genomic DNA from the proband (III-2) and her mother (II-2), compared with that from the father (II-1) (Fig. 2B), suggesting the inherited deletion of the *PRKAR1A* gene in the family.

Identification of the breakpoints on 17q24

To identify the range of deletion at 17q24 in the leukocyte genomic DNA, we measured DNA copy number at each locus in the vicinity of the *PRKAR1A* gene (Fig. 3A) and found that the deletion is in the vicinity of the *LOC732538* gene to intron 1 in the *FAM20A* gene (data not shown). We designed sets of four primers in intron 1 of the *FAM20A* gene and in the vicinity of the *LOC732538* gene (P1–P4 and p1–p4 in Fig. 3B and C) to detect DNA copy number in the loci. The qPCR analyses narrowed the breakpoints to a 1.3 kb region between P2 and P3 in intron 1 of the *FAM20A* gene (Fig. 3B) and to 14 kb between p2 and p3 surrounding the *LOC732538* gene (Fig. 3C).

To identify the breakpoints, cloning of a region encompassing the deletion junction was attempted (Supplementary Fig. 1A, B, and C, see section on supplementary data given at the end of this article). The cloned DNA contained sequences of intron 1 of the *FAM20A* gene and the *LOC732538* gene, and a 24 bp region in which they overlapped (Supplementary Fig. 1D and E), suggesting that each breakpoint should be located in the overlapping region and the deletion size in genomic DNA was ~0.5 Mb. The deletion size and the location were consistent with results obtained from comparative genomic hybridization (CGH) array analysis (Supplementary Fig. 2). Finally, we designed a primer set to detect the deletion and confirmed that the family members (II-2, III-1, and III-2), except for the proband's father (II-1), had the germline deletion by the presence of 325 bp PCR products (Fig. 3D). Her grandmother's leukocytes were unavailable (Supplementary Materials and methods).

Discussion

The most common type of endocrine tumor in CNC is PPNAD, which was detected in 25–30% of patients (2). In this family, her mother developed bilateral adrenal tumors, while pathological findings of right resected tumors were unavailable. Although almost all CNC patients (75%) exhibit asymptomatic elevations in serum GH, IGF1, or PRL level, acromegaly with pituitary adenomas occurs in a smaller population of patients (~10%) (14). Gigantism is frequent in CNC; in addition to two adolescents with gigantism published in individual

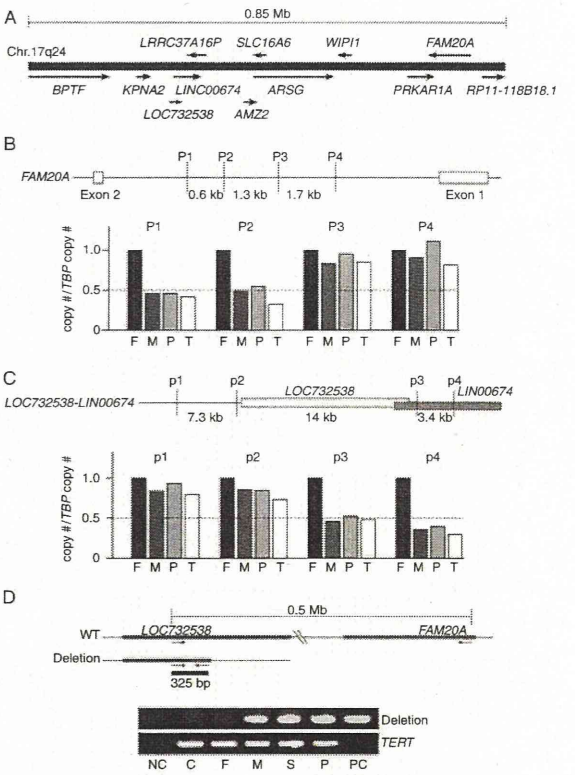

Figure 3

Deletion analyses at several loci on 17q24. (A) Schematic diagram of the 17q24 region. Arrows indicate the orientation of genes in the 5′ to 3′ direction. (B) Relative DNA copy number at positions in intron 1 of *FAM20A* downstream of the *PRKAR1A* gene compared with the *TBP* gene. Using the set of four primers (P1–P4) indicated in the upper diagram, relative DNA copy number at four positions in intron 1 of *FAM20A* was measured by qPCR in leukocytes from the proband (III-2, P), father (II-1, F), and mother (II-2, M) and in the tumor (T). (C) Relative DNA copy number at loci in the vicinity of the *LOC732538* gene compared with the *TBP* gene. Using the set of four primers (p1–p4) in each position, relative DNA copy number was measured in the same way. (D) Identification of the large deletion on 17q24 in family members. The estimated size of PCR products amplified from a WT allele and an allele with the deletion using a primer set (arrow) to detect the deletion (upper diagram). Representative electropherogram of PCR products for leukocyte DNA from the proband (III-2, P), her father (II-1, F), mother (II-2, M), and sister (III-1, S). PCR using a primer set for the *TERT* gene on 5p15.33 as an internal control was also carried out. NC, no template control; C, control genomic DNA; PC, a plasmid including DNA encompassing a deletion junction. A full colour version of this figure is available via http://dx.doi.org/10.1530/EJE-14-0685.

case reports (15, 16), several large series of patients with CNC and GH-secreting pituitary tumors have been published, which have included cases with gigantism (5).

To our knowledge, this is the first proven CNC-related pituitary adenoma with the combination of a large inherited deletion on 17q24 and a somatic frameshift *PRKAR1A* mutation. This study suggests that the standard sequencing method alone is insufficient to detect the genetic abnormalities in leukocytes and tumors. In this study, we estimated the existence of a deletion at the germline level in the family because of the weaker sequencing peaks of the normal allele in addition to somatic frameshift mutation in the tumor (Fig. 2A). However, if the tumor had a large biallelic deletion, we might misdiagnose it as normal due to the sequencing peak derived from contaminated normal tissue. Therefore, even if no mutations were detected in both leukocytes and tumors by sequencing analysis, measurement of DNA copy number at the locus of tumor suppressor gene may be necessary. Owing to the expense of CGH array and the commercial unavailability of multiplex ligation-dependent probe amplification for the *PRKAR1A* gene, qPCR of genomic DNA may be the first-line method for detecting a large deletion.

In ~75% of CNC patients, germline mutations in the *PRKAR1A* gene have been reported (17). Large deletions of the gene loci at 17q24.2–24.3 have recently been detected in 7.7 (18) and 21.6% (6) of *PRKAR1A* mutation-negative patients with CNC. The mutations and large deletions leading to *PRKAR1A* haploinsufficiency have been considered to cause CNC. However, whether haploinsufficiency of *PRKAR1A* is sufficient for the development of pituitary adenomas in CNC remains unknown. Owing to the low frequency of pituitary adenomas in CNC, reports on biallelic inactivation of *PRKAR1A* in pituitary tumors have been scarce. In the first report about inactivating mutations of the *PRKAR1A* gene in CNC, one of the two GH-producing pituitary adenomas showed germline mutation and somatic loss of heterozygosity (LOH), while another adenoma was uninformative (4). Bossis *et al.* (11) and Takano *et al.* (12) reported LOH in pituitary adenomas with *PRKAR1A*-inactivating germline mutation. On the other hand, CGH revealed that loss of 17q was not observed in four pituitary adenomas from patients with CNC (19); however, this method may be inadequate to identify whether these tumors have inactivating mutations or a small deletion. In this study, we demonstrated the biallelic inactivation of the *PRKAR1A* gene in a CNC-related pituitary adenoma and loss of the protein expression in the tumor cells (Fig. 1D).

In conclusion, we found somatic inactivating mutation in GH-producing adenoma in a family with a large inherited deletion of the *PRKAR1A* locus. This suggests that the complete loss of *PRKAR1A* might be necessary for the development of at least some pituitary adenomas in CNC.

Supplementary data

This is linked to the online version of the paper at http://dx.doi.org/10.1530/EJE-14-0685.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Clinicopathological characteristics and therapeutic outcomes in thyrotropin-secreting pituitary adenomas: a single-center study of 90 cases

Clinical article

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Object. The aim of this study was to analyze clinicopathological characteristics and treatment outcomes in a large single-center clinical series of cases of thyrotropin (TSH)–secreting pituitary adenomas.

Methods. The authors retrospectively reviewed clinical, pathological, and treatment characteristics of 90 consecutive cases of TSH-secreting pituitary adenomas treated with transsphenoidal surgery between December 1991 and May 2013. The patient group included 47 females and 43 males (median age 42 years, range 11–74 years).

Results. Sixteen tumors (18%) were microadenomas and 74 (82%) were macroadenomas. Microadenomas were significantly more frequent in the more recent half of our case series (12 of 45 cases) ($p = 0.0274$). Cavernous sinus invasion was confirmed in 21 patients (23%). In 67 cases (74%), the tumors were firm elastic or hard in consistency. Acromegaly and hyperprolactinemia were observed, respectively, in 14 (16%) and 11 (12%) of the 90 cases. Euthyroidism was achieved in 40 (83%) of 48 patients and tumor shrinkage was found in 24 (55%) of 44 patients following preoperative somatostatin analog treatment. Conventional transsphenoidal surgery, extended transsphenoidal surgery, and a simultaneous combined supra- and infrasellar approach were performed in 85, 2, and 3 patients, respectively. Total removal with endocrinological remission was achieved in 76 (84%) of 90 patients, including all 16 (100%) patients with microadenomas, 60 (81%) of the 74 with macroadenomas, and 8 (38%) of the 21 with cavernous sinus invasion. None of these 76 patients experienced tumor recurrence during a median follow-up period of 2.8 years. Stratifying by Knosp grade, total removal with endocrinological remission was achieved in 34 of 36 patients with Knosp Grade 0 tumors, all 24 of those with Grade 1 tumors, 12 of the 14 with Grade 2 tumors, 6 of the 8 with Grade 3 tumors, and none of the 8 with Grade 4 tumors. Cavernous sinus invasion and tumor size were significant independent predictors of surgical outcome. Immunoreactivity for growth hormone, prolactin, or both hormones was present in 32, 9, and 24 patients, respectively. The Ki-67 labeling index was less than 3% in 71 (97%) of 73 tumors for which it was obtained and 3% or more in 2. Postsurgery pituitary dysfunction was found in 15 patients (17%) and delayed hyponatremia was seen in 9.

Conclusions. TSH-secreting adenomas, particularly those in the microadenoma stage, have increased in frequency over the past 5 years. The high surgical success rate achieved in this series is due to relatively early diagnosis and relatively small tumor size. In addition, the surgical strategies used, such as extracapsular removal of hard or solid adenomas, aggressive resction of tumors with cavernous sinus invasion, or extended transsphenoidal surgery or a simultaneous combined approach for large/giant multilobulated adenomas, also may improve remission rate with a minimal incidence of complications.

(http://thejns.org/doi/abs/10.3171/2014.7.JNS1471)

Key Words • **hyperthyroidism** • **somatostatin analogs** • **pituitary surgery** • **syndrome of inappropriate TSH secretion** • **transsphenoidal surgery** • **thyrotropin-secreting pituitary adenoma**

Abbreviations used in this paper: ACTH = adrenocorticotropic hormone; ADH = antidiuretic hormone; FSH = follicle stimulating hormone; FT3 = free triiodothyronine; FT4 = free thyroxine; GH = growth hormone; IGF-1 = insulin-like growth factor 1; IQR = interquartile range; LH = luteinizing hormone; PRL = prolactin; SITSH = syndrome of inappropriate TSH secretion; TRH = thyrotropin-releasing hormone; TSH = thyrotropin (thyroid stimulating hormone).

Thyrotropin (TSH)-secreting pituitary adenomas are rare tumors that account for less than 2% of all pituitary adenomas.[1,27] Development of ultrasensitive measurement methods combined with advanced

This article contains some figures that are displayed in color online but in black-and-white in the print edition.

pituitary imaging techniques have helped in the early diagnosis of TSH-secreting adenomas.[3,4] Transsphenoidal surgery is the first-line treatment for these tumors. However, most studies, even recent papers describing remission rates of 0% to 55%,[7,9,22,35,38] report poor surgical outcomes due to relatively high frequencies of large adenomas, invasive adenomas, and/or markedly fibrous adenomas, although a steady decrease in the proportion of macroadenomas has been reported.[4,32]

Most TSH-secreting pituitary adenomas express a variable number of somatostatin receptors. Administering somatostatin analogs as a primary treatment or adjuvant to surgery is highly effective in reducing TSH secretion or shrinking these tumors,[3,4,11,13,31,32] although published reports are limited by small sample sizes. The purpose of the current study was to investigate the clinicopathological features and outcomes of surgical treatment in patients with TSH-secreting tumors treated at our center.

Methods

Patient Population

A total of 92 patients diagnosed with TSH-secreting adenoma based on clinical and neuroimaging findings underwent pituitary surgery at Toranomon Hospital between December 1991 and May 2013. Two patients were excluded from the study because no TSH-secreting tumor was found. In one of these cases, a microadenoma was found during surgery, but it was immunoreactive for growth hormone (GH) and prolactin and not for TSH. In the other case, which involved a 15-year-old girl, an adenoma was suspected on the basis of MRI but no tumor was found during surgery and thyroid hormone resistance was verified by postoperative molecular biological analysis.[14] The hospital records of each of these 90 patients were reviewed.

These 90 cases of TSH-secreting adenomas represented only 2.7% of the 3276 pituitary adenomas that were surgically treated at our hospital during the overall study period. During the last 5 years of the study period, however, the rate increased to 4.0%, with 54 of 1351 cases of surgically-treated pituitary adenomas being TSH-secreting tumors.

This study was approved by the ethics committee at Toranomon Hospital, Tokyo.

Endocrine Studies

Blood basal levels of the anterior pituitary hormones (GH, prolactin, TSH, follicle-stimulating hormone [FSH], and luteinizing hormone [LH], α-subunit), and their target hormones (insulin-like growth factor 1 [IGF-1], free thyroxine [FT4], free triiodothyronine [FT3], testosterone, free testosterone, and estradiol) were measured pre- and postoperatively. Pituitary stimulation tests were also performed in some cases pre- and postoperatively using a combination of thyrotropin-releasing hormone (TRH) (500 μg), LH-releasing hormone (100 μg), and corticotropin-releasing hormone (100 μg). A chemiluminescent enzyme immunoassay (Lumipulse f or Lumipulse Presto α, Fujirebio Inc.) was used to measure TSH (normal range 0.54–4.26 μIU/ml), FT4 (normal range 0.72–1.52 ng/dl), and FT3 (normal range 2.29–4.17 pg/ml). The α-subunit was also measured using a commercial immunometric assay (normal range 0.16–0.36 ng/ml in males, 0.04–0.66 ng/ml in females). The tumor was considered to be TRH-responsive if the TSH level increased more than twice the basal level in response to the TRH stimulation test. Cosecretion of GH was identified by supranormal IGF-1 levels and a lack of GH suppression in response to a 75-g glucose tolerance test. Associated hyperprolactinemia was identified when tumor cells showed prolactin immunopositivity, whereas an immunonegative response to prolactin was considered a stalk-section effect. GH hypersecretion was considered to be in complete remission when fulfilling the conditions of a normal basal GH level, normal GH suppression (GH nadir < 0.4 ng/ml) during glucose tolerance testing, and normal IGF-I levels based on age and sex.[12] GH, IGF-1, and prolactin assays were performed according to our previously reported methods.[37] Octreotide (100 μg administered subcutaneously) or bromocriptine (2.5 mg by mouth) tests were performed to investigate TSH responses to somatostatin analogs or dopamine agonists. TSH was considered suppressed if it decreased to less than 50% of the basal level.

Plasma TSH, FT3, and FT4 levels were measured 2–4 times during the 2-week hospital stay after surgery and at 3 and 6 months after surgery. Patients were followed up every 6 months at our outpatient department or by the referring hospitals.

MRI Study and Tumor Size

MRI studies were obtained before and after surgery and once a year thereafter using a 1.5- or 3.0-T scanner with injection of gadolinium. The maximum diameter of each tumor was measured, and the tumors were classified as microadenomas (maximum diameter < 10 mm) or macroadenomas (maximum diameter ≥ 10 mm). Tumor volume was calculated according to the Di Chiro and Nelson formula: volume = height × length × width × 0.5233.[20] Lateral tumor growth was assessed by Knosp classification.[16] A thyroid ultrasound study was also performed preoperatively in some patients to assess the condition of the thyroid gland.

Surgical Procedures

In all cases, surgery was performed by the same surgeon (S.Y.), who has performed more than 2500 pituitary surgeries. The same transsphenoidal surgical procedure was basically used for each case. Endoscopy was employed as an adjuvant to microscopic surgery after the year 2000, and purely endoscopic surgery has been advocated since 2012. The tumors were aggressively attacked and as much tumor tissue was removed as possible, even in the presence of cavernous sinus invasion, by wide opening of the sella to expose the floor of the sinus regardless of microscopic or endoscopic approach. Our surgical procedure for removing these tumors was similar to that for removing intracranial meningiomas. If the tumor had a pseudo-capsule or was firm elastic or hard in consistency,

it was carefully dissected from the surrounding normal pituitary and/or medial wall of the cavernous sinus and shrunk by blunt or sharp piece-by-piece intracapsular removal to enhance further dissection of tumor from the surrounding normal structures (Fig. 1). Cavernous sinus invasion can be roughly divided into 2 types: partial invasion and diffuse invasion. Tumor invades the medial wall of the cavernous sinus and protrudes into it to some extent in the former type, and such partial cavernous sinus invasion was strictly confirmed in this study by direct observation of the entire medial wall when we dissected and excised tumor from the medial wall of the cavernous sinus. In contrast, tumor extends into the lateral cavernous sinus compartment and encases the internal carotid artery completely in the latter type, which corresponds to tumors classified as Knosp Grade 4 on MRI and easily judged as invading the cavernous sinus. In addition, we performed extended transsphenoidal surgery or a simultaneous transsphenoidal and transcranial combined approach to treat large/giant multilobulated invasive adenomas.[25]

Criteria for Remission and Recurrence

Patients were considered to be in complete remission if the tumor was completely removed during surgery, as determined by postoperative MRI, and the serum TSH, FT3, and FT4 levels were normalized. In cases of associated cosecretion of GH and/or prolactin, remission was not considered to be complete if GH and/or prolactin levels were not normalized, even if the case fulfilled the criteria of remission of TSH-secreting adenoma. The patient was considered to have a recurrence if clinical, biochemical, and/or neuroimaging signs of tumor activity were detected after complete remission.

Histological and Immunocytochemical Studies

Specimens were obtained during surgery and fixed in 10% buffered formaldehyde, dehydrated in graded ethanol, embedded in paraffin, and studied using routine histological methods such as immunohistochemistry. Microsections were stained with hematoxylin and eosin. Immunocytochemical studies were performed as previously described.[36] Antigen retrieval was not performed routinely for immunostaining of anterior pituitary hormones. However, specimens from 3 tumors that showed negative immunostaining for TSH underwent additional proteinase K treatment (DAKO, 1:50, 9 minutes). Ki-67 labeling was performed using antibodies (MIB-1; clone 30–9 [product no. 790–4286, Ventana]) for assessing the aggressiveness of the tumor. We counted a mean of 1000 tumor cells per case, and the results were expressed as a percentage of tumor cells with positively stained nuclei. Only nuclei with a strong positive label were counted.

Statistical Analysis

Data were expressed as mean ± SD. Statistical analysis was performed using JMP software (version 9.0.2, SAS Institute Inc.). Logistic regression analysis was performed for all continuous data. Categorical variables were analyzed using chi-square tests. Multivariate stepwise logistic regression analysis was performed to detect independent predictors of complete remission rate using factors that had significant associations in univariate analysis. A p value less than 0.05 was considered statistically significant.

Results

Patient Characteristics

Demographic and clinical characteristics of our patients, including tumor characteristics, are summarized in Table 1. The median age of our patients at the time of surgery was 42 years (range 11–74 years). Forty-seven patients (52%) were female. Signs and symptoms of hyperthyroidism were found in 83 patients (Table 1). Six patients had been diagnosed with primary hyperthyroidism before being referred to our hospital. One of these patients had a previous history of radioactive iodine thyroid ablation, and 5 had undergone antithyroid agent treatment. Visual disturbance was noted in 13 patients (14%). Acromegalic features were observed in association with hyperthyroidism in 14 patients (16%), and menstrual irregularity was found in 16 of 30 female patients less than 50 years old. Of the 11 cases of hyperprolactinemias, 6 were due to hypersecretion of prolactin from the tumor itself. Multiple endocrine neoplasia was noted in only 1 patient (1%).

Sixteen patients (18%) had a microadenoma, and 74 patients (82%) had a macroadenoma (Table 1). Microadenomas occurred more frequently in the more recent half of our cases (occurring in 12 of 45 cases) than in the first half of our cases (occurring in 4 of 45) ($p = 0.0274$). Most

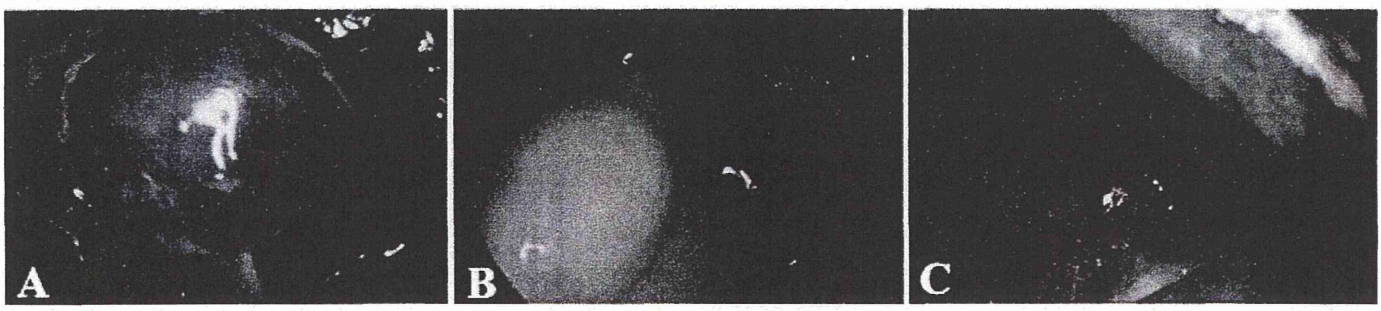

Fig. 1. Endoscopic resection of a TSH-secreting macroadenoma (14 × 12 × 10 mm) in a 50-year-old man. **A:** Intraoperative view showing a fibrous hard tumor. **B:** Dissection of the tumor from the surrounding normal pituitary. The tumor volume was gradually decreased. **C:** Image obtained after removal of the tumor, showing complete resection.

TABLE 1: Clinical and demographic characteristics of 90 patients with TSH-secreting pituitary adenomas*

Variable	Value
age in yrs	
median	42
range	11–74
IQR	33–55
sex	
female	47
male	43
previous thyroidectomy†	1
previous medication‡	5
thyrotoxicosis	83§
palpitation, tachycardia	46/83 (55%)
arrhythmia	11/83 (13%)
cardiac failure	3/83 (4%)
general fatigue	23/83 (28%)
weight loss	18/83 (22%)
excessive sweating	34/83 (41%)
headache	19/83 (23%)
tremor	15/83 (18%)
psychotic state	3/83 (4%)
goiter	44/52 (85%)¶
hypertension	8 (10%)
menstrual disorder	16/30 (53%)**
acromegaly	14 (16%)
hyperprolactinemia	11 (12%)
PRL level in ng/ml††	
median	59.7
IQR	49.7–76.4
visual disturbance	13 (14%)
tumor characteristics	
microadenoma	16 (18%)
macroadenoma	74 (82%)
max diameter in mm	
median	16
IQR	10–25
tumor vol in cm^3	
median	1.3
IQR	0.3–3.6
cavernous sinus invasion	21 (23%)
Knosp grade	
0	36
1	24
2	14
3	8
4	8
elastic firm or hard mass	67 (74%)
multiple endocrine neoplasms	1 (1%)

(continued)

TABLE 1: Clinical and demographic characteristics of 90 patients with TSH-secreting pituitary adenomas* ***(continued)***

* PRL = prolactin.
† Radioactive iodine thyroid ablation.
‡ Past history of antithyroid drug administration under the diagnosis of primary hyperthyroidism.
§ Seven patients showed no signs or symptoms of thyrotoxicosis.
¶ Ultrasonographic examination of the thyroid was performed in 52 cases.
** Only 30 female patients were less than 50 years old.
†† Number of tumors with PRL hypersecretion from tumor that was confirmed by histology.

macroadenomas were intrasellar or they extended up to the chiasmatic cistern. Tumor invasion into the cavernous sinus was confirmed in 21 patients (23%) during surgery. Sixty-seven tumors (74%) were firm elastic or hard in consistency.

Baseline Laboratory Findings

Syndrome of inappropriate TSH secretion (SITSH) (normal or high serum TSH level despite high serum FT4 and/or FT3 levels) was found in 87 patients. Of the 3 patients with euthyroidism, one had taken an antithyroid drug (TSH level 98.4 μIU/ml), one had been treated with thyroid hormone after radioactive I^{131} thyroid ablation, and one had undergone repeat surgery for the regrowth of a TSH-secreting adenoma that had been surgically treated in another hospital. Excluding these 3 patients, the median TSH level was 3.08 μIU/ml (interquartile range [IQR] 1.82–4.33, normal range 0.54–4.26). TSH was above normal in 26 patients (30%) and normal in 61 patients (70%). The median FT4 level was 2.11 ng/dl (IQR 1.71–2.73 ng/dl, normal range 0.72–1.52 ng/dl) and FT3 was 5.85 pg/ml (IQR 4.85–7.81 pg/ml, normal 2.29–4.17 pg/ml). Serum α-subunit levels were measured in 58 patients and were above normal in 44 (76%). There were no significant correlations between serum TSH values and FT4 levels (coefficient of correlation 0.58), TSH values and tumor volumes (coefficient 0.16), or FT4 levels and tumor volumes (coefficient 0.02). Six (13%) of 45 patients had a TSH response (more than twice the basal level) to TRH loading and 19 patients had a TSH response more than 1.5 times the basal level. TSH decreased to less than 50% of the basal level in 49 (71%) of 69 patients administered 100 μg octreotide subcutaneously and 4 (17%) of 24 patients administered 2.5 mg bromocriptine orally.

Preoperative Medical Treatment

Somatostatin analogs have been commercially available since July 1989, but they have not been approved as a treatment for TSH-secreting adenoma in Japan. Therefore, somatostatin analogs were not administered in some cases because of economic reasons. Moreover, they were also not used in cases of small adenomas associated with a slight elevation of serum FT4 level and no marked signs and symptoms of thyrotoxicosis. As a result of a variety of circumstances as mentioned above, preoperative octreotide treatment was administered in 48 cases in this

series. In 25 of these 48 cases, the patients were treated with short-acting octreotide (administered subcutaneously 3 times a day, total daily dose 300 μg, for 1 week before surgery), and in the other 23 cases, they were treated with long-acting octreotide (administered intramuscularly at a dosage of 20 mg either 1 month before surgery or 1 and 2 months before surgery). In an additional case, treatment with long-acting octreotide was not initiated because the patient experienced severe abdominal pain after subcutaneous injection of octreotide. In the 48 patients who received octreotide, the median duration between the start of octreotide treatment and surgery was 34.1 days. Preoperative normalization of FT4 was achieved in 40 (83%) of the 48 patients treated with octreotide and tumor shrinkage by more than 20% was also found in 24 (55%) of 44 patients in whom pre- and post-treatment MR images were available. Stratification by presence or absence of hormonal normalization or tumor shrinkage showed no significant difference in pretreatment serum TSH ($p = 0.93$ for hormonal normalization, $p = 0.16$ for tumor shrinkage) and FT4 levels ($p = 0.77$, $p = 0.17$), existence of GH cohypersecretion ($p = 0.34$, $p = 0.73$), result of octreotide loading test ($p = 0.06$, $p = 0.78$), tumor size (maximum tumor diameter) ($p = 0.51$, $p = 0.27$), and tumor consistency ($p = 0.32$, $p = 0.20$). Patients who could not be treated with or did not respond sufficiently to preoperative octreotide were treated with antithyroid drugs and/or inorganic iodine a few days before surgery to prevent development of perioperative thyroid storm.

Surgical Outcomes

A total of 84 patients received primary surgery, and 6 underwent repeated surgery due to the recurrence or persistence of the tumor (after primary surgery performed in another hospital). Conventional transsphenoidal surgery, extended transsphenoidal surgery, or a simultaneous combined supra- and infrasellar approach was performed in 85, 2, and 3 patients, respectively (Figs. 1 and 2). Surgery was defined as successful if total removal of the tumor and complete endocrinological remission were achieved. By this definition, surgery was successful in 76 patients (84%), including all 16 (100%) of the patients with microadenomas (including one with cavernous sinus invasion), 60 (81%) of the 74 patients with macroadenomas, and 8 (38%) of the 21 patients with cavernous sinus invasion. With respect to Knosp grade, surgery was successful in 34 (94%) of 36 patients with Knosp Grade 0 tumors, all 24 (100%) of those with Grade 1 tumors, 12 (86%) of the 14 with Grade 2 tumors, 6 (75%) of the 8 with Grade 3 tumors, and none (0%) of the 8 with Grade 4 tumors. Following the successful surgeries, serum TSH levels typically decreased below the normal range within 1–3 days, reached their lowest point 1–2 weeks later, and gradually increased back to the normal range after several months. In contrast, serum FT4 levels tended to be normal or above normal during the early postoperative period (1–2 weeks) and decreased below the normal range for up to 6 months after surgery. Postoperative thyroid hormone replacement (50 μg/day levothyroxine sodium hydrate) was needed in 11 (14%) of the 76 patients with successful surgery; however, it was needed only on a temporary basis in 9 of these cases. Only 2 patients still required thyroid hormone replacement at final follow-up. No recurrence of the tumor and/or reappearance of SITSH occurred in the 76 patients in whom surgery was successful (median follow-up period 2.8 years).

The surgeries in the remaining 14 patients were considered to be unsuccessful because the criteria for remission were not met. The details of clinical characteristics and follow-up results of these 14 patients are summarized in Table 2. One of these 14 patients (Case 7) died of malignant hyperthermia syndrome 2 weeks after a simultaneous combined approach for the treatment of her lobulated giant adenoma (no cavernous sinus invasion, Knosp Grade 0). Eight patients had a pure TSH-secreting adenoma, 5 had a TSH- and GH-secreting adenoma, and one had a TSH- and prolactin-secreting adenoma. Cavernous sinus invasion was diagnosed in all but 1 case, although in another case (Case 10), the tumor was assessed as Knosp Grade 0 on the basis of the preoperative MRI study. Euthyroidism was achieved in 10 patients in this group of 14, although in 9 of these 10 cases postoperative MRI showed some residual tumor in either the cavernous sinus (in 8 cases) or the suprasellar region (in Case 7); the patient in Case 10 had no residual tumor. Postoperative endocrine testing showed persistence of abnormal GH dynamics in 4 of the 5 patients (Cases 10–13 in Table 2) with TSH- and GH-secreting adenomas, although testing demonstrated euthyroidism postoperatively in 4 cases also (Cases 9, 10, 12, and 13). In 11 of 14 cases, the patients received adjuvant treatments such as postoperative medication and/or radiation. Residual tumor masses and hypersecretion of hormones were controlled adequately in 9 patients, whereas SITSH was still present in 1 patient (Case 2) and a supranormal IGF-1 level persisted in 1 patient (Case 13) following the treatments. Of the surviving 2 patients who did not undergo adjuvant treatment postoperatively (Cases 5 and 6), one did not experience tumor regrowth or SITSH (Case 5), whereas the other (Case 6) showed SITSH (slight elevation of FT4 levels) 2.5 years after surgery.

Analysis of Preoperative Factors Influencing Surgical Results

The association between preoperative variables (age, sex, GH level, year of surgery, concomitant GH secretion, preoperative TSH, FT3, and FT4 levels, visual disturbance, tumor size, cavernous sinus invasion, Knosp grade, and tumor consistency) and surgical outcome is presented in Tables 3 and 4. The patients were classified into 2 groups based on the surgical results: a successful-surgery group (76 patients), in which the criteria for cure were fulfilled, and an unsuccessful-surgery group (14 patients). Univariate analysis showed that cosecretion of GH, visual disturbance, tumor size, Knosp grade, and cavernous sinus invasion were significantly correlated with surgical outcome, whereas age, sex, year of surgery, tumor consistency, and preoperative serum TSH, FT3, and FT4 levels were not. According to multivariate analysis using the logistic regression model, cavernous sinus invasion was the strongest predictor of the surgical outcome, although tumor size was also an independent predictor.

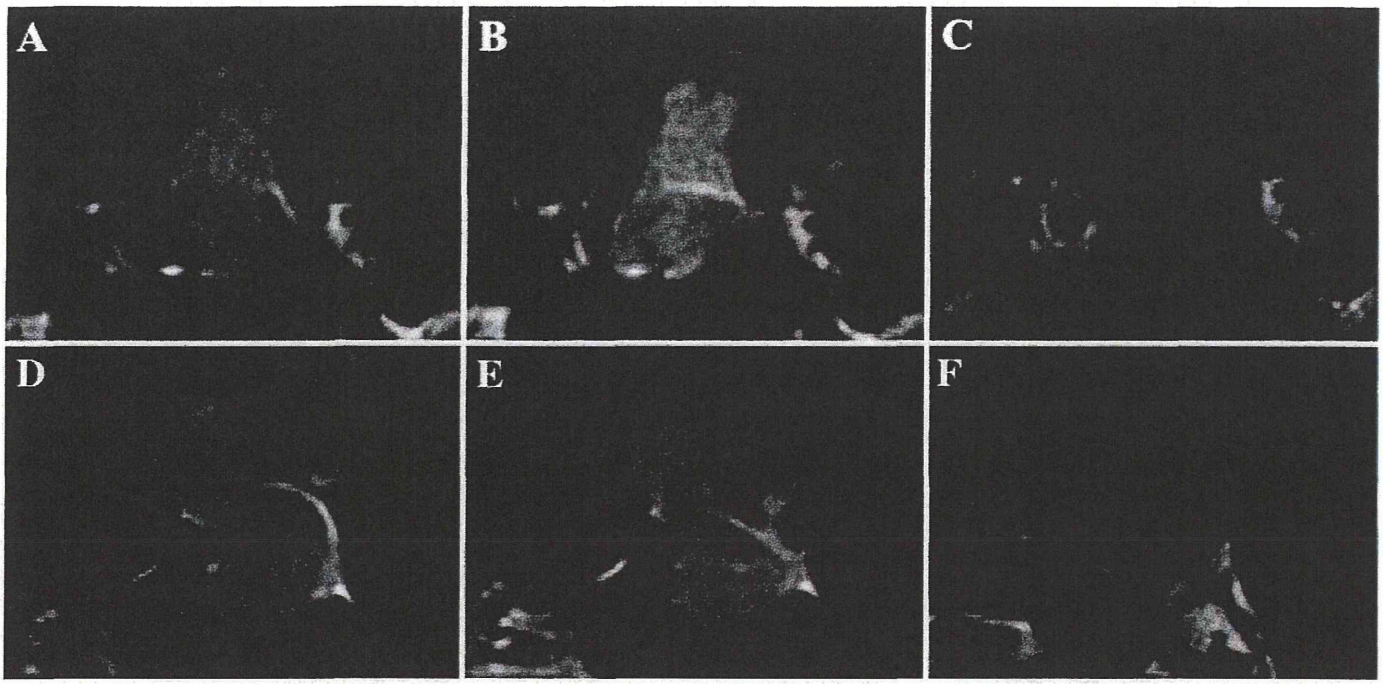

Fig. 2. Coronal (A–C) and sagittal (D–F) T1-weighted contrast-enhanced MR images obtained in a 43-year-old man with SITSH and GH hypersecretion. **A and D:** Initial images show a macroadenoma with suprasellar extension anteriorly. **B and E:** Images obtained after 2 treatments with a long-acting formulation of octreotide showing tumor shrinkage. The patient's visual disturbance also improved. **C and F:** Images obtained after complete tumor resection by extended transsphenoidal surgery and normalization of hormone hypersecretion.

Surgical Complications

One patient died of malignant hyperthermia syndrome 2 weeks after surgery despite appropriate intensive care. In addition, 3 patients died during the follow-up period of conditions unrelated to the adenoma surgery (liver insufficiency, cerebral infarction, and lung cancer, with death occurring 3, 4, and 6 years after surgery, respectively). Postoperative CSF leak occurred in 3 patients. One documented case of meningitis (1.1%) occurred secondary to the CSF leakage and 2 patients required reoperation for repair of CSF leakage. Diabetes insipidus occurred in 10 patients (11%) in the immediate postoperative period and was permanent in 2 (2.2%) patients, who required long-term desmopressin treatment. Nine patients developed delayed hyponatremia postoperatively due to inappropriate antidiuretic hormone (ADH) secretion. One patient had anosmia and one had acute sinusitis after surgery.

Postoperative Pituitary Function

Follow-up pituitary function was investigated in all but the 1 patient who died 2 weeks after surgery. A total of 74 (83%) of 89 patients remained eupituitary after surgery. Of the 15 patients with pituitary impairment, 5 patients were considered to have panhypopituitarism, 6 had GH deficiency, 2 had LH/FSH and adrenocorticotropic hormone (ACTH) deficiency, 1 had ACTH deficiency, and 1 had ADH deficiency.

Histological Findings

Specimens from all but 3 of our patients showed positive immunostaining for TSH, and pretreatment with proteinase K led to positive immunostaining in the 3 TSH-negative cases. GH, prolactin, and both GH and prolactin were detected in specimens from 32, 9, and 24 patients, respectively. Ki-67 immunohistochemistry was carried out in 73 cases, and the Ki-67 labeling index was less than 1% in 40 patients (55%), between 1% and 3% in 31 patients (42%), and greater than 3% in 2 patients (3%). The Ki-67 labeling index did not differ significantly between successful and unsuccessful surgical outcomes ($p = 0.257$) or noninvasive and invasive tumors ($p = 0.460$).

Discussion

Clinical Characteristics

According to Beck-Peccoz et al., the number of reported TSH-secreting adenomas has tripled in the last decade, primarily due to the introduction of ultrasensitive TSH immunometric assays and improved practitioner awareness.[3,4] TSH-secreting tumors account for less than 2% of all pituitary adenomas, according to some reports.[1,27] However, a recent epidemiological investigation in Sweden showed an increased incidence of TSH-secreting adenoma from 0.05 per 1 million per year in 1990–1994 to 0.26 per 1 million per year in 2005–2009.[27] These tumors represented only 2.7% of 3276 pituitary adenomas that were surgically treated at our hospital during the overall study period. However, the proportion of tumors that were TSH-secreting adenomas increased to 4.0% (54 of 1351 cases) during the last 5 years of our study period. The increase in the number of surgical cas-

TABLE 2: Clinical characteristics and follow-up results in 14 patients with unsuccessful surgery*

Case No.	Age (yrs), Sex	Tumor Type	Max Tumor Size (mm)	CSI (KG)	Surgery	Surgical Outcome			
						Thyroid Function	GH/PRL	Residual Tumor	
1	66, F	TSH	22	+(4)	TSS	SITSH	−	+	octreot
2	56, F	TSH	15	+(4)	TSS	euthyroid	−	+	GKS
3	46, F	TSH	29	+(4)	TSS	SITSH	−	+	octreot
4	34, M	TSH	20	+(4)	TSS	euthyroid	−	+	Cyberk
5	40, F	TSH	30	+(3)	TSS	euthyroid	−	+	none
6	31, F	TSH	26	+(4)	TSS	euthyroid	−	+	none
7	51, F	TSH	46	−(0)	combined	SITSH	−	+	none
8	42, F	TSH	41	+(2)	combined	euthyroid	−	+	octreot
9	51, M	TSH+GH	29	+(4)	combined	euthyroid	CR	+	Cyberk
10	50, M	TSH+GH	30	+(0)	TSS	euthyroid	NC	−	octreot
11	28, F	TSH+GH	47	+(4)	TSS	SITSH	NC	+	octreot
12	25, M	TSH+GH	57	+(4)	TSS	euthyroid	NC	+	octreot
13	40, F	TSH+GH	29	+(3)	TSS	euthyroid	NC	+	caberg
14	20, M	TSH+PRL	31	+(2)	TSS	euthyroid	CR	+	Cyberk

* Combined = simultaneous combined supra- and infrasellar approach; CR = complete remission of GH or prolactin hypersecretion; CSI = cavern Knife surgery; KG = Knosp grade; NC = not complete remission; octreotide = long-acting octreotide; peg = pegvisomant; SITSH = syndrome of ina

† The patient died of cerebral infarction 4 years after surgery.

‡ The patient died of malignant hyperthermia syndrome 2 weeks after surgery.