Isolated Adrenocorticotropin Deficiency Concomitant with Graves' Disease: A Case Report and Literature Review

Nobumasa Ohara^{1,2}, Masanori Kaneko¹, Hideyuki Kuriyama³, Kazuhiro Sato³,
Hideki Katakami⁴, Yutaka Oki⁵, Kenzo Kaneko¹ and Kyuzi Kamoi^{6,7}

Abstract

A 73-year-old Japanese woman with untreated Graves' hyperthyroidism developed glucocorticoid-induced adrenal insufficiency (AI) after a supraphysiological dose of prednisolone therapy for bronchial asthma. Days later, she had high plasma adrenocorticotrophic hormone (ACTH) levels and was expected to recover from glucocorticoid-induced AI. Her plasma ACTH levels remained high over 3 months during a physiological dose of hydrocortisone replacement. However, she suffered a further decrease in her serum cortisol level and was diagnosed with isolated adrenocorticotropin deficiency (IAD), in which bioinactive ACTH likely caused the high ACTH value. IAD should be considered as an unusual disorder associated with Graves' disease, especially in older patients.

Key words: isolated adrenocorticotropin deficiency, glucocorticoid-induced adrenal insufficiency, Graves' disease, bronchial asthma, type 2 diabetes mellitus, empty sella turcica

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Introduction

Adrenocorticotrophic hormone (ACTH), a 39-amino-acid bioactive peptide, is derived from anterior pituitary corticotroph cells after enzymatic post-translational modifications of the 241-amino-acid preproprotein, proopiomelanocortin (POMC) (1, 2). The major factor stimulating ACTH release is corticotropin-releasing hormone (CRH) that is secreted from the hypothalamus.

Adrenal insufficiency (AI) is a potentially life-threatening disorder resulting from glucocorticoid deficiency, with or without loss of mineralocorticoids or adrenal androgens (3). Clinical manifestations of AI include nonspecific symptoms such as fatigue, lethargy, anorexia, and hypoglycemia. According to the underlying mechanism, AI can be classified as primary (Addison's disease), secondary, or tertiary.

Glucocorticoid-induced AI is the most common form of

tertiary AI and results from suppression of both hypothalamic CRH and pituitary ACTH by supraphysiological doses of exogenous glucocorticoids (4). Spontaneous recovery typically occurs during the administration of physiological doses of corticosteroid replacement, such as oral hydrocortisone at daily doses of ≤ 20 mg (3-5). Patients with glucocorticoid-induced AI have low plasma ACTH levels, however, they may exhibit transiently high plasma ACTH levels during the recovery phase (6).

Secondary AI results from pituitary disease that hampers the release of ACTH (3), and patients usually have low plasma ACTH levels. However, there are reported cases of secondary AI in which bioinactive ACTH, which produce a high measured plasma ACTH value, are present (7-9). Some of these are high molecular weight peptides, such as pro-ACTH and POMC, which are also referred to as "big ACTH" (10, 11).

Isolated adrenocorticotropin deficiency (IAD) is a rare,

¹Department of Endocrinology and Metabolism, Nagaoka Red Cross Hospital, Japan, ²Department of Endocrinology and Metabolism, Uonuma Institute of Community Medicine, Niigata University Medical and Dental Hospital, Japan, ³Department of Respiratory Medicine, Nagaoka Red Cross Hospital, Japan, ⁴Division of Clinical Research Sciences, Department of Medicine, Teikyo University Chiba Medical Center, Japan, ⁵Department of Family and Community Medicine, Hamamatsu University School of Medicine, Japan, ⁶Department of Internal Medicine, Ojiya General Hospital, Japan and ⁷Center of Diabetes, Endocrinology and Metabolism, Joetsu General Hospital, Japan

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Correspondence to Dr. Nobumasa Ohara, oharan@med.niigata-u.ac.jp

heterogeneous pituitary disease characterized by secondary AI with normal secretion of pituitary hormones other than ACTH (12). Patients with IAD often have organ-specific autoimmune disorders, such as Hashimoto's thyroiditis (13). A few cases of IAD associated with Graves' disease have also been reported (14-17).

We herein report the case of an elderly patient with Graves' disease who developed IAD in association with glucocorticoid-induced AI; she expressed a form of bioinactive ACTH that differed from "big ACTH." In addition, previously reported cases of concomitant IAD and Graves' disease are reviewed.

Measurement of adrenocorticotrophic hormone in the plasma

All measurements of plasma ACTH in the clinical setting were performed using a two-site electro-chemiluminescence immunoassay (ECLIA) (Roche Diagnostics, Tokyo, Japan) (18). This assay employs a monoclonal antibody against the N-terminal fragment 9-12 and a monoclonal antibody against the C-terminal fragment 36-39 of ACTH (19).

The basal ACTH levels in plasma samples collected in March and August 2013, and February 2015, were also measured using an immunoradiometric assay (IRMA) (LSI Medience, Tokyo, Japan) (20), a fluorescence enzyme immunoassay (FEIA) (Tosoh, Tokyo, Japan) (19, 21), and an immune complex transfer enzyme immunoassay (ICT-EIA) I (19, 22).

Measurement of γ 3-melanocyte-stimulating hormone levels in the plasma

To quantitatively assess "big ACTH" levels in the plasma, we measured the γ 3-melanocyte-stimulating hormone (MSH) levels. This assay does not detect the authentic ACTH 1-39 peptide but sensitively detects both POMC and POMC-derived peptides, including pro-ACTH (19, 23).

Analysis of the molecular size of adrenocorticotrophic hormone in the plasma

The molecular size of ACTH in the patient's plasma was analyzed using gel exclusion chromatography. The fractionating procedure for the measurement of ACTH with a radioimmunoassay (RIA) was performed as described previously (24). A non-extracted plasma sample (0.5 mL) was applied to Sephadex G75 (1×50 cm) gel chromatography; the ACTH concentration was determined by a specific conventional RIA. The ACTH RIA uses a rabbit polyclonal antiserum against the N-terminal fragment 1-24 of ACTH. The anti-serum of the RIA system identifies ACTH 1-24-containing peptides equally, including ACTH 1-24, ACTH 1-39, pro-ACTH, and POMC.

exertional dyspnea, and anorexia. She had a maternal family history of cerebral infarction. The patient gave birth twice in her twenties and had no history of obesity, head trauma, or pituitary disease. The patient had been treated with theophylline and antihypertensive drugs for bronchial asthma and essential hypertension, respectively, since 30 years of age. She was diagnosed with type 2 diabetes mellitus at 68 years of age, for which she followed dietary therapy comprising 1,400 kcal/day, and her HbA1c (National Glycohemoglobin Standardization Program, NGSP) (25) level was approximately 6%. Around the same time, she started inhaled corticosteroid therapy with ciclesonide at 200 μ g/day for her bronchial asthma, which was well controlled without additional systemic corticosteroids. The patient developed fatigue, lethargy, and appetite loss in October 2012, and her body weight decreased from 40 kg to 29 kg within 1 month. She visited her primary care physician in February 2013 after experiencing wheezing and exertional dyspnea for a few days. She received a regimen of oral prednisolone therapy (3 days of 10 mg/day and 5 days of 5 mg/day) for exacerbation of her bronchial asthma. On day 8 of the corticosteroid regimen, the patient was transferred to our hospital for a detailed examination of her persisting fatigue, lethargy, and anorexia.

On this physical examination, the patient was 148 cm tall and weighed 29 kg (body mass index, 13.2 kg/m²). Her consciousness was clear, and her body temperature, blood pressure, and pulse rate were 36.9°C, 126/45 mmHg, and 99 beats per minute, respectively. She presented with no headache, visual or vision disturbance, cough, or wheezing. No heart murmurs or chest rales were detected. There was no exophthalmos or skin pigmentation, however, she had mild struma without pain, moist skin, and bilateral finger tremor. Laboratory data showed primary hyperthyroidism [thyroid-stimulating hormone (TSH), <0.01 μ U/mL; free triiodothyronine (FT₃), 18.2 pg/mL; and free thyroxine (FT₄), >8.0 ng/dL]. Ultrasonography showed a diffusely enlarged thyroid gland and a technetium-99m thyroid scintigraphy showed diffusely elevated uptake in the goiter. According to these findings, the patient was diagnosed with Graves' disease (26) and thus was started an oral antithyroid drug treatment with thiamazole (15 mg/day).

The next day, she was taken by ambulance to our hospital because she had fallen into a coma. Her body temperature, blood pressure, and pulse rate were 36.5°C, 133/40 mmHg, and 79 beats per minute, respectively, and her blood chemistry (Table 1) revealed hypoglycemia. The serum levels of immunoreactive insulin and C-peptide were appropriately inhibited, and counterregulatory hormones, such as growth hormone and catecholamines, responded appropriately. However, her serum cortisol and plasma ACTH levels were relatively decreased. After recovery from hypoglycemia upon continuous intravenous glucose infusion, the patient recovered consciousness and was admitted for further endocrine examinations.

The patient was considered to have glucocorticoid-

Case Report

A 73-year-old Japanese woman presented at our hospital in mid-February 2013 due to a 4-month history of fatigue,

Table 1. Laboratory Findings from the Emergency Room Visit (at 3:00 PM) after Transportation to Our Hospital in February 2013.

Hematology		
Red blood cells	421 × 10 ⁴ /μL	(427–571)
Hemoglobin	11.8 g/dL	(12.4–17.2)
Hematocrit	35.9 %	(38.7–50.3)
White blood cells	7,700 /μL	(4,000–9,000)
Platelets	14.8 × 10 ⁴ /μL	(12.0–30.0)
Chemistry		
Casual plasma glucose	0.78 mmol/L	(3.9–7.8)
Serum immunoreactive insulin	< 0.5 μU/mL	(2.2–12.4)
Serum C-peptide	< 0.2 ng/mL	(1.1–3.3)
HbA1c (NGSP)	6.0 %	(4.6–6.2)
Total protein	7.3 g/dL	(6.7–8.3)
Albumin	3.9 g/dL	(3.8–5.3)
Aspartate aminotransferase	21 IU/L	(13–33)
Alanine aminotransferase	16 IU/L	(8–42)
Urea nitrogen	16.1 mg/dL	(8.0–20.0)
Creatinine	0.41 mg/dL	(0.60–1.00)
Sodium	140 mmol/L	(137–147)
Potassium	4.5 mmol/L	(3.5–4.7)
Chloride	101 mmol/L	(98–108)
C-reactive protein	5.07 mg/dL	(< 0.30)
Thyroid-stimulating hormone	< 0.01 μU/mL	(0.30–4.30)
Free triiodothyronine	11.21 pg/mL	(2.00–4.90)
Free thyroxine	> 8.00 ng/dL	(0.70–1.80)
Growth hormone	8.7 ng/mL	(0–0.5)
Insulin-like growth factor 1	107 ng/mL	(38–207)
Plasma ECLIA-ACTH	58.9 pg/mL	(7.2–63.3)
Serum cortisol	14.5 μg/dL	(10.0–25.0)
Serum DHEA-S	225 ng/mL	(70–1770)
Plasma renin activity	0.3 ng/mL/h	(0.1–2.0)
Serum aldosterone	5.6 ng/dL	(3.0–15.9)
Plasma noradrenalin	1.73 ng/mL	(0.06–0.46)
Plasma adrenalin	0.17 ng/mL	(0–0.07)
Plasma dopamine	0.23 ng/mL	(0–0.014)

Blood samples were taken from the patient in a hypoglycemic coma. The reference range for each parameter is shown in parentheses.

ACTH: adrenocorticotropic hormone, ECLIA: electro-chemiluminescence immunoassay, DHEA-S: dehydroepiandrosterone sulfate, NGSP: National Glycohemoglobin Standardization Program

induced AI and was prescribed 10 mg oral hydrocortisone on the day of admission, with continuing oral hydrocortisone replacement at 5 mg/day (5 mg in the morning) from the second morning, to recover her adrenal function; she continued oral thiamazole and inhaled ciclesonide (Fig. 1). Her fasting plasma glucose levels were maintained between 4.2 and 5.8 mmol/L after discontinuation of intravenous glucose infusion, and her systolic blood pressure and pulse rate were approximately 120 mmHg and 80 beats per minute, respectively.

On day 4 of this admission, the patient had low basal serum cortisol and high plasma ACTH levels (Table 2A). She showed incomplete endogenous cortisol release after a single-dose administration of synthetic ACTH 1-24 (Table 2B). However, adequate cortisol release was observed during a prolonged synthetic ACTH 1-24 load (Table 2C). Dynamic tests for secretion of pituitary hormones showed no TSH response and normal release of growth hormone (GH), prolactin, luteinizing hormone (LH), and follicle-stimulating hormone (FSH). Although her plasma ACTH level, both before and after a CRH load or the administra-

tion of growth hormone-releasing peptide (GHRP)-2, was high, no cortisol response was observed (Table 2D and E). These findings suggested diagnoses of either the recovery phase of glucocorticoid-induced AI, secondary AI accompanied by bioinactive ACTH, or the early phase of Addison's disease in the presence of primary hyperthyroidism due to Graves' disease. The patient continued oral thiamazole at 15 mg/day and oral hydrocortisone replacement at 10 mg/day (5 mg in the morning and 5 mg in the evening) (Fig. 1). Magnetic resonance imaging of the pituitary gland revealed an empty sella turcica (Fig. 2). Abdominal computed tomography detected no abnormalities in the adrenal glands, which did not contain a tumor or exhibit atrophy or calcification (Fig. 3). The patient tested negative for anti-pituitary cell antibody, thyroid peroxidase antibody (7 IU/mL; reference range, <16 IU/mL), thyroglobulin antibody (12 IU/mL; reference range, <28 IU/mL), and anti-adrenocortical antibody (titer <1:10), but tested positive for both thyroid-stimulating hormone receptor antibody (37.7 IU/L; reference range, <1.0 IU/L) and thyroid stimulating antibody (693%; reference range, <180%). In addition, she tested negative for glutamic acid decarboxylase antibody (<1.3 U/mL), insulin antibody (<125.0 nU/mL), gastric parietal cell antibody (titer <1:10), anti-nuclear antibody (titer <1:40), and rheumatoid factor (<5 IU/mL). Human leukocyte antigen (HLA) typing showed the presence of A*02:26, B*15:40, and C*01:02:08:01 class I genes and DRB1*09:01/15:01, DQB1*03:03/06:02, DQA1*01:02/03:02, and DPB1*02:01/05:01 class II genes.

To further explore the plasma ACTH level, ACTH in a plasma sample collected at 8 AM on day 21 of admission (Table 2D) was assayed using IRMA, FEIA, and ICT-EIA I, all of which showed that the ACTH level was high (Table 3). In contrast, γ3-MSH was undetectable (Table 2D, 3).

The size of ACTH was determined by gel exclusion chromatography of the plasma sample collected at 8 AM on day 21 of admission. The elution profile (Fig. 4) featured a single peak at approximately the authentic ACTH 1-39 position (fractions 28 to 30). "Big ACTH," which would appear at fraction numbers 14 to 20 (8, 9, 24), was not observed.

The patient experienced improvements in fatigue and moist skin, and regained her previous vigor and appetite. She was discharged 27 days after admission, in March 2013. Two months later, the patient's plasma ACTH levels before and after a CRH load remained high, but she still exhibited cortisol deficiency (Table 2F) and continued hydrocortisone replacement.

In August 2013, her body weight returned to 40 kg, her essential hypertension reappeared, and the patient restarted antihypertensive medication. Dynamic tests performed in a euthyroid state showed normal release of GH, TSH, prolactin, LH, and FSH. The patient's plasma ACTH levels increased above 90 pg/mL after a CRH load, however, no cortisol response was observed (Table 2G). Her basal serum dehydroepiandrosterone-surface (DHEA-S) level decreased, while her basal serum aldosterone level remained normal

Figure 1. Clinical course. Reference ranges for body weight, HbA1c (NGSP), and plasma adrenocorticotrophic hormone (ACTH) levels measured by an electro-chemiluminescence immunoassay (ECLIA), plasma renin activity (PRA), serum cortisol, dehydroepiandrosterone sulfate (DHEA-S), aldosterone, thyroid-stimulating hormone (TSH), free triiodothyronine (FT₃), free thyroxine (FT₄), and thyroid-stimulating hormone receptor antibody (TRAb) are shown in square brackets. All of the blood samples were taken at 8:00 AM with the patient in the supine position, except for those in February 2013, when the samples were taken at 3:00 PM with the patient in a hypoglycemic coma. The reference range for body weight (kg) was calculated as follows: [body height (m)²×18.5] to [body height (m)²×25]. * The patient received a regimen of 8 days of oral prednisolone (10 mg/day for 3 days followed by 5 mg/day for 5 days) for an exacerbation of her bronchial asthma.

(Fig. 1).

In December 2014, her HbA1c levels were elevated to above 7%, and she started treatment with an oral hypoglycemic agent. In February 2015, dynamic tests performed in a subclinical primary hypothyroid state showed an excessive TSH response and normal release of GH, prolactin, LH, and FSH. Significant plasma ACTH release was observed after a CRH load, while no cortisol release was detected (Table 2H). These serial changes in the patient's pituitary and adrenocortical hormone levels, including the fall in serum cortisol but continued aldosterone secretion during 2 years of hydrocortisone replacement (Fig. 1), were not consistent with Addison's disease or a recovery process from glucocorticoid-induced AI and rather indicated a diagnosis of IAD, in which biochemically measurable ACTH with low bioactivity that differed from "big ACTH" might have been present.

The patient continued treatment with hydrocortisone replacement and antithyroid drugs, and her subsequent clinical course has remained uneventful without any additional complications.

Discussion

An elderly Japanese woman with a 4-month history of fatigue, exertional dyspnea, lethargy, and anorexia was diagnosed with thyrotoxicosis due to Graves' disease during 1-week treatment with a supraphysiological dose (5–10 mg/day) of oral prednisolone for her exacerbated bronchial asthma, which had been treated with inhaled corticosteroid therapy with a physiological dose (200 μg/day) of ciclesonide (27). The day after completing this corticosteroid regimen, she developed a hypoglycemic coma with mild glucocorticoid-induced AI (Table 1) and underwent intravenous glucose infusion with physiological doses of oral hydrocortisone (replacement therapy). A few days later, she had a low serum cortisol and a high plasma ACTH level (Table 2A). Because the patient was anticipated to recover from glucocorticoid-induced AI, she was continued on hydrocortisone replacement, and her plasma ACTH level remained elevated for >3 months. However, both her serum cortisol and DHEA-S (28) levels further decreased during that period (Fig. 1). In addition, 2 years later, her plasma ACTH levels before and after a CRH load appeared to be

Table 2. Endocrinological Investigation.**A. Morning and midnight serum cortisol and plasma ACTH levels in February 2013 (day 4 after admission)**

	8:00 AM	12:00 PM
Plasma ECLIA-ACTH (pg/mL)	80.4	45.4
Serum cortisol (μg/dL)	6.5	2.0

Blood samples were taken under hydrocortisone replacement therapy at 5 mg/day (5 mg at 8:30 AM).

B. Rapid ACTH stimulation test in February 2013 (day 7 after admission)

	Before	30 min	60 min
Serum cortisol (μg/dL)	5.8	9.0	10.2
Serum aldosterone (ng/dL)	3.6	5.7	4.9
Plasma ECLIA-ACTH (pg/mL)	67.7	62.7	63.4

Synthetic ACTH 1-24 (cosyntropin hydroxide 0.25 mg) was intravenously administered in the morning (8:00 AM). The test was conducted after cessation of hydrocortisone replacement therapy (5 mg/day) for a day.

C. Prolonged ACTH stimulation test in February 2013 (days 13 to 16 after admission)

	Before	3 days	Reference range
Serum cortisol (μg/dL)	5.0	26.3	(10.0–25.0)
Urinary free cortisol excretion (μg/day)	8.3	255.0	(11.2–80.3)
Serum aldosterone (ng/dL)	5.0	5.4	(3.0–15.9)
Plasma renin activity (ng/mL/h)	1.0	0.2	(0.1–2.0)
Plasma ECLIA-ACTH (pg/mL)	87.3	11.0	(7.2–63.3)

Blood and urine samples were taken each morning (8:00 AM) of the 2 days before and after intramuscular administration of synthetic ACTH 1-24 (cosyntropin zinc hydroxide 1.0 mg/day) for 3 days. Hydrocortisone replacement therapy (5 mg/day) was briefly discontinued the day before and for the duration of test.

D. CRH/GRF/TRH/LHRH stimulation test in March 2013 (day 21 after admission)

	Time (min)					
	0	15	30	60	90	120
Plasma ECLIA-ACTH (pg/mL)	77.2	78.5	88.3	94.4	100.6	88.3
Plasma γ3-MSH (pg/mL)	< 30.0	< 30.0	< 30.0	< 30.0	< 30.0	< 30.0
Serum cortisol (μg/dL)	7.1	7.1	6.7	7.2	7.5	7.1
Serum TSH (μU/mL)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Serum GH (ng/mL)	1.2	10.5	17.1	21.0	11.9	6.2
Serum prolactin (ng/mL)	12.4	27.6	36.9	32.9	26.7	23.1
Serum LH (mIU/mL)	10.4	16.2	22.6	27.0	29.8	29.6
Serum FSH (mIU/mL)	31.8	33.8	37.1	36.8	39.9	41.3

The following were intravenously administered in the morning (8:00 AM): CRH 100 μg, GRF 100 μg, TRH 500 μg, and LHRH 100 μg. The test was conducted after cessation of hydrocortisone replacement therapy (5 mg/day) for a day. The patient had a serum level of estradiol of 5.0 pg/mL (reference range, <39.0 pg/mL). As shown in Table 1, the patient had primary hyperthyroidism due to Graves' disease.

E. Data from the GHRP-2 stimulation test performed on March 2013 (day 24 after admission).

	Time (min)				
	0	15	30	45	60
Plasma ECLIA-ACTH (pg/mL)	75.8	117.1	108.1	88.3	89.5
Serum cortisol (μg/dL)	7.9	9.5	9.2	9.4	9.2
Serum GH (ng/mL)	1.5	33.8	31.2	21.2	13.8
Serum prolactin (ng/mL)	12.1	18.3	17.8	16.8	16.4

GHRP-2 (100 μg) was intravenously administered in the morning (8:00 AM). The test was conducted after cessation of hydrocortisone replacement therapy (5 mg/day) for a day.

normal under the same hydrocortisone replacement regimen, but she still exhibited cortisol and DHEA-S deficiency (Table 2H). These findings were not explained by a recovery process from glucocorticoid-induced AI, but rather indicated a diagnosis of secondary AI accompanied by bioinactive ACTH. Because a dynamic test performed in a euthyroid state confirmed normal secretion of pituitary hormones, with the exception of the inconsistency between the high plasma ACTH levels and no cortisol response (Table 2G), our patient likely developed IAD with a substantial amount of biochemically measurable ACTH with little steroidogenic bioactivity.

Primary AI is characterized by a deficiency of all adrenal hormones, including the mineralocorticoids. As our patient had normal serum aldosterone levels throughout her clinical course (Fig. 1), it is unlikely that our patient had primary AI (3). In addition, the adequate cortisol response to an exogenous ACTH load (Table 2C) indicated that the adrenal gland ACTH receptors were intact.

Table 4 shows a summary of previously reported patients who exhibited concurrent IAD and Graves' disease. These cases include adults of both sexes who were diagnosed nearly simultaneously with both IAD and Graves' disease that were manifested as fatigue and appetite loss, with or

F. Data from the CRH/GRF/TRH/LHRH stimulation test performed in May 2013

	Time (min)					
	0	15	30	60	90	120
Plasma ECLIA-ACTH (pg/mL)	65.6	78.2	84.9	104.0	96.3	95.1
Serum cortisol (μg/dL)	6.1	5.5	5.7	6.3	6.2	6.2
Serum TSH (μU/mL)	2.91	24.80	35.82	30.41	22.25	16.76
Serum GH (ng/mL)	1.9	9.8	7.4	3.5	1.9	1.2
Serum prolactin (ng/mL)	9.9	42.7	48.0	32.5	23.6	18.9
Serum LH (mIU/mL)	11.4	16.4	20.6	25.1	25.5	24.3
Serum FSH (mIU/mL)	28.3	29.2	30.4	31.1	31.2	34.6

The following were intravenously administered in the morning (8:00 AM): CRH 100 μg, GRF 100 μg, TRH 500 μg, and LHRH 100 μg. The test was conducted after cessation of hydrocortisone replacement therapy (10 mg/day) for half a day. The patient was clinically euthyroid, under treatment for Graves' disease with oral thiamazole 10 mg/day.

G. CRH/GRF/TRH/LHRH stimulation test in August 2013

	Time (min)					
	0	15	30	60	90	120
Plasma ECLIA-ACTH (pg/mL)	51.8	66.2	73.8	94.1	85.8	79.1
Serum cortisol (μg/dL)	3.6	3.2	3.3	4.1	4.4	4.0
Serum TSH (μU/mL)	2.11	15.17	22.69	17.45	13.16	9.71
Serum GH (ng/mL)	0.5	5.8	4.3	2.1	1.6	1.4
Serum prolactin (ng/mL)	9.6	36.9	46.5	31.5	24.0	18.6
Serum LH (mIU/mL)	11.9	15.6	20.7	25.2	27.1	25.0
Serum FSH (mIU/mL)	31.6	32.6	34.1	34.5	38.1	37.8

The following were intravenously administered in the morning (8:00 AM): CRH 100 μg, GRF 100 μg, TRH 500 μg, and LHRH 100 μg. The test was conducted after cessation of hydrocortisone replacement therapy (10 mg/day) for half a day. The patient was clinically euthyroid, under treatment for Graves' disease with oral thiamazole 7.5 mg/day.

H. CRH/GRF/TRH/LHRH stimulation test in February 2015

	Time (min)					
	0	15	30	60	90	120
Plasma ECLIA-ACTH (pg/mL)	31.6	44.5	44.6	65.2	60.1	52.7
Plasma γ3-MSH (pg/mL)	< 30.0	< 30.0	< 30.0	< 30.0	< 30.0	< 30.0
Serum cortisol (μg/dL)	3.4	3.2	2.9	3.3	3.3	3.2
Serum TSH (μU/mL)	6.02	54.34	73.07	55.44	39.87	26.94
Serum GH (ng/mL)	0.74	10.10	7.85	4.87	2.13	0.97
Serum prolactin (ng/mL)	10.6	50.1	57.5	38.7	26.4	19.4
Serum LH (mIU/mL)	8.8	12.0	16.3	19.6	20.8	18.7
Serum FSH (mIU/mL)	18.3	19.5	19.1	20.2	20.7	20.4

The following were intravenously administered in the morning (8:00 AM): CRH 100 μg, GRF 100 μg, TRH 500 μg, and LHRH, 100 μg. The test was conducted after cessation of hydrocortisone replacement therapy (10 mg/day) for half a day. The patient had primary subclinical hypothyroidism, under treatment for Graves' disease with oral thiamazole 10 mg/day.

ACTH: adrenocorticotropic hormone, CRH: human corticotropin-releasing hormone, ECLIA: electro-chemiluminescence immunoassay, FSH: follicle-stimulating hormone, GH: growth hormone, GHRP: growth hormone-releasing peptide, GRF: growth hormone-releasing factor, LH: luteinizing hormone, LHRH: gonadotropin-releasing hormone, MSH: melanocyte-stimulating hormone, TRH: thyrotropin-releasing hormone, TSH: thyroid-stimulating hormone

without hypoglycemic attack. The patients exhibited various plasma ACTH levels measured by different assays, and some patients had empty sella turcica.

At the time of IAD development, our patient had a high plasma ACTH level, as measured using various bioassays, including ECLIA, IRMA, FEIA, and ICT-EIA I (18-22) (Table 3). A gel filtration analysis with conventional ACTH-RIA did not detect "big ACTH" in the plasma, but detected peptides that were roughly the same size as authentic ACTH 1-39 (Fig. 4). These findings were similar to those of previously reported cases of secondary AI in which high plasma ACTH levels, but not "big ACTH", were found using different assays (6-9). In the present case, the absence of "big ACTH" was confirmed by the finding that γ3-MSH was undetectable (23) (Table 3). Several studies have shown that conventional ACTH-RIA employing polyclonal antibodies can detect not only authentic ACTH 1-39, pro-ACTH, and POMC, but also ACTH fragments with the loss of several

N-terminal amino acids, such as ACTH 7-39 (29). A recent study also suggested that the ACTH-ECLIA and FEIA can identify ACTH fragments with complete C-terminal regions, even when several N-terminal amino acids are absent (30). However, the biological activity of the ACTH molecule is dependent on the first 24 N-terminal amino acids (ACTH 1-24), but the shorter peptides of less than 20 amino acids are ineffective (1, 2, 31). Thus, our patient might have exhibited ACTH fragments with a deficit of several N-terminal amino acids in the plasma.

An empty sella turcica is defined as an intrasellar herniation of the suprasellar subarachnoid space with compression of the pituitary gland (32). It can be caused by pituitary surgery or radiation, but many cases are of unknown etiology. There are some reported cases of an empty sella turcica in IAD patients with or without Graves' disease (14, 16, 33). Our patient was found to have an empty sella turcica (Fig. 2) without any verifiable causes, concomitant with

Figure 2. Magnetic resonance imaging of the pituitary gland (February 2013). Gadolinium-enhanced T1-weighted magnetic resonance imaging of the brain (A: coronal plane; B: sagittal plane) showed an enlarged sella turcica filled with cerebrospinal fluid, indicating that the sella was empty. No abnormalities were evident in the hypothalamus or hypophyseal stalk.

Figure 3. Computed tomography of the adrenal glands (February 2013). A plain computed tomography scan of the abdomen revealed no abnormality in either the right or left adrenal gland (long and short arrows, respectively).

Graves' disease, while an etiological relationship between the patient's empty sella turcica and IAD remained uncertain.

Polyglandular autoimmune syndrome (PGAS) type 3 is a group of endocrine or nonendocrine organ-specific autoimmune disorders, including autoimmune thyroid diseases, for which genetic factors are involved in the pathogenesis (34). Our patient had an HLA-DRB1*09:01-DQB1*03:03-DQA1*03:02 haplotype, which increases the susceptibility of Japanese individuals to PGAS type 3 (35). Therefore, although our patient tested negative for the anti-pituitary cell antibody, the presence of Graves' disease suggested an autoimmune etiology for her IAD.

The typical symptoms of thyrotoxicosis include fatigue, exertional dyspnea, hyperactivity, and body weight loss with or without an increased appetite; however, older patients may sometimes present with lethargy and anorexia (36). Our patient did not experience any improvement in her fatigue, lethargy, or anorexia during the 1-week treatment regimen with supraphysiological doses of oral prednisolone therapy

for her bronchial asthma and thereafter developed severe hypoglycemia accompanied by mild AI after completion of the prednisolone therapy. Therefore, she did not have clinical AI prior to prednisolone therapy, and her fatigue, lethargy, and anorexia were likely attributable to Graves' hyperthyroidism. On the other hand, 1 week of supraphysiological prednisolone administration seldom causes glucocorticoid-induced AI (37). However, both hyperthyroidism and the prior use of physiological doses of corticosteroids, irrespective of the route of administration, may increase susceptibility to glucocorticoid-induced AI by increasing the rate of glucocorticoid metabolism (38) or by potentially exerting antisecretory effects on CRH and ACTH (1, 2). Additionally, malnutrition caused by anorexia *per se* can cause hypoglycemia (39). Taken together, a combination of previously inhaled ciclesonide, the presence of thyrotoxicosis and anorexia, and the taking of transient supraphysiological doses of prednisolone, might have induced severe hypoglycemia accompanied by mild glucocorticoid-induced AI in the present case (Table 1).

Our patient had an adequate plasma ACTH to serum cortisol ratio of approximately 4 (6, 40) during the hypoglycemic coma, and the ACTH to cortisol ratio rapidly increased to >10 within a few days (Table 1, 2A). On the other hand, abnormal processing of POMC in the anterior pituitary can occur at both the translational and the (enzymatic) post-translational levels (41). These findings suggest that, although the precise mechanism is unknown, our elderly patient developed IAD, following mild glucocorticoid-induced AI, and synthesized a bioinactive ACTH that might have been aberrantly (enzymatically) processed after translation.

GHRP-2, a synthetic agonist of ghrelin, is a potent growth hormone secretagogue and also stimulates the secretion of ACTH and cortisol in humans (42). The GHRP-2 test has been shown to be a diagnostic tool for secondary AI (43, 44). At the time of IAD development, our patient exhibited a delayed (and slight) ACTH release to exogenous CRH (Table 2D), and in contrast, a rather rapid ACTH re-

Table 3. Basal Levels of Plasma ACTH Measured Using Different Bioassays, and the Levels of Plasma γ 3-MSH.

	March 2013	August 2013	February 2015	Unit	Reference range
ECLIA-ACTH	77.2	51.8	31.6	pg/mL	7.2–63.3
FEIA-ACTH	64.1	44.5	28.0	pg/mL	7.7–63.1
IRMA-ACTH	59.5	42.5	25.9	pg/mL	7.4–55.7
ICT-EIA I-ACTH	81.1	42.6	21.6	pg/mL	4.0–45.0
RIA- γ 3-MSH	< 30.0	< 30.0	< 30.0	pg/mL	< 30.0

Blood samples were taken in the morning (8:00 AM), after cessation of hydrocortisone replacement for more than half a day, in March 2013 (day 21 after admission); in August 2013; and in February 2015, as shown in Table 2D, G, and H, respectively.

ACTH: adrenocorticotrophic hormone, ECLIA: electro-chemiluminescence immunoassay, FEIA: fluorescence enzyme immunoassay, ICT-EIA: immune complex transfer enzyme immunoassay, IRMA: immunoradiometric assay, MSH: melanocyte-stimulating hormone, RIA: radioimmunoassay

Figure 4. The gel filtration profile of the patient's plasma (March 2013). The arrows indicate the elution positions of authentic adrenocorticotrophic hormone (ACTH) 1-39, void volume (Vo), and total volume (Vt). The numbers on the x axis represent the fraction numbers.**Table 4. Summary of Reported Patients who Presented with Concomitant Graves' Disease and Isolated Adrenocorticotropin Deficiency.**

Case	Sex	Major symptoms	Graves' disease			Isolated adrenocorticotropin deficiency							Ref.
			Age at diagnosis (years)	TRAb or TSAb	Palpable struma	Exophthalmos	Age at diagnosis (years)	Basal serum cortisol levels (μ g/mL)*	Basal plasma ACTH levels (pg/mL)*	Pituitary autoantibodies	MRI findings of pituitary gland	Complicating disorders	
1	Female	Fatigue, appetite loss, palpitation, hypoglycemia	43	Positive	(+)	(–)	43	Undetectable	Undetectable	Negative	Empty sella	None	14
2	Female	Fatigue, appetite loss	47	Positive	(+)	(+)	47	4.1	< 5	Negative	N.A.	Systemic lupus erythematosus	15
3	Male	Fatigue, body weight loss	60	Positive	(–)	(–)	60	0.9	6.8	Negative	Empty sella	None	16
4	Male	Appetite loss, palpitation	73	Positive	(–)	(–)	73	3.9	9.0	N.A.	No abnormalities	None	17
5	Female	Fatigue, lethargy, appetite loss, hypoglycemia	73	Positive	(+)	(–)	73	5.0	87.3	Negative	Empty sella	Bronchial asthma, hypertension, T2DM	Present case

*Basal cortisol and adrenocorticotrophic hormone (ACTH) levels were measured at the time of diagnosis of isolated adrenocorticotropin deficiency by different bioassays (6) from case to case.

The case 3 patient underwent treatment with oral thiamazole and cortisone acetate for hyperthyroidism and adrenal insufficiency from the age of 47 years until the diagnoses of isolated adrenocorticotropin deficiency and Graves' disease.

MRI: magnetic resonance imaging, N.A.: not available, TRAb: thyroid-stimulating hormone receptor antibody, TSAb: thyroid stimulating antibody, T2DM: type 2 diabetes mellitus

lease was observed after GHRP-2 loading (Table 2E), neither of which triggered a significant cortisol response. These findings suggest that GHRP-2 may have stimulated the impaired corticotroph cells via pathways other than the CRH receptor, under conditions of almost full endogenous CRH stimulation.

In conclusion, our elderly patient with Graves' hyperthyroidism and anorexia developed hypoglycemia accompanied by mild glucocorticoid-induced AI after a supraphysiological dose of oral prednisolone therapy for bronchial asthma. Shortly thereafter, she developed IAD with high plasma ACTH measurements, for which bioinactive ACTH that was not "big ACTH," such as ACTH fragments with a deficit of several N-terminal amino acids, was likely responsible. The present case suggests that IAD may develop in association with glucocorticoid-induced AI. In addition, IAD should therefore be considered as an unusual complicating disorder in patients with Graves' disease, especially in older adults.

The authors state that they have no Conflict of Interest (COI).

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