CLINICAL STUDY

Relationship between cytokeratin staining patterns and clinico-pathological features in somatotropinomae

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Abstract

Objective: Somatotropinomae are classified as densely and sparsely granulated adenomae, which typically exhibit a perinuclear pattern (PP) and a dot pattern (DP) in cytokeratin (CK) immunostaining respectively. Some exhibit a mixed pattern (MP). We studied the relationship between these somatotropinoma subtypes and their clinico-pathological features.

Methods: The study population consisted of 141 Japanese acromegalic patients. We evaluated their clinical presentation and their response to provocation tests with TRH and LHRH and to suppression (octreotide) test. Tumour tissues were subjected to immunostaining for CAM-5.2, MIB-1, CD34, E-cadherin (CDH1) and p53 (TP53). In 43 cases (30 non-DP and 13 DP), we analysed *gsp* mutations (constitutively activating mutations of the $G_s\alpha$ protein that is encoded by *GNAS* gene).

Results: The 141 adenomae were categorised into three subtypes based on their CK staining patterns; 30 (21.3%) exhibited DP, 83 (58.9%) exhibited PP, and 28 (19.9%) exhibited MP. Compared with the other subtypes, DP adenomae were significantly larger, and their E-cadherin expression and response to TRH, LHRH and octreotide challenge were lower. The postoperative cure rate tended to be lower in DP adenomae. *gsp* mutations were detected in 25 of 43 cases examined (58.1%); 20 of the 30 non-DP (66.7%) and 5 of the 13 DP tumours (38.5%) were affected by the mutation.

Conclusion: DP somatotropinomae exhibit characteristic features. Compared with the non-DP subtypes, DP adenomae manifested a larger tumour size, a lower incidence of abnormal responses to TRH and LHRH challenge, a poor response to octreotide test and a lower expression of E-cadherin. *gsp* mutation was not exclusive for non-DP somatotropinomae.

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Introduction

Human pituitary adenomae are benign neoplasms that account for about 15–20% of all intracranial neoplasms (1–3). Based on their electron microscopic features, somatotropinomae or GH-producing adenomae (GHomae) that produce acromegaly and gigantism are classified into two subtypes (1, 4, 5), i.e. densely and sparsely granulated (DG and SG) tumours. The DG subtype manifests abundant closely packed secretory granules of large to medium size. Most tumour cells are polyhedral or elongated with spherical euchromatic nuclei containing conspicuous nucleoli. In the cytoplasm, well-developed Golgi apparatus harbouring immature developing granules and elongated cristae in the mitochondria are observed (1, 4, 5). These features are distinct from SG subtype adenoma cells whose cytoplasm contains scarcely distributed small secretory granules and fibrous bodies. The perinuclear pattern (PP) and dot pattern (DP) in cytokeratin (CK)

immunostaining observed under light microscopy can be good surrogate for the electron microscopic features of the DG and SG subtype adenomae respectively (1, 6). However, there are some adenomae that exhibit a combined feature of PP and DP or the mixed pattern (MP) (7–11).

The fibrous body is an intracytoplasmic globular aggregation of filaments containing CK, which belongs to the intermediate filament protein family (6, 7, 12–14). Apart from its role in the formation of cell frameworks, CK has other important functions, and disorganised CK distribution may result in the disruption of cellular functions (14). The CK immunostaining pattern is related to epithelial malignancies and reflects ongoing cell activity (15). However, the molecular background and pathophysiology of the two GHoma phenotypes remain to be elucidated.

The *gsp* mutation is a somatic activating mutation of the α subunit of the heterotrimeric G_s protein encoded by the *GNAS* gene (16, 17). It is thought to be an early

event in tumourigenesis and is detected in ~40–50% of sporadic GHoma (18–22). There have been few studies on the impact of *gsp* mutations on the GHoma subtypes (19, 23); this mutation has been documented only in DG GHoma (19, 23).

In this study, we retrospectively evaluated clinico-pathological features of GHoma with their respective CK pattern in acromegalic patients. The relationships between the CK pattern and *gsp* mutation were investigated as well.

Materials and methods

Subjects

The study population consisted of 141 Japanese patients with GHoma who underwent transsphenoidal adenomectomy in our hospitals. Before surgery, none had been treated with dopamine receptor subtype-2 (D_2) agonists, somatostatin analogues (except for the single challenge test) or irradiation.

Acquisition of clinical data

Clinical data and the patient's age and sex, symptoms, hormonal profiles and tumour morphologies were obtained from clinical records and preoperative magnetic resonance imaging (MRI) scans.

Hormonal evaluation

Plasma GH and insulin-like growth factor 1 (IGF1) levels were measured for routine preoperative endocrine evaluation. The GH responses to TRH (500 µg, i.v.), LHRH (100 µg, i.v.) and octreotide (50 µg, s.c.), expressed as the ratio (%) of the maximum or minimum GH level to the basal GH level, were evaluated on different days. An abnormal response to TRH or LHRH was defined as an increase in the GH level to 200% or more of the baseline value. A good response to the octreotide test was defined as a $\geq 90\%$ or greater decrease in the GH level from the baseline during testing. Postoperative cure was defined according to the Cortina consensus criteria (24), i.e. as a GH nadir during postoperative oral glucose tolerance test (OGTT, < 1 ng/ml) and normalisation of serum IGF1 based on the standard value for the Japanese population. Of the 141 patients, 127 underwent postoperative OGTT.

Imaging studies

The maximum tumour diameter was measured on preoperative MRI scans. The tumour volume (cm^3) was estimated using the formula: $0.5 \times \text{width} \times \text{length} \times \text{height}$ (25). Cavernous sinus invasion by the tumour was judged to be present when the preoperative MRI scan showed an internal carotid artery surrounded by the tumour (Knosp's grade IV lateral growth) (26) or

when there was intraoperative microscopic and/or endoscopic evidence of disruption of the medial wall of the cavernous sinus and lateral extension of the tumour through the window.

Patients with a documented history of pituitary apoplexy were excluded from this study. GH-producing indices were calculated based on the ratio of the preoperative GH level (ng/ml) per cm^3 of the tumour volume (ng/ml per cm^3) (8).

Histological studies

Surgically isolated adenomae tissues were fixed in 10% formaldehyde and embedded in paraffin before cutting into thin slices (3 µm thick) for light microscopy and light microscopic immunohistochemistry. The 3 µm thick sections were stained with haematoxylin and eosin (H&E) reagent (Figs 1A and 2A). Although we had intended to exclude acidophilic adenomae with hyperprolactinaemia characterised by clear cytoplasmic vacuoles and dot-like CK positivity to eliminate possible contamination with acidophilic stem cell adenomae (1), we could not identify this rare type of adenomae. Immunohistochemistry was performed with the avidin–biotin–peroxidase complex method, where diaminobenzidine was used for antigen visualisation. The specific antibodies are listed in Table 1. Microwave antigen retrieval was performed in citrate buffer (pH 6.0). Positivity for CAM-5.2, anti-CK reagent, was classified into three patterns: i) DP (dot-like CK positivity limited to fibrous body), ii) PP (diffuse CK-positive response in cytoplasm mainly with perinuclear preference) and iii) MP (adenomae with various mixed ratios of

Figure 1 Histological examination of a somatotropinoma (GHoma) with a dot pattern (DP) in a 47-year-old woman. Bar: 50 µm. (A) Haematoxylin and eosin (H&E) staining revealed that the neoplastic cells were acidophilic. (B) CAM-5.2 immunostaining was used to categorise this tumour as a DP subtype based on the intracytoplasmic globular aggregation of intermediate filaments containing cytokeratin. (C) The tumour showed diffuse immunoreactivity with GH. (D) The tumour did not display membranous expression of E-cadherin.

Figure 2 Histological examination of a GHoma with a perinuclear staining pattern (PP) in a 57-year-old man. Bar: 50 µm. (A) H&E staining revealed that the tumour was acidophilic. (B) CAM-5.2 immunostaining was used to categorise this tumour as a PP subtype based on the perinuclear distribution of cytokeratin. (C) The tumour was diffusely immunoreactive with GH. (D) Membranous immunoreactivity of E-cadherin was diffusely observed in this tumour.

DP and PP). Cells immunoreactive to GH, prolactin (PRL), TSH and p53 were counted and expressed as a percentage of stained cells of more than 500 adenomae cells at 400× magnification. Cells immunoreactive to E-cadherin (CDH1) were also quantitatively assessed and expressed as a percentage of cells that stained positive on the plasma membrane. The microvascular density (MVD) was assessed by counting the number of lumen with CD34 positive staining at 400× magnification. MIB-1 index reflecting proliferating cell ratio was calculated from more than 500 cells.

gsp gene mutation study

For *gsp* mutation analysis, 43 frozen GHoma specimens were available. Their number was restricted because in some cases the amount of tumour tissue was too small

Table 1 List of antibodies used for immunohistochemical detection.

Antibodies agents	Sources	Nature of antibodies	Dilutions
CAM-5.2	Becton Dickinson ^a	Monoclonal	— ^b
GH	DAKO ^c	Polyclonal	1:100
PRL	Immunotech ^d	Monoclonal	1:100
βTSH	Immunotech	Monoclonal	1:100
KI-67	DAKO ^c	Monoclonal	1:50
CD34	DAKO ^c	Monoclonal	— ^b
E-cadherin	Cell Signalling ^e	Monoclonal	1:25
p53	Beckman Coulter ^d	Monoclonal	— ^b

^aFranklin Lakes, NJ, USA.
^bNo dilution.
^cGlostrup, Denmark.
^dMarseille, France.
^eDanvers, MA, USA.

to be prepared as frozen sample or because protocols for the acquisition of frozen tissues were not uniform. Two authors (K T and J Y-T), blinded to clinical and pathological data, subjected the available frozen tissues to mutation analysis. The mean diameter of the 43 tumours used for mutation analysis and of all 141 tumours was similar (19.6 ± 8.1 mm (s.d.) and 18.7 ± 9.6 mm respectively).

The specimens were preserved under -80°C until RNA extraction. Total RNA contents were extracted from the adenomae tissues, and the first-strand cDNA was synthesised from 5 µg total RNA by RT-PCR after removing contaminating genomic DNA as reported previously (18). To amplify the specific G_{α} -subunit cDNA fragment (249 bp), including codons 201 and 227 of the G_{α} gene, we designed a sense primer and an antisense primer corresponding to nucleotide sequences 562–585 (5′-GTGTATCAAGCAGGCTGACTATGTG-3′) and 793–819 (5′-CAGGCGTTGTTCTGGTT-3′) respectively. Using these primers, the PCR was performed to amplify the aforesaid first-strand cDNA, although the temperature for annealing was changed to 60°C for 40 cycles. The PCR product was purified and directly sequenced using ABI PRISM software (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

The SPSS software program (version 15.0, IBM Co., Chicago, IL, USA) was used for statistical analysis of the results. The data was analysed by the contingency *C* test followed by Fisher’s exact test, the Kruskal–Wallis test and/or the Mann–Whitney *U* test where appropriate. Differences of $P < 0.05$ were considered statistically significant.

Ethical considerations

This retrospective study was approved by the Kagoshima University Hospital Ethical Committee (reference no: 2008-144).

Results

As we found that the clinical features of MP and PP adenomae were similar, we present our findings as comparisons between DP and non-DP (i.e. combined MP and PP) GHomae.

Patient profiles

The gender distribution in the 141 patients, 64 males and 77 females, was comparable; their age ranged from 9 to 75 years (48.9 ± 13.5 , mean $\pm$ s.d.; Table 2). The mean preoperative GH level was 62.1 ± 183.7 ng/ml. Abnormal responses to TRH and LHRH tests were observed in 75 and 16.7% of 128 patients respectively; 12 (30.8%) of 39 patients had a good response to the

Table 2 Demographic and clinical data of subjects.

Variable	
Sex	Male 64, female 77
Age (years)	48.9 ± 13.5
Preoperative GH level (ng/ml)	62.1 ± 183.7
Tumor diameter (mm)	18.7 ± 9.6
Microadenomae	21 (14.9%)
Macroadenomae	120 (85.1%)
Tumor volume (cm ³) ± s.d.	6.6 ± 13.1
GH-producing index (ng/ml per cm ³)	21.9 ± 36.1
TRH test (<i>n</i> : 128)	
Abnormal response	96 (75%)
Normal response	32 (25%)
LHRH test (<i>n</i> : 128)	
Abnormal response	21 (16.7%)
Normal response	107 (83.3%)
Octreotide test (<i>n</i> : 39)	
Good response	12 (30.8%)
Poor response	27 (769.2%)
Surgical outcome (<i>n</i> : 127)	
Cured	72 (56.7%)
Non-cured	55 (43.3%)

Abnormal response, increases of the GH level to 200% or more; a good response, ≥90% decreases in GH levels from the preoperative basal GH level during examination; cured, nadir GH during glucose tolerance test <1.0 ng/ml and normalised IGF1 level (based on standard value for normal Japanese population).

octreotide test. Of the 141 patients, 21 (14.9%) manifested microadenomae, the other 120 (85.1%) harboured macroadenomae. Postoperative cure was obtained in 72 (56.7%) of 127 patients who underwent postoperative OGTT.

CK patterns and clinical features

Based on the classification criteria described in Materials and methods, of the 141 tumours, 30 (21.3%) were DP (Fig. 1A and B), 83 (58.9%) were PP (Fig. 2A and B), and 28 (19.9%) were MP subtype tumours (Table 3). The mean age tended to be lower in patients with DP adenomae than those with non-DP adenomae (45.0 ± 16.1 vs 50.1 ± 12.6 years, $P=0.098$). The mean maximal tumour diameter of DP, PP and MP adenomae was 23.5 ± 11.5 , 17.5 ± 8.9 and 16.8 ± 8.2 mm respectively; DP GHomae were significantly larger than non-DP tumours ($P=0.006$). The volume of DP GHomae was also significantly larger than that of non-DP adenomae (11.7 ± 18.4 vs 5.3 ± 10.9 cm³, $P=0.006$). GH-producing index tended to be lower in DP adenomae (17.3 ± 22.2 ng/ml per cm³) than in non-DP adenomae (23.2 ± 39.1 ng/ml per cm³) ($P=0.145$). On the other hand, with respect to tumour invasiveness, there was no significant difference between the tumour types.

Significantly more patients with non-DP tumours than those with DP tumours manifested an abnormal response to TRH (81.2 vs 51.9%, $P=0.003$) and LHRH

challenge (19.8 vs 3.7%, $P=0.034$) and a good response to octreotide (42.9 vs 0%, $P=0.008$).

Of the 127 patients who underwent postoperative OGTT, 72 (56.7%) obtained postoperative cure. Although the postoperative cure rate was better in patients with non-DP adenomae than those with DP adenomae (60.4 vs 42.3%), the difference was not statistically significant ($P=0.076$).

Immunohistochemistry

Most of the adenoma cells were diffusely immunoreactive with GH (Figs 1C and 2C; Table 4). Between DP and non-DP adenomae, there was no significant difference in the percentage of GH-immunopositive cells, nor in the percentage of cells immunopositive for other anterior pituitary hormones. The MIB-1 index was higher in DP GHomae than in non-DP GHomae (0.72 ± 1.6 vs 0.20 ± 0.39 %, $P=0.086$). The expression of MVD was almost the same between the two groups. Although not statistically significant ($P=0.101$), the expression of p53 was slightly higher in DP GHomae. Lastly, the expression of E-cadherin involving the CK network (27–29) was significantly ($P<0.001$) lower in DP GHomae than in non-DP GHomae (Figs 1D and 2D).

gsp mutations

The mutation was detected in 25 of 43 (58.1%) tumours examined. Incidence of *gsp* mutation tended to be higher in non-DP (66.7%) adenomae than in DP (38.5%) adenomae ($P=0.083$; Table 5). There were four different mutations; they were Arg to Cys in codon 201 (68%), Arg to Ser in codon 201 (8%), Gln to Leu in codon 227 (20%) and Gln to Arg in codon 227 (4%).

The clinico-pathological features (including age, sex, basal GH level and MIB-1 index) of the 43 patients whose tumours were subjected to mutation analysis were not significantly different, irrespective of their mutation status. Also, there was no significant difference with respect to immunoreactivity to GH, PRL or TSH (data not shown). On the other hand, the tumour size was significantly smaller ($P=0.006$) and the GH-producing index was significantly larger ($P=0.007$) in adenomae with *gsp* mutations than those without *gsp* mutations. In addition, while all tumours with the mutation manifested an abnormal response to TRH challenge, this was the case in 50% of adenomae without *gsp* mutations ($P<0.001$). We observed a good response to octreotide in 44.4% of adenomae with *gsp* mutations and in 18.2% of tumours without the mutation; the difference was not significant ($P=0.217$), possibly because our sample size of cases who underwent octreotide test ($n=20$) was too small for statistical evaluation.

Table 3 Summary of clinico-pathological data.

	Adenomae type			<i>P</i> value	non-DP adenomae (n=111)	<i>P</i> value (DP versus non-DP)
	DP (n=30)	MP (n=28)	PP (n=83)			
Age (years; mean $\pm$ s.d.)	45.0 $\pm$ 16.1	48.7 $\pm$ 12.9	50.6 $\pm$ 12.5	0.228 ^a	50.1 $\pm$ 12.6	0.098 ^b
Sex						
Male	11 (36.7%)	13 (46.4%)	40 (48.2%)	0.550 ^c	53 (47.7%)	0.273 ^d
Female	19 (63.3%)	15 (53.6%)	43 (51.8%)		58 (52.3%)	
Basal GH level (ng/ml; mean $\pm$ s.d.)	115.7 $\pm$ 326.9	51.5 $\pm$ 65.8	46.2 $\pm$ 130.5	0.249 ^a	47.5 $\pm$ 117.3	0.184 ^b
Size (mm; mean $\pm$ s.d.)	23.5 $\pm$ 11.5	16.8 $\pm$ 8.2	17.6 $\pm$ 8.9	0.020^a	17.4 $\pm$ 8.7	0.006^b
Microadenomae	2 (6.7%)	3 (14.9%)	16 (19.3%)	0.197 ^c	19 (17.1%)	0.124 ^d
Macroadenomae	28 (93.3%)	25 (89.3%)	67 (80.7%)		92 (82.9%)	
Volume (cm ³ ; mean $\pm$ s.d.)	11.7 $\pm$ 18.4	4.6 $\pm$ 10.5	5.8 $\pm$ 11.04	0.021^a	5.3 $\pm$ 10.9	0.006^b
GH-producing index (ng/ml per cm ³ ; mean $\pm$ s.d.)	17.3 $\pm$ 22.2	23.8 $\pm$ 39.4	23.1 $\pm$ 39.2	0.255 ^a	23.2 $\pm$ 39.1	0.145 ^b
Invasion in macroadenomae ^e						
Invasive	10 (35.7%)	4 (16%)	24 (35.8%)	0.167 ^c	28 (30.4%)	0.599 ^d
Non-invasive	18 (64.3%)	21 (84%)	43 (64.2%)		64 (69.6%)	
TRH test						
Normal response	13 (48.1%)	1 (4.2%)	18 (23.4%)	0.001^c	19 (18.8%)	0.003^d
Abnormal response	14 (51.9%)	23 (95.8%)	59 (76.6%)		82 (81.2%)	
LHRH test						
Normal response	26 (96.3%)	19 (79.2%)	62 (80.5%)	0.132 ^c	81 (80.2%)	0.034^d
Abnormal response	1 (3.7%)	5 (20.8%)	15 (19.5%)		20 (19.8%)	
Octreotide test						
Good response	0	3 (50%)	9 (42.9%)	0.033^c	12 (42.9%)	0.008^d
Poor response	11 (100%)	4 (50%)	12 (57.1%)		16 (57.1%)	
Surgical outcome						
Cured ^f	11 (42.3%)	12 (52.2%)	49 (62.8%)	0.167 ^c	61 (60.4%)	0.076 ^d
Non-cured	15 (57.7%)	11 (47.8%)	29 (37.2%)		40 (39.6%)	

Non-dot pattern, perinuclear pattern+mixed pattern; GH-producing index, basal GH level per cm³ volume; bold numerals, *P* < 0.05.

^aKruskal–Wallis test.

^bMann–Whitney test.

^cContingency *C* test.

^dFisher's exact test.

^eNo microadenomae showed evident cavernous sinus invasion confirmed by intraoperative exposure.

^fCured: nadir GH during glucose tolerance test < 1.0 ng/ml and normalised IGF1 level (based on standard value for normal Japanese population).

Discussion

The classification of GHoma based on their CK immunostaining patterns results in three subtypes, i.e. DP, PP and MP tumours (7–11). We found some different clinico-pathological features in DP adenomae than in non-DP adenomae.

CK staining patterns and clinico-pathological features

The age tended to be lower in patients with DP than those with non-DP GHoma (*P* = 0.98). This finding coincides with the report of Mazal *et al.* (11) and Obari *et al.* (9). With respect to the tumour size, our findings were similar to the findings reported by Mazal *et al.* (11) who also observed that DP adenomae were significantly larger than non-DP adenomae. While the difference was not statistically significant (*P* = 0.086), the MIB-1 index, indicative of the proliferative activity of tumour cells, tended to be higher in our patients with DP adenomae than those with non-DP adenomae. According to Mazal *et al.* (11), it was significantly higher in DP tumours than in PP tumours, suggesting that the DP subtype may be

more highly proliferative. One of our patients manifested an atypical GHoma that regrew rapidly and recurred postoperatively; the MIB-1 index was ~10%, and 23% of the cell nuclei were positive for p53. Based on CK immunostaining, this tumour was classified as a DP subtype adenoma. In our study, more DP tumours than non-DP tumours expressed p53, although the difference was not statistically significant (*P* = 0.101). Our patients with DP adenomae were younger than those with non-DP tumours, possibly because the former subtype is more highly proliferative and consequently, these tumours tend to be larger. Besides excessive GH secretion, cranial nerve signs and headache may be clues for a diagnosis of acromegaly, and these non-hormonal manifestations may appear earlier in patients with DP adenomae due to their larger size.

The expression of E-cadherin was significantly lower in DP adenomae than in non-DP adenomae (*P* < 0.001); a finding that coincides with observations reported by others (8, 10). The E-cadherin cell adhesion system is involved in cell–cell interactions via coupling to cytoskeleton-associated desmosomal proteins (27). As E-cadherin is critical for the anchoring of CK filaments to the cell surface, the loss of E-cadherin expression on

Table 4 Summary of immunohistochemistry data. Results are mean $\pm$ s.d.

	Adenomae type			<i>P</i> value ^a	non-DP adenomae (n=111)	<i>P</i> value ^b (DP versus non-DP)
	DP (n=30)	MP (n=28)	PP (n=83)			
GH (%)	> 90%	> 90%	> 90%	N/A	> 90%	N/A
PRL (%)	6.6 $\pm$ 11.2	18.0 $\pm$ 21.4	11.4 $\pm$ 18.0	0.113	13.1 $\pm$ 19.1	0.226
β TSH (%)	0.19 $\pm$ 1.04	0.18 $\pm$ 0.97	1.04 $\pm$ 4.49	0.374	0.82 $\pm$ 3.92	0.371
MIB-1 index (%)	0.72 $\pm$ 1.6	0.29 $\pm$ 0.63	0.17 $\pm$ 0.25	0.162	0.20 $\pm$ 0.39	0.086
MVD	32.8 $\pm$ 23.4	27 $\pm$ 24.3	35 $\pm$ 23.7	0.365	33.4 $\pm$ 24.0	0.927
E-cadherin (%)	9.9 $\pm$ 25.1	54.3 $\pm$ 26.3	70.2 $\pm$ 35.3	<0.001	66.5 $\pm$ 33.9	<0.001
p53 (%)	1.21 $\pm$ 4.25	0.48 $\pm$ 0.79	0.28 $\pm$ 0.75	0.101	0.33 $\pm$ 0.76	0.363

MIB-1 index, percentage of positive nucleus for KI-67 staining of more than 500 cells; MVD, microvascular density; N/A, not applicable. Bold numerals, $P < 0.05$.

^aKruskal–Wallis test.

^bMann–Whitney test.

the cell membrane results in CK collapse (28) and may facilitate the formation of a fibrous body that stains with CAM-5.2 in GHomae. As the disruption of cell–cell contact is a key step in tumour progression, the loss of E-cadherin expression has been observed in various carcinomas (29). The disruption of cell–cell contact due to impaired E-cadherin expression may be a factor in the growth of DP adenomae. Other factors influencing tumour growth, such as apoptotic process, cell–cell junction and intercellular communication, should be further investigated to understand the linking mechanisms between CK expression and tumour growth.

DP adenomae did not have the tendency to be more invasive than the other types; a finding that is inconsistent with the reports by Mazal *et al.* (11) and Obari *et al.* (9), where both groups determined invasion by Knosp grading (26). However, we evaluated the invasion into cavernous sinus based on intraoperative

findings under microscopic and/or endoscopic observations in addition to preoperative MRI findings. The discrepancy may have resulted from the difference in defining ‘invasion’.

With respect to hormone production, we found no significant difference in immunoreactivity to GH, TSH and PRL between DP and non-DP adenomae. Although statistically insignificant, the GH-producing index was lower in DP adenomae than in non-DP adenomae in our study. This could have been due to the larger tumour size and smaller number and size of secretion granules (electron microscopic features) of DP adenomae than those of PP adenomae (1). Accordingly, Nishioka *et al.* (8) have also reported that an increased percentage of fibrous body is associated with lower basal GH levels per unit volume of tumour tissue and decreased immunoreactivities to GH and PRL.

Table 5 A summary of implication of *gsp* gene mutation on clinico-pathological features.

Clinico-pathological data	Mutated type	Wild-type	<i>P</i> value
Cytokeratin (CK) pattern ^a			
Dot pattern (n=13)	5 (38.5%)	8 (61.5%)	0.083 ^b
Non-dot pattern (n=30)	20 (66.7%)	10 (33.3%)	
Age (mean $\pm$ s.d.)	50.2 $\pm$ 13.9	44.7 $\pm$ 13.7	0.218 ^c
Sex			
Male	13 (52%)	5 (27.8%)	0.101 ^b
Female	12 (48%)	13 (72.2%)	
Basal GH levels (ng/ml; mean $\pm$ s.d.)	44.1 $\pm$ 41.1	43.1 $\pm$ 54.8	0.313 ^c
Size (mm; Mean $\pm$ s.d.)	16.3 $\pm$ 4.8	24.2 $\pm$ 9.4	0.006^c
Volume (cm ³ ; mean $\pm$ s.d.)	2.8 $\pm$ 2.3	10.1 $\pm$ 8.9	0.007^c
GH-producing index (ng/ml per cm ³ ; mean $\pm$ s.d.)	26.5 $\pm$ 29.9	12.2 $\pm$ 17.3	0.007^c
TRH test			
Normal response	0 (0%)	9 (50%)	<0.001^b
Abnormal response	25 (100%)	9 (50%)	
LHRH test			
Normal response	23 (92.0%)	13 (72.2%)	0.095 ^b
Abnormal response	2 (8%)	5 (27.8%)	
Octreotide test			
Good response	4 (44.4%)	2 (18.2%)	0.217 ^b
Poor response	5 (55.6%)	9 (81.8%)	
MIB-1 index (%; mean $\pm$ s.d.)	0.22 $\pm$ 0.31	0.45 $\pm$ 1.11	0.697 ^c

^aPercentage with a cytokeratin (CK) pattern. Bold numerals, $P < 0.05$.

^bFisher's exact test.

^cMann–Whitney test.

We routinely perform TRH and LHRH tests on patients with GHoma to assess the preoperative and postoperative secretory dynamics of the pituitary hormones: TSH, PRL, LH and FSH. Abnormal GH responses to these tests provide additional evidence for acromegaly, and persistent abnormal responses to TRH challenge in cured acromegals are thought to be predictive of recurrence (30). In this study, the incidence of abnormal response to preoperative TRH or LHRH challenge test was significantly ($P < 0.05$) lower in DP adenomae than in non-DP adenomae. Ectopic TRH receptors in GHoma cells play an important role in the abnormal GH response to TRH (31). Physiologically, TRH receptors act via the inositol 1,4,5-triphosphate pathway to release TSH and PRL (32, 33). However, ectopically expressed TRH receptors cross-couple with cAMP and regulate GH secretion by somatotroph cells (34), and the abnormal GH response is more frequently observed in adenomae with *gsp* mutation than those without the mutation (35) as is in our series. As *gsp* mutation was more frequently encountered in non-DP adenomae in this study, it is thus rational to expect TRH receptor prevailed more frequently in DG GHoma than in SG ones (31). Ectopic expression of TRH receptors may couple with tumourigenesis of GHoma (triggered by *gsp* mutation) and amplified cAMP signalling (36, 37).

The response rate to the prolonged administration of octreotide was reported to be significantly higher in patients with DG adenomae than those with SG adenomae (38, 39). In our series, the response to octreotide was higher in patients with non-DP adenomae than those with DP adenomae. In fact, all patients manifesting a good octreotide response had abnormal responses to TRH challenge, while this was true for 55.6% of patients with a poor response to octreotide (data not shown in table). The difference in the frequency of positive response to octreotide test may be partially due to the different *gsp* mutation prevalence between DP and non-DP adenomae. Somatostatin receptor subtype-2 (SSTR2), to which octreotide preferentially binds, is overexpressed by the constitutive activation of adenylyl cyclase in GHoma with *gsp* mutation (40). Nakashima *et al.* (41) have reported that adenomae with *gsp* mutation are more likely to display plasma membrane-dominant staining of SSTR2A. Elucidating the differences in expression and distribution of somatostatin receptors and their intracellular signalling between DP and non-DP GHoma cells are themes that need further investigation.

Postoperative cure rate was better in non-DP adenomae (60.4%) than in DP adenomae (42.3%) in our series ($P = 0.076$), which would be a reflection of the smaller volume in non-DP adenomae. Lack of the statistical significance may be due to relatively lower GH productivity of DP adenomae. Because of the lower GH productivity, patients with limited residual adenomae tissue with DP might be judged as 'cured' by the current consensus criteria of cure (42).

***gsp* mutations and CK staining pattern**

Although the number of adenomae available for analysis of the relationship between *gsp* mutations and the CK staining pattern was limited ($n = 43$), we found that 38.5% of the DP tumours manifested the mutation. This finding differs from the observations reported by Asa *et al.* (23), suggesting that in GHoma the CK architecture may not be related directly with *gsp*-induced tumourigenesis.

The rate of the *gsp* mutation in our series (58.1%) is comparable to the rate reported in Caucasian patients (19–21) and in a recent series of 100 Japanese patients (53.0%) (18). In studies reported in 1993, the incidence of *gsp* mutations in Japanese acromegals was low as 9.3 and 4.4% (43, 44); however, re-evaluation performed 10 years later by the same authors indicated that the corrected incidence was 53.1% (45).

Findings on the relationship between the presence of *gsp* mutations and tumour size have been discordant (19–21, 46). In our study, adenomae with *gsp* mutations were significantly smaller ($P < 0.01$) than tumours without *gsp* mutations. We posit that the smaller size of our non-DP adenomae may be partly attributable to the higher incidence of *gsp* mutations in that adenoma subtype.

Conclusion

DP somatotropinomas exhibit characteristic features. Compared with the non-DP subtypes, the DP tumours were larger; patients with DP tumours had a lower incidence of abnormal responses to TRH and LHRH challenge and a poorer response to octreotide test. Lastly, the E-cadherin expression level was lower in DP adenomae than in non-DP adenomae, a factor that may explain their larger size. Both DP and non-DP tumours manifested *gsp* mutations. Additional investigations are necessary to elucidate the molecular background of the observed differences in the CK staining patterns and to understand their relationship with tumourigenesis.

Declaration of interest

We declare that no authors have any conflict of interest with respect to financial relationships, stock options, board memberships, consulting agreements, advisory council memberships, honoraria, ownership interests and royalties, and that none has served as a spokesperson or expert witness, or given testimony. There are no conflicts that could be perceived as prejudicing the impartiality of the research reported.

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