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Brief Report

Clinical Usefulness of the Growth Hormone–Releasing Peptide-2 Test for Hypothalamic-Pituitary Disorder

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Abstract

Context: Growth hormone deficiency (GHD) develops early in patients with hypothalamic-pituitary disorder and is frequently accompanied by other anterior pituitary hormone deficiencies, including secondary adrenal insufficiency (AI). A growth hormone–releasing peptide-2 (GHRP2) test, which is widely used for the diagnosis of patients with GHD, is thought to induce release of not only growth hormone (GH) but also ACTH. However, its clinical usefulness in hypothalamic-pituitary disorder is unclear.

Objective: We aimed to determine the clinical utility of the GHRP2 test in patients with hypothalamic-pituitary disorders, particularly for AI concomitant with GHD.

Methods: The GHRP2 test, a cosyntropin stimulation test, corticotropin-releasing hormone (CRH) tests, and/or insulin tolerance tests (ITTs) were performed on 36 patients with hypothalamic-pituitary disorder.

Results: Twenty-two (61%) had severe GHD, and 3 (8%) had moderate GHD by GHRP2. There was no difference in baseline ACTH and cortisol between non-GHD, moderate GHD, and severe GHD participants. However, a cosyntropin stimulation test and subsequent CRH tests and/or ITTs revealed that 17 (47%) had secondary AI and 16/17 (94%) cases of secondary AI were concomitant with severe GHD. ROC curve analysis demonstrated that the ACTH response in the GHRP2 test was useful for screening pituitary-AI, with a cutoff value of 1.55-fold (83% sensitivity and 88% specificity). Notably, the combination of ACTH response and the peak cortisol level in the GHRP2 test using each cutoff value (1.55-fold and 10 μg/dL, respectively) showed high specificity (100%) with high accuracy (0.94) for diagnosis of pituitary-AI.

Conclusion: We recommend measuring ACTH as well as GH during the GHRP2 test to avoid overlooking or delaying diagnosis of secondary AI that frequently accompanies GHD.

Key Words: growth hormone–releasing peptide-2, growth hormone deficiency, pituitary adrenal insufficiency

Abbreviations: ACTH, adrenocorticotropic hormone; AI, adrenal insufficiency; AUC, area under the curve; AVP, arginine vasopressin; CRH, corticotropin-releasing hormone; GH, growth hormone; GHD, growth hormone deficiency; GHRP2, growth hormone-releasing peptide-2; ITT, insulin tolerance test; ROC, receiver operating characteristic.

Most of the adult-onset growth hormone deficiency (GHD) were known to be caused by a pituitary or parasellar tumor, and/or the consequences of treatment of the tumor including surgery or radiation therapy [1]. Therefore, patients with GHD may have additional anterior pituitary hormone deficits, notably rendering them at risk of developing adrenocorticotropic hormone (ACTH) deficiency [2]. Since secondary adrenal insufficiency (AI), including ACTH deficiency, is a life-threatening disorder [3, 4], prompt diagnosis and management of this disease are essential. However, a diagnosis of secondary AI can be missed due to normal or slightly altered ACTH and cortisol levels [5]. Many patients with equivocal basal cortisol levels may require a dynamic test for correct diagnosis and then cosyntropin stimulation test, corticotropin-releasing hormone (CRH) test, or an insulin tolerance test (ITT) may be first options being most validated in clinical use [6, 7]. However, a cosyntropin stimulation test is helpful if the test is abnormal, but a normal cortisol response does not rule out secondary AI, especially in the first 6 months of disease before adrenal atrophy is established [8, 9]. A systematic review and meta-analysis of the accuracy of cosyntropin stimulation test to diagnose secondary AI reported a high specificity but low sensitivity of 64% to 83% [10]. ITT has generally been regarded as the reference standard test for assessment hypothalamic-pituitary-adrenal axis including acute secondary AI [8, 9]. However, the result cannot be interpreted unless adequate hypoglycemia is achieved, and the test should not be undertaken in patients

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with a history of heart disease, epilepsy, or unexplained blackout; caution must also be shown in older patients [11]. In addition, the cutoffs for the CRH test are less well defined. Therefore, a need exists for another low-risk, practical, sensitive, specific, and reproducible test for the investigation of secondary AI [7, 12]. A growth hormone-releasing peptide-2 (GHRP2) is used for the evaluation of the capacity of growth hormone (GH) secretion, and for the diagnosis of GHD. Additionally, GHRP2 has also been considered to be a unique test that stimulates both somatotroph and corticotrope cells and induces ACTH release through GH secretagogue receptors in the hypothalamus [13-18]. There is a possibility that the GHRP2 test could be used in the detecting of secondary AI. However, it is not clear what the additional benefits are beyond the traditional tests of cosyntropin stimulation, CRH, and ITT. The present study proposes how to use the GHRP2 test as a supplement to the traditional tests in patients with concomitant AI and GHD.

Methods

Patient Characteristics and Clinical Definitions

This study was approved by the Human Research Ethics Committee at Chiba University (approval number: 3652). Thirty-six patients (17 male and 19 female; age 18-81 years) were referred to Chiba University Hospital between 2010 and 2019 for the evaluation of hypopituitarism: pituitary adenoma (7 cases), Rathke cleft cyst (5 cases), surgery of the pituitary adenoma (9 cases), radiation therapy of the head and neck carcinoma (4 cases of germinoma, 1 case of neuroblastoma, and 1 case of upper pharynx cancer), hypophysitis (5 cases), pituitary stalk interruption (2 cases), pituitary apoplexy (1 case) and Sheehan's syndrome (1 case). Thirty-six patients were hospitalized and all received GHRP2 and cosyntropin stimulation tests, 35 received a CRH test, and 28 received ITT (Table 2). GHD was diagnosed based on a GHRP2 test and classified as non-GHD (peak GH > 16 ng/mL), moderate GHD (9 ng/mL < peak GH ≤ 16 ng/mL) and severe GHD (peak GH ≤ 9 ng/mL) as previously reported [19, 20]. The patients were evaluated for AI (adrenal, pituitary, or hypothalamic) using the results of a cosyntropin stimulation test followed by a CRH test and/or ITT according to the guidelines advocated by the Japanese Society of Endocrinology [6]. Briefly, normal response in a cosyntropin stimulation test was defined as peak serum cortisol level higher than 18 μg/dL [6, 21]. A positive response in CRH test and ITT were defined as more than 2-fold increase in ACTH level compared with baseline [6, 14] and peak serum cortisol level higher than 18 μg/dL [6]. Pituitary-AI was diagnosed with negative responses in both cosyntropin stimulation and CRH test. Hypothalamic-AI was diagnosed with a negative cortisol response in both cosyntropin stimulation test and ITT but a positive response in CRH test. Adrenal-AI was diagnosed with a negative response in cosyntropin stimulation test but positive ACTH response in CRH test and/or ITT.

Provocation Tests and Hormonal Assays

The provocation tests were performed following an overnight fast and 30 minutes of rest in a supine position. Serum GH, plasma ACTH, and serum cortisol were measured by electrochemiluminescence immunoassay (ECLIA), immunoradiometric assay (Mitsubishi Chemical), and radioimmunoassay (RIA), respectively.

Cosyntropin Stimulation Test

Blood samples were collected before injection and at 30 and 60 minutes after intravenous bolus injection of a 250-μg dose of synthetic 1-24 ACTH (cosyntropin).

GHRP2 Test

Blood samples were collected before injection and at 15, 30, and 60 minutes after intravenous bolus injection of a 100-μg dose of GHRP2. In this provocation test, plasma ACTH and serum cortisol were measured by immunoradiometric assay (Mitsubishi Chemical) and radioimmunoassay (RIA), respectively.

CRH Test

Blood samples were collected before injection and at 15, 30, 60, 90, and 120 minutes after intravenous bolus injection of a 100-μg dose of CRH.

Insulin Tolerance Test

Blood samples were collected before injection and at 15, 30, 60, 90, and 120 minutes after intravenous bolus injection of human regular insulin at a dose of 0.05 to 0.20 U/kg body weight. Only samples with plasma glucose levels below 50 mg/dL in ITTs after the injection were included in further analyses.

Data Analyses

The Shapiro-Wilk test showed that the endocrinological data were not normally distributed. Hence, pairwise comparisons were performed using the Mann-Whitney U-test. A value of $P < 0.05$ was considered statistically significant. Statistical analysis was performed using SPSS Statistics for Windows (SPSS Inc., Chicago, IL, USA). Receiver operating characteristic (ROC) analyses were used to assess the cutoff points for predicting various categories of AI after GHRP2 administration. Sensitivity and specificity were calculated at cutoff values providing the maximum Youden index (Sensitivity + Specificity − 1). The area under the ROC curve (AUC) was also calculated. In general, an AUC of 0.5 suggests no discrimination, 0.7 to 0.8 is considered acceptable, 0.8 to 0.9 is considered good, and above 0.9 is considered excellent.

Results

Severe GHD Was Associated With a High Rate of Secondary AI

A GHRP2 test was performed on 36 patients with suspected hypopituitarism; among them, 22 (61%) were found to have severe GHD and 3 (8%) were diagnosed with moderate GHD (Table 1). There was no difference and wide scatter in baseline ACTH and cortisol values between non-GHD, moderate GHD, and severe GHD participants (Table 2, Fig. 1A and 1B). However, a cosyntropin stimulation test and subsequent CRH test and/or ITT revealed that there were 6 cases of pituitary-AI and 10 cases of hypothalamic-AI among severe GHD patients, and there was 1 case of pituitary-AI among non-GHD patients (Table 1, Fig. 1C). Namely, 94% (16/17) of cases of secondary AI were found in severe GHD patients.

Table 1. Clinical data of the patients

No.	Type of GHD	Type of AI	Age (y)	Sex	Etiology	Impaired function	Hormone replacement	MRI findings
#1	Non-GHD	Non-AI	60	F	Pituitary adenoma (GHoma)	LH/FSH	-	8.3 mm sellar mass
#2	Non-GHD	Non-AI	61	F	Pituitary adenoma (GHoma)	-	-	22 mm ectopic pituitary mass in the left cavernous sinus
#3	Non-GHD	Non-AI	18	M	Rahke`s cleft cyst	-	-	6 mm non-enhancing cystic area in the pituitary
#4	Non-GHD	Non-AI	45	F	Rahke`s cleft cyst	-	-	8 mm non-enhancing cystic area in the pituitary
#5	Non-GHD	Non-AI	30	F	Surgery of pituitary adenomawith pituitary apoplexy(NF)	-	-	Pituitary apoplexy with high T1 and low T2 signal (BS) →No abnormalities (AS)
#6	Non-GHD	Non-AI	32	F	Surgery of pituitary adenoma (CD)	-	-	7 mm sellar mass (BS) →No abnormalities (AS)
#7	Non-GHD	Non-AI	50	M	Surgery of pituitary adenoma (CD)	-	-	No abnormalities (BS) →No abnormalities (AS)
#8	Non-GHD	Non-AI	34	F	Radiation therapy of germinoma	AVP	Desmopressin acetate	Destruction of the local normal parts of the pituitary gland
#9	Non-GHD	Non-AI	31	F	Hypoysitis	-	-	Enlargement of the pituitary gland
#10	Non-GHD	Non-AI	64	F	Hypoysitis	AVP	Desmopressin acetate	Disappearance of T1-weighted high intensity signal of pituitary posterior lobe
#11	Non-GHD	Pituitary-AI	37	F	Hypoysitis	ACTH/PRL	Glucocorticoid	Empty sella
#12	Moderate GHD	Non-AI	58	M	Rahke`s cleft cyst	GH	-	9 mm non-enhancing cystic area in the pituitary
#13	Moderate GHD	Non-AI	45	F	Radiation therapy of neuroblastoma	GH	-	14 mm heterogeneous enhancing mass in the frontal lobe
#14	Moderate GHD	Non-AI	48	F	Hypoysitis	GH/LH/FSH	-	Enlargement of the pituitary gland and the thickening of the pituitary stalk
#15	Severe GHD	Pituitary-AI	66	M	Pituitary adenoma (NF)	GH/ACTH/TSH/LH/FSH	Glucocorticoid Levothyroxine sodium	22 mm sellar mass with suprasellar extension
#16	Severe GHD	Pituitary-AI	56	F	Pituitary adenoma (NF)	GH/ACTH/TSH/LH/FSH	Glucocorticoid Levothyroxine sodium	20 mm sellar mass with suprasellar and third ventricle extension
#17	Severe GHD	Pituitary-AI	66	M	Pituitary adenoma (NF)	GH/ACTH/TSH/LH/FSH	Glucocorticoid Levothyroxine sodium	17 mm sellar mass with cystic change
#18	Severe GHD	Pituitary-AI	18	M	Radiation and chemotherapy of germinoma	GH/ACTH/TSH/LH/FSH/PRL/AVP	Glucocorticoid Levothyroxine sodium Sex steroid Desmopressin acetate	10 mm peripherally slight enhancing cystic suprasellar mass
#19	Severe GHD	Pituitary-AI	43	F	Radiation and surgery of germinoma	GH/ACTH/TSH/LH/FSH/AVP	Glucocorticoid Levothyroxine sodium Sex steroid Desmopressin acetate	Empty sella with 5 mm suprasellarcystic area
#20	Severe GHD	Pituitary-AI	81	M	Hypoysitis	GH/ACTH/TSH/LH/FSH	Glucocorticoid Levothyroxine sodium Growth hormone	Enlargement of the pituitary gland and the thickening of the pituitary stalk
#21	Severe GHD	Hypothalamic-AI	59	F	Pituitary adenoma (NF)	GH/ACTH	Glucocorticoid	15 mm sellar mass

Table 1. Continued

No.	Type of GHD	Type of AI	Age (y)	Sex	Etiology	Impaired function	Hormone replacement	MRI findings
#22	Severe GHD	Hypothalamic-AI	26	M	Congenital abnormalities (injury sustained during a birth)	GH/ACTH/TSH/LH/FSH/PRL	Glucocorticoid Levothyroxine sodium Sex steroid Growth hormone	Undetectable of the normal pituitary gland and the pituitary stalk
#23	Severe GHD	Hypothalamic-AI	40	M	Congenital abnormalities (injury sustained during a birth)	GH/ACTH/TSH/LH/FSH	Glucocorticoid Levothyroxine sodium Sex steroid Growth hormone	Remarkably small pituitary gland
#24	Severe GHD	Hypothalamic-AI	26	M	Radiation therapy of germinoma	GH/ACTH/TSH/LH/FSH/PRL/AVP	Glucocorticoid Levothyroxine sodium Growth hormone Sex steroid Desmopressin acetate	A small enhancing area in the sella and corpus callosum
#25	Severe GHD	Hypothalamic-AI	40	M	Radiation therapy of upper pharynx cancer	GH/ACTH/TSH	Glucocorticoid Levothyroxine sodium	No abnormalities
#26	Severe GHD	Hypothalamic-AI	71	F	Rathke cleft cyst	GH/ACTH/TSH/LH	Glucocorticoid Levothyroxine sodium	17 mm non-enhancing cystic area in the pituitary
#27	Severe GHD	Hypothalamic-AI	20	M	Rathke cleft cyst	GH/ACTH/TSH/LH/PRL/AVP	Glucocorticoid Levothyroxine sodium Growth hormone Desmopressin acetate	28 mm non-enhancing cystic area in the pituitary
#28	Severe GHD	Hypothalamic-AI	29	M	Surgery of pituitary adenoma (NF)	GH/ACTH/TSH	Glucocorticoid Levothyroxine sodium	38 mm dumbbell-shaped sellar mass, extending into the cavernous sinus (BS) →15 mm residual mass in the right side of the pituitary gland around the internal carotid artery (AS)
#29	Severe GHD	Hypothalamic-AI	39	M	Surgery of pituitary adenoma (NF)	GH/ACTH/TSH/LH/FSH/AVP	Glucocorticoid Levothyroxine sodium Growth hormone Sex steroid Desmopressin acetate	Enlargement of the pituitary gland and the thickening of the pituitary stalk (BS) →Empty sella(AS)
#30	Severe GHD	Hypothalamic-AI	64	F	Surgery of pituitary adenoma (NF)	GH/ACTH/TSH/LH/FSH/PRL	Glucocorticoid Levothyroxine sodium	Outside report; details not available(BS) →Empty sella(AS)
#31	Severe GHD	Non-AI	66	F	Pituitary adenoma (NF)	GH/TSH/PRL	Levothyroxine sodium	27 mm homogenously enhancing sellar mass
#32	Severe GHD	Non-AI	31	F	Surgery of pituitary adenoma (NF)	GH/LH/FSH	Sex steroid	Outside report; details not available (BS) →Empty sella(AS)
#33	Severe GHD	Non-AI	49	M	Surgery of pituitary adenoma (NF)	GH/LH/FSH	Growth hormone	26 mm sellar mass, compressing the optic chiasm (BS) →No abnormalities(AS)
#34	Severe GHD	Non-AI	70	M	Surgery of pituitary adenoma (NF)	GH/TSH	Levothyroxine sodium	29 mm sellar mass with niveau (BS) →15 mm residual sella mass (AS)
#35	Severe GHD	Non-AI	64	M	Pituitary apoplexy	GH/LH/FSH	-	Pituitary apoplexy with low T1 and low T2 signal
#36	Severe GHD	Non-AI	33	F	Sheehan syndrome	GH	-	Empty sella

Abbreviations: ACTH, adrenocorticotropic hormone; AI, adrenal insufficiency; AS, after surgery;AUC, area under the curve; AVP, arginine vasopressin; BS, before surgery; CD, Cushing's disease; CRH, corticotropin-releasing hormone; F, female; FSH, follicle-stimulating hormone; GH, growth hormone; GHD, growth hormone deficiency; LH, luteinizing hormone; M, male; NF, non-functioning; PRL, prolactin; TSH, thyroid-stimulating hormone; y, years.

Table 2. Baseline and peak of GH, ACTH, and cortisol in each stimulation test

No.	Baseline			GHRP2 test			Cosyntropin stimulation test	CRH test		ITT test	
	GH (ng/mL)	ACTH (pg/mL)	Cortisol (μg/dL)	Peak GH, ng/mL	Baseline→Peak ACTH, pg/mL (fold)	Peak Cortisol, μg/dL	Peak Cortisol, μg/dL	Baseline→Peak ACTH, pg/mL (fold)	Peak Cortisol, μg/dL	Baseline→Peak ACTH, pg/mL (fold)	Peak Cortisol, μg/dL
#1	1.01	6.2	11.6	32.3	6.2 → 12 (1.9)	15.2	39.9	5.2 → 94.5(18.2)	48.9		
#2	0.9	5.8	11.8	76.2	5.8 → 22.9 (3.9)	23.3	33.8			8.9 → 22.2 (2.5)	12.3
#3	0.13	16.9	10.4	124	16.9 → 118 (7)	24.3	29.8	17 → 68.9 (4.1)	22.3	33.5 → 143 (4.3)	22.7
#4	0.27	6.5	8	48.5	6.5 → 10.1 (1.6)	10.5	23.5	9.2 → 50.2 (5.5)	19.5	8.4 → 18.4 (2.2)	17.6
#5	0.2	12.3	7.3	31.8	12.3 → 41.9 (3.4)	17.4	25.8	9.1 → 59.4(6.5)	24.9	13.3 → 43.8 (3.3)	18.6
#6	2.01	59.7	22.1	24	59.7 → 245 (4.1)	38.3	20.3	44.4 → 165 (3.7)	29.1	35.1 → 51.3 (1.7)	20.4
#7	0.24	7.2	<1.0	33.7	7.2 → 38.6 (5.4)	5.9	59.9	7.2 → 64.6 (9)	19.3	5.1 → 41.2 (8.1)	17.8
#8	0.35	<5.0	9.4	19.7	<5.0 → 9.8	12.6	26.8	7.2 → 43.7(6.1)	29.1		
#9	0.09	<5.0	6.7	21.5	<5.0 → 6.3	8.7	24.2	<5.0→ 100	16.2	<5.0 → 52.6	20.4
#10	0.15	6.8	9.4	48.9	6.8 → 13.1 (1.9)	9.4	31.2	7.7 → 32.5 (4.2)	24.9	11.6 → 272 (23.4)	27.5
#11	0.53	<5.0	0.1	29.7	<5.0 → <5.0 (0)	0.1	1.3	<5.0 → <5.0 (0)	0.2	<5.0 → <5.0 (0)	0.3
#12	0.02	18.9	9.2	15.17	18.9 → 26.5 (1.4)	15.2	30.1	11.6 → 21.8 (1.9)	16.6	13.7 → 28.5 (2.1)	20.7
#13	0.37	9.5	5.6	15.8	9.5 → 79.6 (8.4)	15.9	20.3	7 → 68.5 (9.8)	17.1		
#14	<0.05	6.8	4.8	10.6	6.8 → 40.6 (6)	12.6	23.2	10.8 → 89.3 (8.3)	16.6	6.6 → 60.5 (9.2)	14.3
#15	0.29	18.2	6.8	4.38	18.2 → 27.8 (1.5)	9.8	10.7	23.4 → 28.4 (1.2)	11.4		
#16	0.17	8.2	5	0.62	8.2 → 57.7 (7)	10.5	13.5	20.1 → 36.3 (1.8)	8.8		
#17	0.09	<5.0	<1.0	1.05	<5.0 → <5.0 (0)	<1.0	13.4	<5.0 → <5.0 (0)	<1.0	<5.0 → <5.0 (0)	<1.0
#18	<0.05	<5.0	<1.0	0.19	<5.0 → <5.0 (0)	<1.0	<1.0	<5.0 → <5.0 (0)	<1.0	<5.0 → <5.0 (0)	<1.0
#19	<0.05	<5.0	<1.0	0.16	<5.0 → <5.0 (0)	<1.0	<1.0	<5.0 → <5.0 (0)	<1.0		
#20	<0.05	<5.0	1.3	0.12	<5.0 → 7.5	1.9	8.5	<5.0 → 6.3	1.7	5.5 → 11.6 (2.1)	1.7
#21	0.07	21.5	8.3	8.62	21.5 → 52.7 (2.5)	14.6	17.7	37.3 → 110 (2.9)	17.6	15.8 → 23.7 (1.5)	13.7
#22	<0.05	8.4	1.1	0	8.4 → 159 (18.9)	3.7	3.5	9.1 → 165 (18.1)	5.5	17.8 → 19 (1.1)	2.1
#23	<0.05	6.3	3.4	0.07	6.3 → 25.9 (4.1)	10.6	10.5	5.8 → 56.4 (9.7)	12.7	<5.0 →6.5	3.9
#24	<0.05	14.3	<1.0	0.5	14.3 → 65.5 (4.6)	1.7	3.5	<5.0 → 30.2	2.6	6.2 → 10 (1.6)	2.2
#25	0.14	15.8	8.1	2.99	15.8 → 25.5 (1.6)	11.2	1	22.5 → 67.4 (3)	17.6	17.1 → 23.2 (1.4)	13.6
#26	0.49	12.7	2.2	1.52	12.7 → 107 (8.4)	11.2	14.6	12.5 → 315 (25.2)	20.9	8.1 → 13.9 (1.7)	8.7
#27	0.1	<5.0	<1.0	0.5	<5.0 → 10	1.1	1.5	<5.0 → 58.5	<1.0	<5.0 → <5.0 (0)	<1.0
#28	0.81	7.2	2.2	6.66	7.2 → 19.5 (2.7)	4.8	7.2	5.8 → 38.3 (6.6)	7.1	<5.0 →9	6.6
#29	0.07	12	2.1	0.6	12 → 81 (6.8)	4.4	13.7	9.4 → 78.7 (8.4)	2.9	7.8 → 12.7 (1.6)	2.3
#30	<0.05	<5.0	<1.0	0	<5.0 → 7.8	<1.0	1	<5.0 → 18.5	<1.0	<5.0 → <5.0 (0)	<1.0
#31	0.34	5.3	0.5	1.11	5.3 → 49.1 (9.3)	3.2	21.5	4.9 → 160 (32.7)	5.9		
#32	<0.05	10.2	8.4	1.73	10.2 → 15.5 (1.5)	11	18.9	22.6 → 40.8 (1.8)	15.3		
#33	<0.05	10.3	10.6	7.9	10.3 → 18.2 (1.8)	13.6	18.2	17.8 → 34.2 (1.9)	11.8	12 → 73.5 (6.1)	19.6
#34	0.09	18.2	5.7	1.09	18.2 → 35.7 (2)	10.8	21.7	17.2 → 31.2 (1.8)	10.2	26 → 115 (4.4)	12.8
#35	0.06	18.9	12.7	1.58	18.9 → 31.9 (1.7)	14.3	22.3	13.1 → 22.9 (1.7)	12.7	11.7 → 24.2 (2.1)	15.5
#36	0.24	13.3	9.6	5.36	13.3 → 20.4 (1.5)	10.2	19.3	13.1 → 25.8 (2.0)	9.9	13.1 → 44.8 (3.4)	16.5

Abbreviations: ACTH, adrenocorticotropic hormone; CRH, corticotropin-releasing hormone; GHRP2, growth hormone-releasing peptide-2; GH, growth hormone; ITT, insulin tolerance tests.

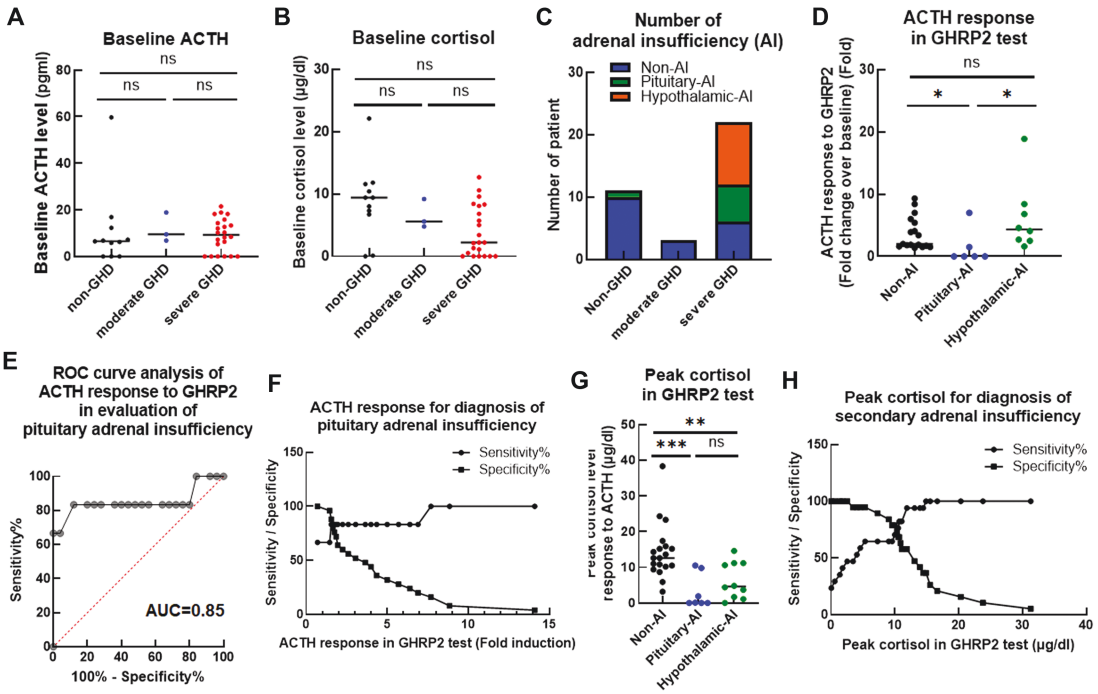

Figure 1. Screening of concomitant second adrenal insufficiency in patients with growth hormone deficiency using GHRP2 test. A and B. Comparison of individual basal ACTH level (A) and basal cortisol level (B) in patients with non-GHD (black circles), moderate GHD (blue circles) and severe GHD (red circles). GHD: growth hormone deficiency. ns: not significant. C. The number of patients with adrenal insufficiency (non-: blue, pituitary-: green and hypothalamic-: orange) in non-GHD, moderate GHD and severe GHD. AI: adrenal insufficiency. D. Comparison of individual ACTH responses to GHRP2 in patients with non-AI (black circles), pituitary-AI (blue circles) and hypothalamic-AI (green circles). $*P < 0.05$. ns: not significant. E and F. ROC curve (E) and plots of the variation in sensitivity (circles) and specificity (squares) (F) for ACTH response in GHRP2 to predict pituitary adrenal insufficiency. AUC: Area under the ROC curve. G. Comparison of individual peak cortisol to GHRP2 in patients with non-AI (black circles), pituitary-AI (blue circles) and hypothalamic-AI (green circles). AI: adrenal insufficiency. $**P < 0.01$ and $***P < 0.001$. ns: not significant. H. ROC curve plotted the variation in sensitivity (circles) and specificity (squares) for peak cortisol levels in GHRP2 to predict secondary adrenal insufficiency.

Clinical Usefulness of the GHRP2 Test for Screening Secondary AI

To examine whether the GHRP2 test can be used for detecting secondary AI, we compared the ACTH response (peak level divided by basal level) and peak ACTH levels after the GHRP2 challenge in non-AI, pituitary-AI, and hypothalamic-AI. The ACTH response in the GHRP2 test was clearly lower in pituitary-AI than in the others, but there was no difference between non-AI and hypothalamic-AI (Table 2, Fig. 1D). Next, the ROC curves for ACTH response in the GHRP2 test were plotted, and the AUC and the most appropriate cutoff values were calculated to classify pituitary-AI. The ACTH responses showed a high AUC of 0.85 (Fig. 1E), and at the cutoff ACTH response of 1.55-fold, the GHRP2 test was useful for the detection of pituitary-AI, with 83% sensitivity and 88% specificity (Fig. 1F). The peak ACTH levels showed a relatively high AUC of 0.81 in predicting pituitary-AI, but the sensitivity was lower than the ACTH response (71.4%). On the other hand, the peak cortisol levels in response to GHRP2 were significantly lower in pituitary- and hypothalamic- (secondary) AI than in non-AI (Fig. 1G). Using the cutoff peak cortisol level of 10 pg/mL, the GHRP2 test was able to predict secondary AI (pituitary-AI and hypothalamic-AI) with 70.5% sensitivity and 78.9% specificity (Fig. 1H). Of note, the combination of the ACTH response and peak cortisol level using each cutoff value (1.55-fold and 10 μg/dL respectively) showed high specificity (71% sensitivity and 100% specificity) with high accuracy (0.94) for diagnosis of pituitary-AI.

Discussion

In this paper, we propose how to utilize the GHRP2 test in patients having hypothalamic-pituitary disorders especially for the clinical diagnosis of AI concomitant with GHD [22, 23]. Although the baseline ACTH and cortisol did not differ between non-GHD, moderate GHD, and severe GHD participants, a dynamic test using cosyntropin stimulation test, corticotropin-releasing hormone (CRH) test, or an insulin tolerance test (ITT) revealed the existence of secondary AI in severe GHD group. In clinical practice, there is the risk that patients with equivocal basal cortisol levels may miss the opportunity to undergo the dynamic tests and subsequently be referred to the hospital for the adrenal crisis at times of illness or other stress. We recommend measuring ACTH and cortisol simultaneously when performing GHRP2 for the diagnosis of GHD. It is not easy to distinguish precise adrenal status by only GHRP2 testing due to the overlap among subjects in 3 groups (non-AI, pituitary-AI, and hypopituitary-AI). However, GHRP2 helps pick up secondary AI, and when combined with traditional dynamic tests, it may provide a more reliable diagnosis and classification of secondary AI. The GHRP2 helps screen for pituitary-AI using the ACTH response with peak cortisol level. Further, hypothalamic-AI might be suspected by low peak cortisol level with normal ACTH response after GHRP2 administration. To the best of our knowledge, the cutoff value of ACTH in the GHRP2 test has not been reported previously. On the other hand, a few reports have mentioned the cutoff value of cortisol in the GHRP2 test. Arimura et al set the cutoff cortisol level at

11.6 μg/dL for secondary AI [17], and Kano et al set the cutoff value at 13-14 μg/dL for both primary and secondary AI [15]. Those cutoff values for cortisol are very close to the value we obtained in this study.

ACTH is regulated primarily by 2 hypothalamic hormones: arginine vasopressin (AVP) [24] and CRH [25], which colocalize mainly in the paraventricular nuclei of the hypothalamus. It has been reported that GHRP2 stimulates the hypothalamic-pituitary-adrenal axis via the GH secretagogue receptors in the hypothalamus and ACTH directly from the pituitary [13, 16, 17, 26]. Some studies have suggested that GHRP2-evoked ACTH stimulation may be mediated by CRH [13, 26], while others propose that GHRP2 stimulates ACTH by a mechanism different from that of CRH or AVP [16, 17]. Importantly, the positive ACTH response to GHRP2 but not to ITT in hypothalamic-PI in our study might indicate that GHRP2 has an ACTH-stimulation pathway different from ITT.

In conclusion, since the GHRP2 test is widely used in the diagnosis of GHD, it is beneficial to use GHRP2 for screening pituitary-AI, which is often concomitant with severe GHD and should not be overlooked to prevent a fatal adrenal crisis.

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Disclosures

The authors have no conflicts of interest to report. The authors declare that there are no competing financial interests.

Data Availability

All data generated or analyzed during this study are included in this published article or is available from the corresponding authors on reasonable request.

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