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【 ORIGINAL ARTICLE 】

Acromegaly Cases Exhibiting Increased Growth Hormone Levels during Oral Glucose Loading with Preadministration of Dipeptidyl Peptidase-4 Inhibitor

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Abstract:

Objective Glucose-dependent insulinotropic polypeptide (GIP) is speculated to worsen growth hormone (GH) hypersecretion in acromegaly and to be a cause of paradoxical increases in GH (PI-GH) during 75-g oral glucose tolerance testing (75-g OGTT). Dipeptidyl peptidase-4 inhibitors (DPP4is), which increase the circulating concentration of active GIP, are frequently administered to diabetic patients, including those with acromegaly. We aimed to determine whether or not the administration of a DPP4i increases GH concentration, especially in patients demonstrating PI-GH during a DPP4i-OGTT, in which a DPP4i was administered immediately before 75-g OGTT.

Methods This prospective cross-sectional study was carried out on acromegalic patients admitted to Hokkaido University hospital between June 2011 and May 2018. The participants underwent both 75-g OGTT and DPP4i-OGTT. For those who underwent surgery, immunohistochemical staining and quantitative polymerase chain reaction (PCR) for the GIP receptor (GIPR) were performed on the resected pituitary adenomas.

Results Twenty-five percent of the participants had PI-GH confirmed (3 of 12 cases). Two of the three participants who demonstrated PI-GH exhibited higher circulating GH concentrations during DPP4i-OGTT than during OGTT. The increase in plasma glucose was reduced during DPP4i-OGTT compared to during 75-g OGTT, suggesting that the increase in GH during DPP4i-OGTT was due not to high glucose concentrations but instead increased GIP caused by the administration of DPP4i. The adenoma from one participant with PI-GH displayed positive immunostaining for GIPR and a higher GIPR messenger ribonucleic acid (mRNA) expression than the others.

Conclusion DPP4i may enhance the GH secretion response during glucose loading, especially in individuals with PI-GH.

Key words: acromegaly, growth hormone, glucose-dependent insulinotropic polypeptide

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Introduction

Acromegaly is associated with a characteristic facial appearance and metabolic abnormalities induced by an excess of growth hormone (GH). In most instances, the condition is caused by a GH-secreting pituitary adenoma.

Glucose inhibits GH secretion (1), and the nadir GH concentration is <0.4 μg/L during 75-g oral glucose tolerance testing (75-g OGTT) in healthy subjects. Oral glucose administration suppresses GH secretion and likewise is the standard method for assessing the inhibitory control of GH release (2). One of the diagnostic criteria for acromegaly is that the GH levels are not suppressed to the normal range (nadir GH level: 1.0 ng/mL) during 75-g OGTT. In addition, one of the criteria for remission of acromegaly is nadir GH levels <0.4 ng/mL during 75-g OGTT (2).

However, previous studies have shown that the GH concentration is >1 μg/L during 75-g OGTT (3) in patients with acromegaly, and 10-30% of acromegalic patients show a paradoxical increase in GH (PI-GH) (4-8). Other studies have shown that GH concentrations are more substantially reduced in individuals with PI-GH than in those without it during octreotide or bromocriptine testing (9), and PI-GH is associated with an increased incidence of remission in response to somatostatin analogues (10). Umahara et al. (5) reported that postprandial GH secretion is higher and that continuous intravenous administration of glucose-dependent insulinotropic polypeptide (GIP), a gastrointestinal hormone, causes an increase in the secretion of GH in acromegalic patients with PI-GH. Those data suggested that incretins, including GIP, might worsen GH hypersecretion in acromegaly with PI-GH.

The incidence of diabetes mellitus in acromegalic patients is 16-56%, with the substantial variation resulting from the heterogeneity of study populations (11-13). In patients with acromegaly, diabetes mellitus is partly responsible for the increase in mortality risk, thus reinforcing the importance of hyperglycemia management (14).

Dipeptidyl peptidase-4 inhibitors (DPP4is) reduce the incretin-degrading enzyme DPP4 and thus increase the concentrations of active incretins, including GIP. They are widely used for treating diabetic patients. GIP may stimulate GH secretion in acromegalic patients, and the use of DPP4i may worsen acromegaly and even induce hyperglycemia in these patients. However, there have been reports of increased GIP in acromegalic patients (15) and increased GIP resistance in acromegalic patients, which in turn increases insulin resistance and causes hyperglycemia (16). Thus, although some reports have suggested an association between acromegaly and incretin, the mechanism underlying this association is not clear.

The present study evaluated whether or not the administration of a DPP4i increases GH concentrations in acromegalic patients demonstrating PI-GH during 75-g OGTT.

Materials and Methods

Patients

This prospective cross-sectional study was carried out on untreated acromegalic patients admitted to Hokkaido University hospital between June 2011 and May 2018. The following exclusion criteria were applied: the presence of a disease that might affect the endocrinologic evaluation, such as an infectious disease, liver disease, or kidney disease; poorly-controlled diabetes; or other issues that the physician in charge regarded as rendering the patient unsuitable for the study.

This study conformed to the principles of the Declaration of Helsinki and the Japanese Ministry of Health, Labor and Welfare's ethical guidelines on clinical research. It was carried out after obtaining written informed consent from the study participants and approval from the Hokkaido University Hospital Ethics Committee (clinical study reference number 018-0101). The study was registered with UMIN (ID #000006516).

GTT

The participants underwent 75-g OGTT, and their GH, blood glucose, serum insulin, and active GIP concentrations were measured at 0, 60, and 120 minutes. On another day, the DPP4i sitagliptin (50 mg) was administered 1 hour before repeated 75-g OGTT, and the same parameters were measured (DPP4i-OGTT). Because more than 80% of DPP4 activity was inhibited 1 hour after oral sitagliptin (50 mg) (17), the protocol was designed for DPP4i to be taken 1 hour before glucose loading. The participants were required to fast after 9:00 p.m. the day before the test. 75-g OGTT and DPP4i-OGTT were performed at intervals of more than 1 day and less than 7 days. Patients with poor glycemic control at admission underwent 75-g OGTT after treatment to improve their glycemic control. Blood samples of active GIP were collected using blood collection tubes containing a DPP4 inhibitor (P800; BD, Tokyo, Japan). The active GIP concentrations were measured with a commercial enzyme-linked immunosorbent assay (ELISA) kit (Human GIP, Active form Assay Kit-IBL; Immuno-Biological Laboratories, Fujioka, Japan) after solid-phase extraction enforcement.

Immunostaining

The resected pituitary adenomas from participants who underwent surgery were analyzed using immunohistochemistry for the GH and GIP receptor (GIPR).

Immunohistochemical staining for GH was eprformed using an automated staining machine (DAKO Autostainer Link 48, Santa Clara, USA). The polyclonal anti-GH antibody (DAKO) was diluted 1:4000 for use as a primary antibody.

To analyze the expression of GIPR, paraffin-embedded tissue was sectioned and deparaffinized using xylene and ethanol. We performed antigen retrieval in pH 6.0 sodium

Table. Clinical and Endocrine Parameters in the Participants.

Case No.	Age (yr)/ sex	HbA1c (%)	IGF-1 (ng/mL)	IGF-1 (SD)	PI-GH	75-g OGTT for GH (μg/L)			75-g OGTT for glusose (mg/dL)				75-g OGTT for GIP (pmol/L)			75-g OGTT for IRI (μU/mL)			
						0'	60'	120'	0'	60'	120'	AUC†	0'	60'	120'	0'	60'	120'	AUC‡
1	63/F	5.7	558	6.67	+	13.80	34.50	33.60	105	212	231	22,800	4.3	17.5	26.5	2.6	35.2	115.2	5,646
2	43/F	6.8	626	7.37	+	66.62	270.42	195.65	108	253	208	24,660	2.3	26.8	6.8	<0.5	40.7	47.8	3,891
3	65/M	6.0	694	8.16	+	13.40	73.40	31.60	123	220	250	24,390	2.3	21.7	21.1	5.0	16.4	43.9	2,451
7	63/F	5.9	607	7.09	-	8.10	9.44	10.01	98	241	229	24,270	7.1	38.8	44.5	4.5	31.9	47.5	3,474
10	65/M	10.3	757	8.75	-	4.90	4.80	5.10	161	379	390	39,270	39.9	173.0	126.0	13.9	24.0	25.1	2,610
11	29/M	5.4	635	6.50	-	26.25	29.17	27.44	96	165	143	17,070	2.3	28.0	20.3	8.5	67.0	52.6	5,853
12	47/M	5.7	568	6.96	-	15.64	16.59	17.18	94	146	160	16,380	3.9	24.7	12.3	4.6	19.7	20.5	1,935

Case No.	DPP4i-OGTT for GH (μg/L)			DPP4i-OGTT for glusose (mg/dL)				DPP4i-OGTT for GIP (pmol/L)			DPP4i-OGTT for IRI (μU/mL)			
	0'	60'	120'	0'	60'	120'	AUC†	0'	60'	120'	0'	60'	120'	AUC‡
1	9.60	29.80	52.90	94	165	163	17,610	9.7	69.1	38.2	5.2	36.3	62.1	4,197
2	77.00	274.30	185.80	106	125	193	16,470	10.4	66.6	44.6	3.8	7.8	45.6	1,950
3	11.10	91.00	36.90	114	190	182	20,280	4.5	44.1	38.0	5.5	44.4	47.8	4,263
7	8.48	11.87	11.95	102	177	160	18,480	14.2	84.4	89.2	6.3	21.2	31.7	2,412
10	9.50	11.50	9.40	148	307	362	33,720	33.3	232.0	166.0	20.6	51.1	65.4	5,646
11	25.33	27.03	26.75	94	150	145	16,170	11.1	53.8	56.6	7.5	79.1	92.0	7,731
12	18.43	22.06	22.07	91	111	137	13,500	19.8	31.6	40.0	3.9	6.9	16.5	1,026

A paradoxical increase in growth hormone (PI-GH) was defined as the situation in which the highest concentration of GH was twice that of the baseline GH concentration during a 75-g OGTT.

HbA1c: hemoglobin A1c, n/a: date not available, IGF-1: insulin-like growth factor-1, PI-GH: paradoxical increase in GH, OGTT: oral glucose tolerance test, DPP4i-OGTT: oral glucose tolerance test with concurrent oral administration of a dipeptidyl peptidase-4 inhibitor, PG: plasma glucose, GIP: glucose-dependent insulinotropic polypeptide, IRI: immunoreactive insulin, AUC: area under the curve.†mg/mL·min, ‡μUI/mL·min.

citrate buffer heated in a microwave and then treated sections with a protein-blocking reagent (Dako Japan Inc, Tokyo, Japan) following treatment with hydrogen peroxide. The sections were then incubated with an anti-GIPR antibody (kindly provided by Prof. Timothy J. Kieffer, University of British Columbia, Canada) overnight at 4 °C, washed, and incubated with biotinylated anti-rabbit IgG antibody (Vector Laboratories, Burlingame, USA) for 2 hours at room temperature. The slides were then stained using 3,3'-diaminobenzidine (Vector Laboratories). A positive control GIPR-immunostained section of mouse pancreas is shown in Supplementary material 1.

Immunohistochemical staining for GH, cytokeratin (CAM 5.2), and somatostatin receptors type 2 (SSTR2) and type 5 (SSTR5) was performed using an automated staining machine (Autostainer Link 48; DAKO). The polyclonal anti-GH antibody (DAKO), CAM5.2 (DAKO), SSTR2 (Abcam, Cambridge, UK), and SSTR5 (Abcam) were diluted 1:4000 for use as a primary antibody.

Quantitative polymerase chain reaction (PCR)

Ribonucleic acid (RNA) was extracted from formalin-fixed, paraffin-embedded adenomas using a PureLink FFPE RNA Isolation Kit (Thermo Fisher Scientific, Waltham, USA), in accordance with the manufacturer's instructions. Two hundred micrograms of RNA from each sample were reverse-transcribed to prepare cDNA using the SuperScript III First-Strand Synthesis System (Thermo Fisher Scientific). Quantitative real-time PCR was then performed using Fast SYBR® Green Master Mix (Thermo Fisher Scientific) and an Applied Biosystems 7,500 Fast Real-Time PCR System (Thermo Fisher Scientific). GIPR messenger RNA (mRNA) expression was normalized to that of GAPDH. The primer sequences used for GIPR and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) amplification are shown in Supplementary material 2.

Study endpoints

The primary endpoint was the difference in GH concentrations between OGTT and DPP4i-OGTT, classified according to the presence or absence of PI-GH during 75-g OGTT. There is currently no agreement concerning the criteria to define the PI-GH response pattern (2). Some previous reports described an increase of more than 20-100% above basal levels (2). In this study, PI-GH was defined as present when the highest GH concentration during OGTT was twice that of the baseline GH. The secondary endpoints were the prevalence of PI-GH and positive immunostaining for GIPR in participants who did or did not demonstrate PI-GH.

Statistical analyses

Continuous data are expressed as means and standard deviations if the variances were equal or as medians (minimum value-maximum value) if the variances were not equal.

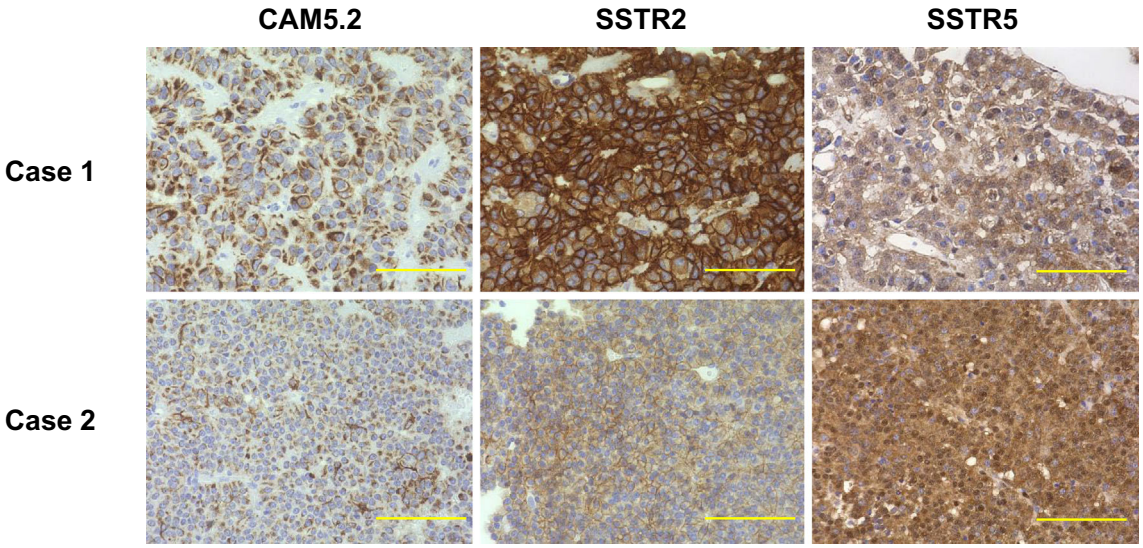

Figure 1. **Immunostaining for cytokeratin, SSTR2, and SSTR5. Representative images of cytokeratin (CAM5.2) and somatostatin receptors type 2 (SSTR2) and type 5 (SSTR5) in immunostained adenoma sections for cases 1 and 2. Scale bar: 100 μm. Case 1: CAM5.2 staining showed a cytoplasmic pattern, suggesting granulated adenoma. SSTR2 reactivity was strongly observed on the cell membrane circumferentially in almost 100% tumor cells, which was compatible with a score of 3 in the immunohistochemical scoring system. SSTR5 reactivity was observed only in cytoplasm, which was compatible with a score of 1. Case 2: CAM5.2 staining showed a cytoplasmic pattern, suggesting granulated adenoma. SSTR2 staining demonstrated partial membranous reactivity in less than 50% of tumor cells, which was compatible with a score of 2 in the immunohistochemical scoring system. SSTR5 reactivity was observed only in the cytoplasm, which was compatible with a score of 1.**

Statistical analyses were carried out using the Ekuseru-Toukei 2015 software program (Social Survey Research Information, Tokyo, Japan).

Results

Representative cases with PI-GH

Case 1

The patient was a 63-year-old woman with a facial appearance characteristic of acromegaly, including a protruding nasal arch, enlarged nose and lips, and giant tongue. Her hemoglobin A1c (HbA1c) value was 5.7%, and insulin-like growth factor 1 (IGF-1) was 558 ng/mL [standard deviation (SD): 6.67]. Magnetic resonance imaging (MRI) revealed a 12-mm mass protruding from within the sella turcica to the upper part of the sella turcica. The baseline and peak GH concentrations were 13.5 and 34.50 ng/mL (a 2.5-fold increase) during 75-g OGTT, showing PI-GH. During the test, the change in the blood glucose value was 126 mg/dL, the change in the GIP concentration was 22.2 pmol/dL, and the change in the immunoreactive insulin (IRI) level was 112.6 μU/mL. A further increase in GH was observed during DPP 4i-OGTT, from the baseline value of 9.60 μg/mL to the peak value of 52.90 μg/mL (a 5.5-fold increase). During DPP4i-OGTT, the change in blood glucose was 71 mg/dL, which was similar to that during 75-g OGTT, but the change in GIP concentration was 59.4 pmol/dL, which were greater than that during 75-g OGTT. (Table). During the bromocriptine (2.5 mg) loading test, the GH concentration was suppressed from the baseline value of 10.60 μg/mL to the nadir of 7.70 μg/mL. During the octreotide (50 μg) loading test, the GH concentration was suppressed from the baseline value of 15.60 μg/mL to the nadir of 0.40 μg/mL.

Transsphenoidal pituitary tumor resection was performed, and the pathology was a GH-producing pituitary adenoma (densely granulated). SSTR2 reactivity was strongly observed at the cell membrane circumferentially in almost 100% of tumor cells, which was compatible with a score of 3 in the immunohistochemical scoring system (18). SSTR5 reactivity was observed only in the cytoplasm, which was compatible with a score of 1 (18) (Fig. 1). Immediately after surgery, the GH and IGF-I levels normalized to 0.9 ng/mL and 115 ng/mL (SD: -0.3), respectively. The GH concentration decreased markedly from the baseline value of 1.00 μg/mL to the peak value of 2.20 μg/mL and the nadir of 0.90 μg/mL during 75-g OGTT. Five years after surgery, no recurrence has been observed.

Case 2

The patient was a 43-year-old woman with a facial appearance characteristic of acromegaly, including a protruding nasal arch, enlarged lips, protruding mandible, and giant tongue. Her HbA1c value was 6.8%, and IGF-1 was 626 ng/mL (SD: 7.37). MRI revealed a 30-mm mass in the sella turcica. The baseline and peak GH concentrations were 66.62 and 270.42 ng/mL (a 4.1-fold increase), respectively,

during 75-g OGTT, showing PI-GH. During the test, the changes in blood glucose was 145 mg/dL, the changes in GIP was 24.5 pmol/dL, and the changes in IRI was 47.3 μU/mL. During DPP4i-OGTT, GH increased from the baseline 77.0 μg/mL to the peak value 274.3 μg/mL (a 3.6-fold), an increase similar to that of OGTT.

During DPP4i-OGTT, the change in the blood glucose value was 68 mg/dL, the change in the GIP concentration was 56.2 pmol/dL, and the change in the IRI level was 41.8 μU/mL (Table). During the bromocriptine (2.5 mg) loading test, the GH concentration was suppressed from the baseline value of 38.25 μg/mL to the nadir of 13.65 μg/mL. During the octreotide (50 μg) loading test, GH concentration was suppressed from the baseline value of 74.86 μg/mL to the nadir of 3.69 μg/mL. Preoperative drug therapy was started with octreotide [long acting repeatable (LAR) 20 mg/4 weeks, later increased to 30 mg/4 weeks]. Six months after the start of drug therapy, her GH concentration was 7.85 ng/mL, and IGF-I was 367 ng/mL (SD: 4.31), and she underwent transsphenoidal pituitary tumor resection.

The pathology was GH-producing pituitary adenoma (densely granulated). SSTR2 staining demonstrated partial membranous reactivity in less than 50% of tumor cells, compatible with a score of 2 in the immunohistochemical scoring system (18). SSTR5 reactivity was observed only in the cytoplasm, which was compatible with a score of 1 (18) (Fig. 1). Immediately after surgery, the GH and IGF-I levels normalized to 0.89 ng/mL and 172 ng/mL (SD: 0.59), respectively. The GH decreased markedly from the baseline value of 1.19 μg/mL, peaking at 2.49 μg/mL, and then reaching a nadir of 1.29 μg/mL during 75-g OGTT. Because the GH and IGF-I levels were elevated to 3.33 ng/mL and 368 ng/mL (SD: 4.33), respectively, at 3 months after surgery, octreotide LAR 20 mg/4 weeks was started. Subsequently, a decrease in IGF-1 levels was observed, and the dosage was reduced to 10 mg/4 weeks. More than four years have passed since then, and the levels of both GH and IGF-I remain within the criteria.

Study in all cases

There were 12 participants (8 women and 4 men) enrolled in this study, and the 7 cases (3 women and 4 men) who underwent immunostaining with anti-GIPR antibody are shown in Table. All 12 participants' data are shown in Supplementary material 3. All participants were treatment-naïve when they underwent both 75-g OGTT and DPP4i-OGTT.

In a comparison between 75-g OGTT and DPP4i-OGTT, the baseline plasma glucose levels remained virtually unchanged, but the plasma glucose area under the curve (AUC) (mg/dL-min) improved in all cases during DPP4i-OGTT. Active GIP levels were increased or enhanced in all cases. Baseline IRI values were similar between the two loading tests, but the peak values and AUC were reduced by DPP4i-OGTT compared with 75-g OGTT.

Regarding cases that demonstrated PI-GH during 75-g OGTT, the GH concentrations substantially increased during DPP4i-OGTT in cases 1 and 3. In case 2, although a marked increase in GH was measured during DPP4i-OGTT, this increment was similar to that measured during 75-g OGTT (Fig. 2).

Cases 4 to 12 did not demonstrate PI-GH. In cases that did not demonstrate PI-GH, the increases in GH concentration were higher during DPP4i-OGTT than during 75-g OGTT in three cases (cases 7, 10, and 12) but not in one other case (cases 11) (Fig. 2). In cases 10 and 12, baseline GH levels were elevated during DPP4i-OGTT. However, in case 10, baseline GIP was not elevated, so factors other than GIP were suspected to be involved. There were no adverse events associated with DPP4i administration.

Regarding immunostaining of GIPR for resected pituitary adenoma, case 1 showed positive immunostaining for GIPR in the cytoplasm of the adenoma, but the adenoma from case 2, who showed a paradoxical increase in GH and no further increase in GH during DPP4-OGTT, was negative for GIPR. The adenomas from cases 7, 10, 11, and 12 were also GIPR-negative (Fig. 3). The GIPR mRNA expression was higher in the adenoma from case 1 than in those from the other cases, including case 2, which is consistent with the results of DPP4i-OGTT and immunostaining for GIPR (Fig. 4).

There were no apparent differences in drug responsiveness or other clinical background features between PI-GH and non-PI-GH cases (data not shown).

Discussion

In this study, we showed for the first time that individuals with PI-GH had an elevated GH secretion during DPP4i-OGTT. Among the seven cases analyzed in the present study, 43% had PI-GH, which was consistent with previous reports describing the occurrence of PI-GH in approximately 10-30% of acromegaly cases (5, 6).

Patients with acromegaly in the present study underwent OGTT combined with oral DPP4i administration, which resulted in an increase in GIP and a decrease in plasma glucose concentrations compared to OGTT. Oral DPP4i may have suppressed the plasma glucose levels as a result of the increased GIP. With respect to the fact that IRI was reduced with DPP4i-OGTT, it is possible that reactive IRI secretion in response to elevated plasma glucose may have been suppressed.

Cases 1 and 2 both showed PI-GH and were of the densely granulated type on CAM5.2 staining. Patients showing PI-GH were reportedly more likely to have the densely granulated type (19, 20), and the two cases in this study were similar to those previously reported. It has also been reported that patients who present with PI-GH have a better response to somatostatin analogues than others (9, 10). The two cases in this study also responded well to octreotide, and the staining of SSTR2 was consistent with this. However, although patients with PI-GH are reported to be older and have smaller tumors than those without it (10), case 2

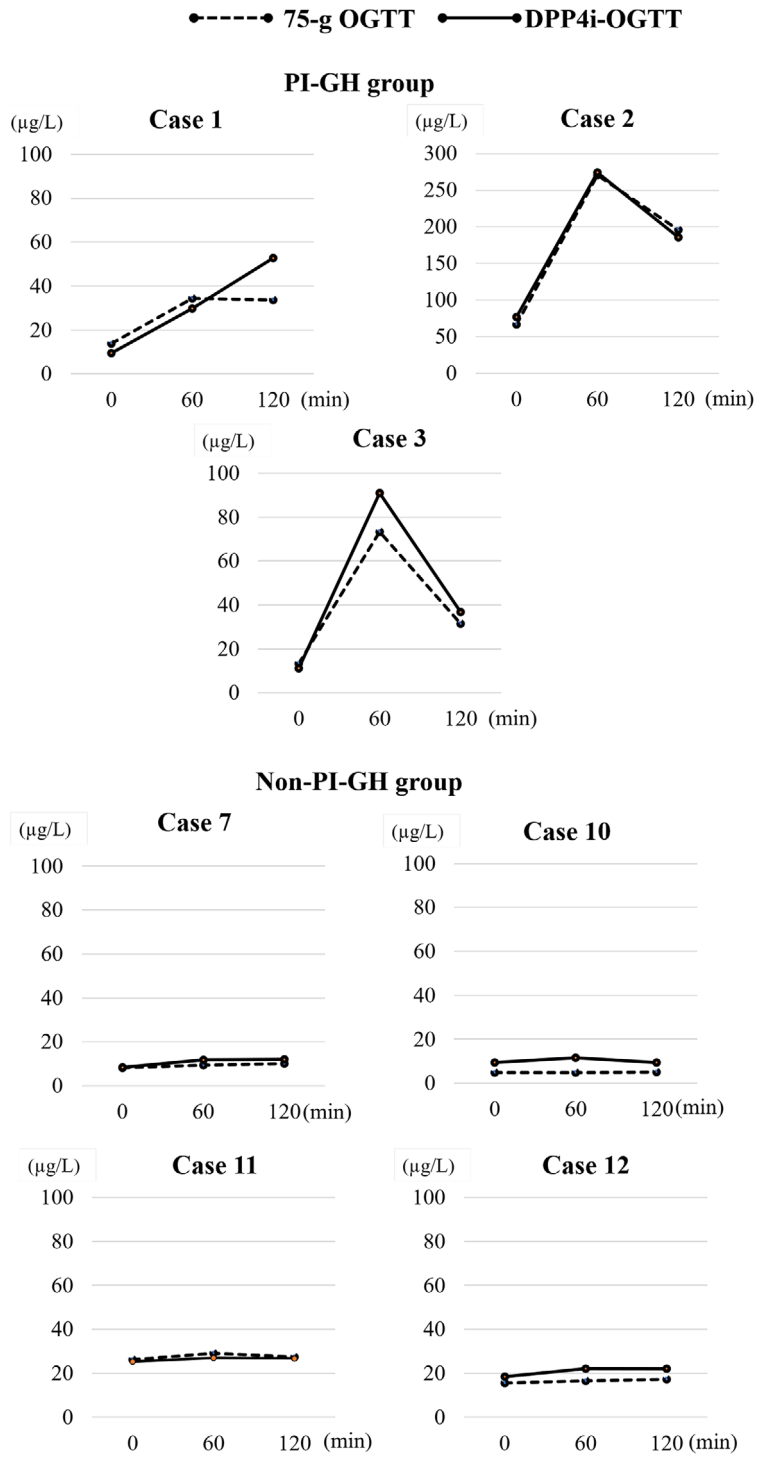

Figure 2. Changes in the GH concentration during 75-g OGTT and DPP4i-OGTT. PI-GH was defined as being present when the peak GH was twice that of the baseline GH during 75-g OGTT. Three of 12 cases showed PI-GH during 75-g OGTT (cases 1-3). In cases 1 and 3, the increase in GH was more marked during DPP4i-OGTT. In case 2, although there was a substantial increase in GH during DPP4i-OGTT, the increment was similar to that during OGTT. In the participants without PI-GH (cases 7, 10, 11, and 12), the increase in GH was higher during DPP4-OGTT than during the 75-g OGTT in three cases but not in one other case. OGTT: oral glucose tolerance testing, DPP4i-OGTT: oral glucose tolerance testing with concurrent oral administration of a dipeptidyl peptidase-4 inhibitor, PI-GH: paradoxical increase in GH

was relatively young and had a larger tumor size, which differed from the general characteristics of PI-GH patients.

Two of the three individuals with PI-GH, including case 1, exhibited elevated GH secretion during DPP4i-OGTT,

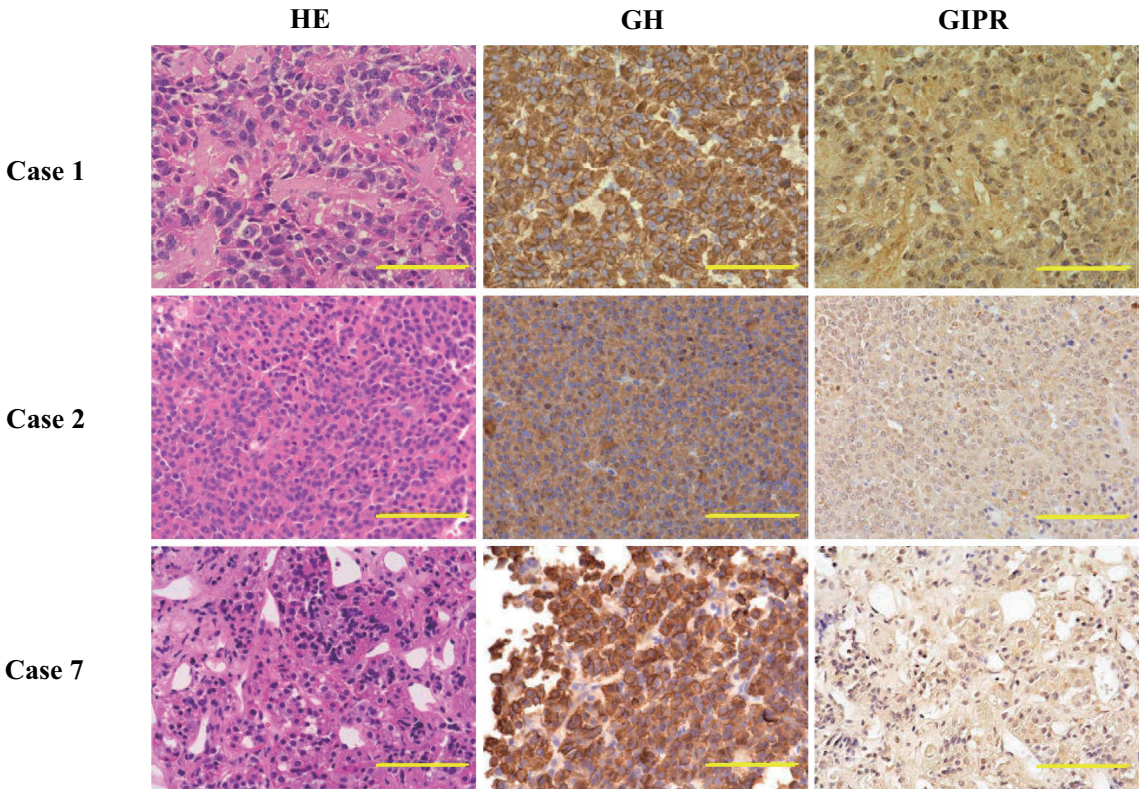

Figure 3. **Immunostaining for GIPR. Representative images of Hematoxylin and Eosin staining, and GH and glucose-dependent insulinotropic polypeptide receptor (GIPR)-immunostained adenoma sections for cases 1, 2, and 7. Scale bar: 100 μm.**

Figure 4. ***GIPR*** **mRNA expression. A quantitative reverse transcription-PCR analysis of the GIPR gene expression in adenomas from cases 1, 2, 4, 6, 7, 9, 10, 11, and 12. The mRNA expression was normalized to that of GAPDH, and the x-axis values are fold-differences normalized to the mean value of the participants without PI-GH. GIPR: gastric inhibitory polypeptide receptor**

suggesting that the administration of DPP4i exacerbates the endocrine abnormalities present in acromegaly. Increases in blood glucose were similar during DPP4i-OGTT; this implied that the GH increase during DPP4i-OGTT was not the result of a lower glucose concentration but instead of the GIP-increasing effect of DPP4i. The action of GIP has been proposed as an explanation for the paradoxical increase in GH during OGTT (7, 8). The administration of GIP to acromegalic patients increased serum GH (7), and another study showed that GIP treatment of GIPR-overexpressing rat GH3 cells caused an increase in GH transcription, mediated via an increase in cyclic adenosine monophosphate (cAMP) (8). Furthermore, GIPR is highly expressed in acromegalic patients who demonstrate PI-GH during OGTT, and GIP administration increases GH secretion more effectively in cultured adenoma cells expressing high levels than in those with low levels of GIPR (21). When these findings are considered along with the present findings concerning GIPR expression in some adenomas from acromegalic patients, one of the factors contributing to PI-GH in acromegalic patients may be the expression of GIPR in the hypophyseal tumor.

However, one of the three individuals with PI-GH (case 2) did not exhibit a further increase in GH during DPP4i-OGTT compared to 75-g OGTT. In addition, GIPR immunostaining was negative in the adenoma from this case, suggesting that factors other than incretins can stimulate GH secretion in individuals with PI-GH.

Among the participants who did not demonstrate PI-GH, some had a higher GH concentration following DPP4i-OGTT than that following OGTT. Because GIPR was not expressed in the adenomas removed from these individuals, hormones other than GIP secreted in response to oral glucose loading may have caused their elevated GH concentrations. DPP4 degrades a number of peptides, including growth hormone-releasing peptide, pituitary adenylate

cyclase-activating polypeptide, and chemokines (22); these substances may thus contribute to the increased GH concentrations during DPP4i-OGTT in individuals not demonstrating PI-GH. GIPR expression was not detected in adenomas from cases 7, 10, and 12, suggesting that other DPP4 substrates are involved. These results imply that DPP4is should be carefully administered to individuals without PI-GH.

Several limitations associated with the present study warrant mention. First, measurements were only made at 0, 60, and 120 minutes during OGTT, which may have missed the timing of the peak values. Second, the mechanism underlying GH secretion during OGTT may differ between early (within about 60 minutes after loading) and late (after about 120 minutes after loading) timepoints; the former involves the inappropriate secretion of GH, while the latter is the reactive secretion of GH due to a reduction in glucose once it has been elevated (23). This mechanistic difference may have influenced the results of this study. Third, GIPR immunostaining and quantitative PCR were not performed on adenomas from all 12 of the cases, as cases 3, 5, and 8 did not undergo pituitary adenomectomy. Finally, this study had a small sample size; further studies involving larger numbers of cases should be conducted to confirm our findings.

DPP4is may increase GH in either PI-GH or non-PI-GH; however, the extent of the increase is particularly high in PI-GH. Substrates of DPP4 other than GIP might be involved in non-PI-GH. In the present study, there was no marked difference between PI-GH and non-PI-GH cases with regard to drug responsiveness or other clinical background features. Patients with acromegaly, regardless of the presence of PI-GH, should thus be carefully monitored for worsening of the disease after treatment with a DPP4i.

Prior to conducting this study, we obtained informed consent from all subjects and approval of the study plan from the Hokkaido University Hospital Ethics Committee (clinical study reference number 018-0101).

Author's disclosure of potential Conflicts of Interest (COI).

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