion, and comparison of the area under the curve (AUC) of the TSH response between patients with and patients without impaired TSH secretion in the RCC and NFA groups. Circles and X marks represent the mean and vertical lines show one standard deviation (SD). #, Difference between the RCC and NFA groups with TSH secretion impairment; §, interaction between the group and time course as evaluated by a repeated measures analysis of variance (ANOVA). †, Mann-Whitney *U* test; ‡, unpaired *t*-test. Patient numbers are shown in parentheses.

of RCC and NFA cases with impaired TSH secretion, respectively, presented a delayed response pattern of which peak was at 60 min and after, while 6% of cases

without impairment did. Since the basal TSH levels varied widely, the cases with the TSH secretion impairment were divided into three groups according to the basal TSH level. Whereas the low basal TSH group consisting of only one RCC case showed a markedly reduced response, in the high and normal basal TSH groups, the responses after 30 min were identical (data not shown).

The responses of LH and FSH to GnRH in cases with impaired gonadotropin secretion were significantly smaller than those in cases without impairment (Supplementary Fig. 2). The reductions in patients with RCCs were greater than those in patients with NFAs. Similar results were obtained even when patients were divided into amenorrheic women, non-amenorrheic women, and men (data not shown).

The GH responses to GHRH (Fig. 4a) and IIH (Fig. 4b) in cases with impaired GH secretion were reduced from responses in cases without impairment, and were identical between the RCC and NFA groups. The comparison between the groups of patients with a normal response to GHRH and a reduced response to IIH and of patients with reduced responses to both tests suggested that apparently smaller reduction in the response to GHRH than in that to IIH was attributable to the former group (data not shown).

In the cases with impaired ACTH secretion, which was defined according to cortisol responses, the ACTH response to CRH (Fig. 5a) was excessive and that to IIH (Fig. 5b) was reduced in the RCC group but these responses were unchanged in the NFA group. Conversely, the cortisol responses to CRH (Fig. 5c) and IIH (Fig. 5d) were both reduced in both groups, although the response to IIH was more diminished in the RCC group. Patients in whom CRH and IIH tests were both performed were divided into three groups according to the cortisol response—patients with reduced responses to both tests, patients with a normal response to CRH and a reduced response to IIH, and patients with normal responses to both (Supplementary Fig. 3). Among the RCC cases, the ACTH response to CRH was excessive in patients with a reduced cortisol response to both tests, and the ACTH response to IIH was more diminished in patients with a reduced cortisol response to IIH alone.

Discussion

Among the subjects in this retrospective study, RCCs were much smaller than NFAs. The prevalence of impaired gonadotropin, PRL, and GH secretion was lower in the RCC group than in the NFA group, as would be expected from the difference in cyst/tumor size, whereas the prevalence of impaired TSH and ACTH secretion did not differ between the two groups.

Fig. 4 GH responses to GHRH (a) and insulin-induced hypoglycemia (IIH) (b) in patients with and without impaired GH secretion, and comparison of the AUC of the GH responses between patients with and patients without impaired GH secretion in the RCC and NFA groups. $^{\#}$, Difference between the RCC and NFA groups with GH secretion impairment; § , interaction between the group and time course, as evaluated by a repeated measures ANOVA. † , Mann-Whitney U test; ‡ , unpaired t -test. Patient numbers are shown in parentheses.

Although larger cysts/tumors caused an individual hormone secretion impairment more frequently and a larger number of impairments in individual cases, small cysts of which size rarely led to impaired hormone secretion in NFAs could frequently cause impairment in RCCs.

In NFAs, the prevalence of impaired secretion of each hormone was in line with previous reports [5, 6, 8-10, 23]. The tumor size was greater in patients with hormone secretion impairment than in those without impairment for every hormone, except ACTH. The numbers of impaired hormones in individual patients were positively correlated with tumor size. These findings seemed consistent with the generally accepted concept that hypopituitarism in pituitary tumors is caused by physical compression. However, the prevalence of TSH and ACTH secretion impairment in RCCs was not signifi-

cantly different from that in NFAs, despite the smaller cyst size. Compared with similar-sized NFAs, small RCCs tended to present more impaired hormones. These findings imply the importance of RCC as a cause of hypopituitarism and seem to suggest that RCCs can impair hormone secretion more easily by different mechanism(s) from NFAs.

To determine characteristics, especially damaged sites, of the impaired hormone secretion in RCCs and NFAs, we examined hormone responses in stimulation tests, which revealed the following features. 1) In most patients with hypothyroidism, the TSH response to TRH, being identical between both groups, was essentially normal. It was reported that more than two-thirds of patients with central hypothyroidism had normal or excessive TSH responses to TRH, many of whom showed delayed and/or prolonged patterns [24]. In the present study, more than half of cases with impaired TSH secretion showed the delayed response. TRH-knockout mice reportedly showed hypothyroidism accompanied with an increased basal TSH level and an increased response to TRH because of biologically inactive TSH [25]. 2) The LH and FSH responses to GnRH in patients with impaired gonadotropin secretion were more severely reduced in RCCs than in NFAs. Considering the lower prevalence of this impairment in RCC, different mechanisms may involve in the occurrence and severity of the impairment. The damaged sites could not be identified without the repeated administration of GnRH [26]. 3) The GH responses to GHRH in patients with impaired GH secretion were identical in the RCC and NFA groups, as were the GH responses to IIH; however, the reduction in the former response was smaller than that in the latter, which was attributable to the inclusion of cases with normal GH responses to GHRH into the patients with impaired GH secretion in an approximately constant ratio through both groups. 4) Among the patients with impaired ACTH secretion, most cases showed reduced cortisol responses to both of CRH and IIH. Meanwhile, the ACTH responses to CRH were not reduced or even excessive in most cases. Since chronically decreased ACTH secretion can reduce adrenal responsiveness to ACTH [27], the decreased cortisol responses to CRH likely indicate not necessarily direct damage to corticotrophs but merely chronic ACTH deficiency independent of damaged sites. The adrenal unresponsiveness seemed prominent in RCCs, the reason for which was unknown.

These findings suggest that the main damaged site of hypopituitarism, particularly with respect to TSH and ACTH secretions, in RCC and NFA was the hypothalamic-pituitary axis, which was observed in more than 90% of patients with impaired hormone secretion. According to the same definition, hyperprolactinemia

Fig. 5 ACTH (a and b) and cortisol (c and d) responses to CRH (a and c) and IIH (b and d) in patients with and without impaired ACTH secretion, and comparison of the AUCs of the ACTH and cortisol responses between patients with and patients without impaired ACTH secretion in the RCC and NFA groups. $\S$, Interaction between the group (RCC with ACTH secretion impairment vs. NFA with ACTH secretion impairment) and time course, as evaluated by a repeated measures ANOVA. $\dagger$, Mann-Whitney U test; $\ddagger$, unpaired t -test. Patient numbers are shown in parentheses.

should be considered as hormone secretion impairment. The GHRH test indicated that the damaged sites were the pituitary gland in 55–60% of patients with impaired GH secretion. However, considering the arginine + GHRH test is accepted as an alternative to the IIH test for diagnosing GH deficiency [21, 28], the GH responses evoked by GHRH could be diminished even in cases with supraphysiological damage.

Both RCC and NFA are space-occupying lesions in the sellar/parasellar sites. If the hypothalamic-pituitary axis is the main damaged site responsible for pituitary dysfunction in both groups, what led to the difference in the relationship between cyst/tumor size and hormone secretion impairment? In comparison to NFAs, the smaller RCCs could cause similar impairments of TSH, PRL and GH secretion and greater impairment of gonadotropin and ACTH. Hypothalamic hormones enter the hypothalamic-pituitary portal vein at the median eminence, and all blood initially passes through the posterior lobe before entering the anterior lobe [29]. Since RCC arises from between the anterior and posterior lobes, it seems probable that an RCC could block the blood flow

entering the anterior lobe more effectively than an NFA. Moreover, it was reported that inflammation existed in the cyst epithelium or the subjacent interstitial tissue of the RCC [30]. It was also reported that high protein concentrations or the mucous nature of the RCC contents were related to high (to iso-) intensity on T1-weighted MR imaging, inflammation of the cyst wall, and pituitary dysfunction [19, 31, 32]. Also in the present study, all but one RCCs with hormone secretion impairment of which sizes (the sum of three dimensions) were less than 37 mm (the size that had no hormone impairment in NFAs) showed high or iso-intensity on T1-weighted MR imaging (data not shown). Thus, inflammation of the surrounding tissue, especially that involving the pituitary stalk, may have contributed to the impaired hormone secretion in relatively small RCCs.

The present study was associated with some limitations. First, this was a retrospective study in a single institution, in which patients were hospitalized (*i.e.*, selected as subjects) according to each physician's decision. Secondly, the histological diagnoses of RCC were made only in less than half of the cases, which was inevi-

table for the inclusion of nonsurgical cases [13, 17]. Diagnostic MR imaging features were based on previously published reports [11, 20] and repeated MR imaging follow-up seemed to help confirm the diagnosis. Thirdly, the same diagnostic criteria for impaired hormone secretion were not uniformly adopted in all patients. Especially, the IHH test, which is the most accepted test for GH and ACTH deficiencies, was performed and valid in approximately 80% and 60% of the cases in the RCC and NFA groups, respectively. The test is contraindicated in patients with coronary heart disease or epilepsy [33]. In cases where the IHH data were unavailable, we substituted the GHRH and CRH tests, which are generally not recommended for the diagnoses of the hormone deficiencies [21]. Theoretically, because these tests directly stimulate the pituitary gland, they cannot diagnose suprahypophysial defects and thus may underestimate the prevalence. However, regarding the CRH test, the adrenal unresponsiveness to ACTH seemed to compensate for the inability to detect suprahypophysial defects as discussed above. The CRH test detected impaired ACTH secretion similarly or more frequently compared with the IHH test. The GHRH test detected impaired GH secretion less frequently compared with the IHH test, but that underestimation cannot explain our main finding regarding the relationship between cyst/tumor size and hormone secretion impairment. Moreover, cyst characteristics other than size, *e.g.*, the MR intensity and histological findings, were not systematically examined. Finally, the interactions between each pituitary hormone could not be excluded. It is well

known that hyperprolactinemia causes hypogonadotropic hypogonadism. Besides, GH deficiency decreases conversion of T4 to T3 [34], and some cases of isolated ACTH deficiency show transient GH deficiency before glucocorticoid replacement [35].

Nevertheless, this is the first study demonstrating that even small RCCs, of which size rarely leads to impaired hormone secretion in NFAs, can frequently cause impairment. This finding suggested that the mechanism of pituitary dysfunction in RCCs might differ from that in NFAs, although the main damaged site was the interrupted hypothalamic-pituitary axis in both groups. Regarding the natural history and surgical outcomes of the RCC, it has been reported that only a limited number of asymptomatic RCCs grow to be symptomatic [13, 15, 17], while the pituitary dysfunction caused by RCC barely shows complete recovery after surgery [15, 17, 36, 37]. However, the present study implies that pituitary dysfunction may be overlooked in patients with small and therefore seemingly asymptomatic RCCs. Further studies should be performed to verify whether surgery or other interventions can improve the prognosis of such cases.

Disclosure

The authors declare no conflict of interest in association with the present study.

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Supplementary Fig. 1 Correlations between the numbers of impaired hormones in individual cases and their cyst/tumor sizes (each separate dimension) in patients with RCC and those with NFA. Symbols are the same as used in Fig. 2.

Supplementary Fig. 2 LH (a) and FSH (b) responses to GnRH in patients with and without impaired gonadotropin secretion, and comparison of the AUCs of the LH and FSH responses between patients with and patients without impaired gonadotropin secretion in the RCC and NFA groups. [#], Difference between the RCC and NFA groups with gonadotropin secretion impairment; [§], interaction between the group and time course, as evaluated by a repeated measures ANOVA. [†], Mann-Whitney *U* test; [‡], unpaired *t*-test. Patient numbers are shown in parentheses.

Supplementary Fig. 3 Comparison of the AUCs of the ACTH (a) and cortisol (b) responses to CRH and IIH between patients with reduced cortisol responses to both CRH and IIH, patients with normal responses to CRH and reduced responses to IIH, and patients with normal responses to both CRH and IIH in the RCC and NFA groups. [¶], $p < 0.01$; ns, not significant by Tukey's *post-hoc* test following significant ANOVA (all $p < 0.0001$ but $p = 0.0018$ for ACTH responses to IIH). The other symbols are the same as used in Fig. 5.

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