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Unique Sex Steroid Profiles in Estrogen-Producing Adrenocortical Adenoma Associated With Bilateral Hyperaldosteronism

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Because of its rarity, our understanding of steroidogenesis in estrogen-producing adrenocortical adenoma, including the response to adrenocorticotropic hormone (ACTH) stimulation, remains limited. A 65-year-old man was referred to us because of primary aldosteronism and a right adrenal tumor. Endocrinological evaluations revealed secondary hypogonadism due to hyperestrogenemia. Adrenal venous sampling (AVS) and subsequent liquid chromatography–tandem mass spectrometry (LC-MS/MS) indicated bilateral hyperaldosteronism and a right estrogen-producing adrenocortical tumor. He subsequently underwent right unilateral adrenalectomy, which resulted in clinical remission of hypogonadism. Subsequent histopathological analysis identified a right estrogen-producing adrenocortical adenoma and multiple, concomitant adrenocortical micronodules. Sequential evaluation of steroid profiles using LC-MS/MS revealed unique hormone production, including adrenal androgens, and less responsiveness to ACTH in the right estrogen-producing adrenocortical adenoma as compared to the nonneoplastic adrenal cortex. This case study revealed unique profiles of steroid production in estrogen-producing adrenocortical adenoma associated with concomitant primary aldosteronism. Sequential steroid profiling analysis using LC-MS/MS in combination with AVS can contribute to the diagnosis of various adrenal disorders.

Key Words: estrogen-producing adrenocortical adenoma, primary aldosteronism, adrenal venous sampling, ACTH, LC-MS/MS

Estrogen-producing adrenocortical tumors (EPATs) are very rare, accounting for 1% to 2% of all adrenal tumors [1, 2]. The great majority of EPAT are adrenocortical carcinomas; thus, only a few adenomas have been reported in the literature. An EPAT causes bilateral painful

Abbreviations: ACTH, adrenocorticotropic hormone; AVS, adrenal venous sampling; CYP11B2, aldosterone synthase; EPAT, estrogen-producing adrenocortical tumor; HU, Hounsfield unit; LAV, left adrenal vein; LC-MS/MS, liquid chromatography–tandem mass spectrometry; MN, micronodule; PA, primary aldosteronism; RAV, right adrenal vein.

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gynecomastia in almost all male patients and vaginal bleeding in women [3]; these characteristic signs contribute to its clinical diagnosis. Estrogen-producing adrenocortical carcinomas were reported to show higher expression levels of aromatase, 17 β-hydroxysteroid dehydrogenase 3, and luteinizing hormone/choriogonadotropin receptor than other adrenocortical carcinomas [4]. However, details about steroidogenesis including its response to adrenocorticotropic hormone (ACTH) have remained unknown.

We here report a male case of EPAT associated with primary aldosteronism (PA). Of particular interest, steroid profiling using liquid chromatography–tandem mass spectrometry (LC-MS/MS) clearly demonstrated unique production of sex steroids, including 11-oxygenated androgens, and their response to cosyntropin both in peripheral and adrenal venous samples (AVS), from both adrenal glands.

1. Case Presentation

A 65-year-old Japanese man was referred to us because he had hypokalemia (3.4 mM) and a right adrenal tumor. He also had a 10-year history of hypertension managed with amlodipine. Clinical parameters were as follows: blood pressure, 143/87 mm Hg; heart rate, 76 beats/min; body height, 165.6 cm; body weight 76.0 kg. On admission, physical examination incidentally revealed bilateral painless gynecomastia, and he also had a 5-year history of reduced libido and erectile dysfunction that he assumed to be physical changes due aging. Laboratory evaluation demonstrated a plasma aldosterone concentration of 18.6 ng/dL, plasma renin activity of 0.3 ng/mL/h, and an aldosterone-to-renin ratio of 62.0, while normokalemia was maintained by oral potassium replacement. In addition, we detected a low level of total testosterone, a high estradiol level, and a low gonadotropin level (Table 1), but a normal prolactin level. The sex hormone binding globulin level was slightly elevated. The patient provided his written informed consent to all necessary investigations.

The aldosterone-to-renin ratio following 50 mg captopril loading was 24.5, which was higher than the cutoff value of 20 for the diagnosis of PA. The cortisol level after 1 mg dexamethasone loading was 2.2 μg/dL. The hypothalamus and pituitary gland showed no remarkable findings in imaging studies. His hypogonadotropic hypogonadism was therefore interpreted as secondary to hyperestrogenemia. Computed tomography demonstrated the presence of a well-circumscribed, right adrenal tumor (37 × 30 mm). The mean attenuation values of the tumor on the unenhanced, early enhanced, and 3-min-delayed enhanced images were 12.78 Hounsfield unit (HU), 152.64 HU, and 80.98 HU, respectively. The absolute and relative percentage washout at 3-minute delayed imaging of this tumor were calculated as 51.23% and 46.95%, respectively. We then performed AVS to precisely identify the sources of hyperaldosteronism and hyperestrogenemia. We collected blood samples from 3 sampling points, both right and left adrenal veins (RAV, LAV), and the external iliac vein as a reference, both at baseline and under cosyntropin stimulation [7]. Plasma specimens were used for aldosterone, cortisol, and sex steroid profiling by LC-MS/MS (ASKA Pharmaceutical Medical Corporation) [8, 9]. We first evaluated sex steroid profiling in adrenal veins (Table 2). Plasma estrone and estradiol levels were markedly higher in the RAV than in the LAV and the external iliac vein, regardless of cosyntropin stimulation. These findings confirmed that the right adrenal tumor was the cause of hyperestrogenemia. Of particular interest was the fact that there were also significant differences in androgen levels, including dehydroepiandrosterone and testosterone, between the RAV and LAV at baseline. However, those androgens responded more markedly to cosyntropin injection in the LAV than in the RAV. The left adrenal gland also produced more 11-oxygenated androgens than the right side in response to cosyntropin stimulation. In addition, aldosterone-to-cortisol ratios under cosyntropin stimulation were 4.45 and 7.94 in the RAV and LAV, respectively, and the laterality index was 1.78, which was lower than the cutoff value of 2.6 for unilateral PA. These clinical evaluations ultimately led to the clinical diagnosis of right EPAT with bilateral hyperaldosteronism.

Table 1. Changes of sex steroid profiles in peripheral blood during the perioperative period

		Before Surgery			After Surgery	
	Reference Interval*a*	**Baseline**	**60 min After ACTH**	**DST1**	**POD 7**	**POD 44**
LH, mIU/mL	0.8-5.7	< 0.07	N/A	N/A	3.55	20.65
FSH, mIU/mL	2.0-8.3	< 0.30	N/A	N/A	6.59	20.27
Estrone, pmol/L	68-213[5]	2757	2927	2993	56	100
Estradiol, pmol/L	46-160[5]	378	451	448	11	55
DHEA, nmol/L	2.5-46.7[6]	11	15	9.3	5.0	4.7
Androstenedione, nmol/L	1.5-8.3[6]	23	24	23	1.6	1.3
Δ5A-diol, nmol/L	N/A	1.6	1.8	1.3	1.1	1.1
Testosterone, nmol/L	9.2-29.1[5]	4.6	5.0	5.4	2.2	8.4
DHT, pmol/L	568-2339[5]	757	826	826	172	585
11-OHAD, nmol/L	N/A	6.0	8.4	0.9	5.5	6.3
11-ketoAD, nmol/L	N/A	1.3	1.3	0.4	1.1	1.3
11-OHT, pmol/L	N/A	296	558	99	526	788
11-ketoT, pmol/L	N/A	1058	728	132	926	1488
SHBG, nmol/L	10-57	59	N/A	N/A	N/A	40
Aldosterone, ng/dL	3-15	18.6	21.9	11.1	15.5	8.1
Renin activity, ng/mL/h	1.0-3.9	0.3	0.3	0.6	0.2	0.2
Serum potassium, mmol/L	3.5-5.0	4.3	N/A	N/A	5.4	4.1
Potassium replacement, mmol/day	N/A	98	N/A	N/A	48	24

Steroid profiles in peripheral blood before and after surgery are shown. Preoperative evaluation suggested adrenocorticotropin-independent production of estrogens and androgens except for DHEA and 11-oxygenated androgens. After surgery, estrogen levels immediately normalized, and gonadotropins gradually increased, whereas adrenal androgens, other than 11-oxygenated androgens, decreased. SHBG was slightly elevated at baseline, and normalized after the surgery. Reference intervals were taken from previous studies [5, 6].

Abbreviations: Δ5A-diol, 5-androstene-3β,17β-diol; 11-ketoAD, 11-ketoandrostenedione; 11-ketoT, 11-ketotestosterone; 11-OHAD, 11-β-hydroxy-androstenedione; 11-OHT, 11-hydroxytestosterone; DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone; DST1, 1 mg dexamethasone suppression test; FSH, follicle-stimulating hormone; LH, luteinizing hormone; N/A, not available; POD, postoperative day; SHBG, sex hormone binding globulin.

[a]Reference intervals for estrogens, testosterone, DHT, androstenedione, and DHEA were referred from references [5] and [6].

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Table 2. Steroid profiling in adrenal venous sampling before and after cosyntropin stimulation

	Right Adrenal Vein (Tumor Side)			External Iliac Vein			Left Adrenal Vein (Nontumor Side)		
	Baseline	Post-ACTH	Fold[a]	Baseline	Post-ACTH	Fold[a]	Baseline	Post-ACTH	Fold[a]
Aldosterone, ng/dL	62.8	473.9	7.5	5.2	9.3	1.8	92.8	3041.6	32.8
Cortisol, ng/dL	10.0	106.4	10.6	9.4	12.8	1.4	21.4	382.9	17.9
Estrone, pmol/L	119088	107874	0.9	3382	3334	1.0	1240	1484	1.2
Estradiol, pmol/L	1439	1090	0.8	719	657	0.9	297	248	0.8
DHEA, nmol/L	307	546	1.8	10	11	1.1	28	1404	50.1
Androstenedione, nmol/L	1095	1391	1.3	16	20	1.3	15	441	29.4
Δ5A-diol, nmol/L	3.1	7.5	2.4	1.3	1.9	1.5	0.9	19	21.1
Testosterone, nmol/L	25	21	0.8	6.0	6.5	1.1	4.0	15	3.8
DHT, pmol/L	723	585	0.8	654	757	1.2	723	620	0.9
11-OHAD, nmol/L	22	218	9.9	4.5	6.4	1.4	65	1323	20.4
11-ketoAD, nmol/L	1.0	3.2	3.2	0.6	1.0	1.7	1.7	15	8.8
11-OHT, pmol/L	394	1478	3.8	887	788	0.9	854	7621	8.9
11-ketoT, pmol/L	496	661	1.3	728	595	0.8	728	926	1.3

Measurement of sex steroid profiles and aldosterone-to-cortisol ratios was performed using blood samples collected from right and left adrenal veins (RAV, LAV) and the external iliac vein, at baseline and under stimulation with cosyntropin. Plasma estrone and estradiol levels in RAV were markedly higher than those in LAV, regardless of cosyntropin stimulation. In addition, androgen levels were higher in RAV than in LAV, but the androgens in LAV responded much more markedly to cosyntropin stimulation than those in RAV. However, the left adrenal gland produced more 11-oxygenated androgens than the right side. In terms of the laterality of hyperaldosteronism, aldosterone-to-cortisol ratios under cosyntropin stimulation were not significantly different between RAV and LAV (4.45 vs 7.94).

Abbreviations: Δ5A-diol, 5-androstene-3β,17β-diol; ACTH, adrenocorticotropin; DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone; 11-ketoAD, 11-ketoandrostenedione; 11-ketoT, 11-ketotestosterone; 11-OHAD, 11-β-hydroxy-androstenedione; 11-OHT, 11-hydroxytestosterone.

[a]Increase fold is calculated as a steroid concentration under cosyntropin stimulation divided by that concentration at baseline.

Subsequently, the patient underwent right adrenalectomy to treat his hyperestrogenemia. The resected right adrenal gland weighed 20.0 g and the tumor measured 41 × 36 × 22 mm. The tumor was yellowish on the cut surface harboring jelly-like foci of degeneration. Histologically, compact cells harboring eosinophilic cytoplasm and an oval nucleus were predominant in the tumor (Fig. 1A). Based on Weiss criteria, the tumor was diagnosed as an adrenocortical adenoma (1 point). Subsequent immunohistochemical analyses revealed that the tumor cells were diffusely positive for 17α-hydroxylase and partially positive for 3β-hydroxysteroid dehydrogenase (Fig. 1B and 1C). Estrogen-related enzymes, including both 17β-hydroxysteroid dehydrogenase 5 and aromatase, were detected in the tumor cells, consistent with a potential of neoplastic estrogen production (Fig. 1D-1G). In addition, dehydroepiandrosterone-sulfotransferase, estrogen sulfotransferase, steroid sulfatase, and 5α reductase were diffusely detected in the tumor (Fig. 1H-1L). However, the tumor showed no aldosterone synthase (CYP11B2) immunoreactivity, whereas multiple CYP11B2-positive adrenocortical micronodules (MNs) were detected in the subcapsular area surrounded by a CYP11B2-negative zona glomerulosa in the adjacent nonneoplastic adrenal gland (Fig. 1M and 1N). The zona fasciculata and the reticularis both were well preserved and expressed dehydroepiandrosterone-sulfotransferase (Fig. 1O). Therefore, the CYP11B2-positive adrenocortical MNs, but not the tumor, were considered to be responsible for aldosterone overproduction in this patient.

Based on the previously stated findings, we ultimately diagnosed this lesion as an estrogen-producing adrenocortical adenoma harboring multiple adrenocortical MNs. After right adrenalectomy, estrogen levels as well as those of androstenedione immediately normalized, whereas the gonadotropins gradually elevated (Table 1). Total testosterone and dihydrotestosterone levels decreased transiently on the seventh postoperative day and then gradually elevated, followed by gradual clinical amelioration of gynecomastia. In terms of PA, the aldosterone level and the required amount of potassium replacement decreased after the operation (Table 1), but both hypertension and hypokalemia persisted. Therefore,

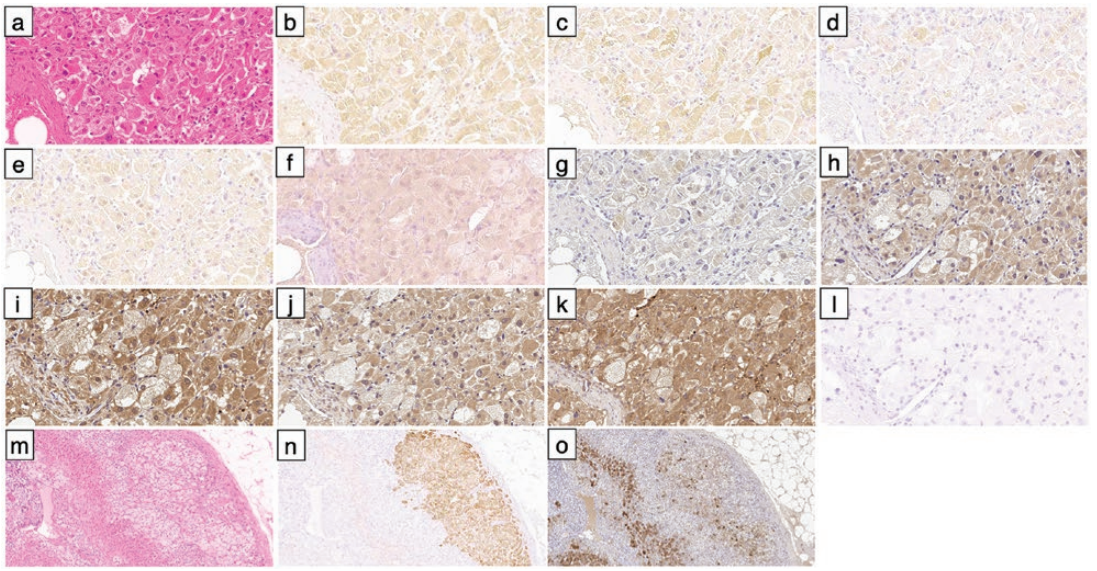

Figure 1. Immunohistochemical staining of the resected adrenal gland. A to L, Right adrenal tumor and M to O, adjacent adrenal gland. A, Hematoxylin-eosin (×200); B, 17α-hydroxylase (diffuse, ×200); C, 3β-hydroxysteroid dehydrogenase (partial, ×200); D, 17β-hydroxysteroid dehydrogenase 5 (focal, ×200); E, aromatase (focal, ×200); F, CYP11B1 (partial, ×200); G, dehydroepiandrosterone-sulfotransferase (diffuse, ×200); H, steroid sulfatase (diffuse, ×200); I, 5α reductase 1 (diffuse, ×200); J, 5α reductase 2 (diffuse, ×200); K, estrogen sulfotransferase (diffuse, ×200); L, aldosterone synthase (CYP11B2) (negative, ×200); M, Hematoxylin-eosin (×200); N, CYP11B2 (focal, ×200); O, dehydroepiandrosterone-sulfotransferase (diffuse, ×200).

we switched the patient's medication from amlodipine to eplerenone for the remaining PA to control his blood pressure and keep his normokalemia stable.

2. Discussion

We report a very rare case of EPAT complicated with bilateral hyperaldosteronism. In this patient, steroid profile analysis using LC-MS/MS revealed not only unique hormone production in the adenoma, but also distinct responses to cosyntropin stimulation between the tumor and the contralateral nonneoplastic adrenal gland. AVS also successfully determined the precise localization both of hyperestrogenemia and hyperaldosteronism.

Furthermore, results of LC-MS/MS analysis revealed a dramatic decrease of adrenal androgen and estrogens levels in peripheral blood following right adrenalectomy. Results of immunohistochemical evaluation also revealed that the tumor cells expressed aromatase, the rate-limiting enzyme of estrogen synthesis, and 17β-hydroxysteroid dehydrogenase 5, the rate-limiting enzyme of testosterone synthesis, indicating that the tumor itself produced estrogens and their precursors (Fig. 2). Considering the perioperative changes of androgens and gonadotropins in peripheral blood, the patient's androgen levels may have been maintained by the tumor, possibly alleviating his symptoms related to hypogonadotropic hypogonadism. EPAT in this case also produced estrogens and its precursors independently from ACTH stimulation, based on both a 1 mg dexamethasone suppression test and a rapid ACTH stimulation test (Table 1). However, his peripheral levels of 11-oxygenated androgens suggested the presence of ACTH-dependent production of these steroids. These 11-oxygenated androgens are considered potential adrenal biomarkers of congenital adrenal hyperplasia, but have not been reported in EPAT [10]. In our patient, AVS followed by LC-MS/MS demonstrated that those androgens were secreted by nonneoplastic adrenal gland tissue and not by EPAT. AVS also revealed an increase in some androgen levels in the right adrenal, under cosyntropin stimulation, which could be influenced by the right adjacent adrenal cortex. ACTH stimulation has been reported to induce the production of most androgens in the adrenal glands of women [11]. In this case, all the androgens measured, except for dihydrotestosterone in the LAV, clearly responded to cosyntropin injection. Those findings suggest the presence of ACTH-dependent steroid synthesis in nonneoplastic

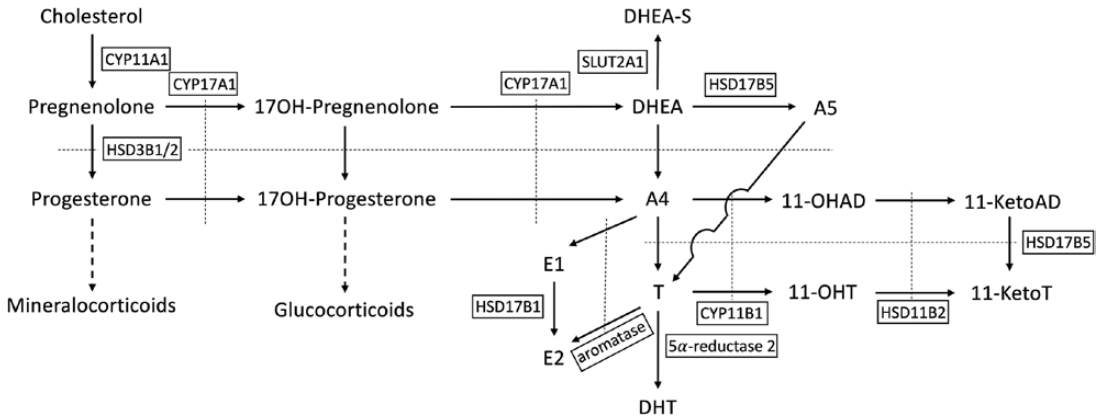

Figure 2. The steroidogenesis pathway of sex hormones. In this patient, immunohistochemical staining of the resected adrenal gland revealed expression of all the steroidogenic enzymes related to estrogens and their precursors, androstenedione and testosterone, in the estrogen-producing adenoma. Analysis of steroid profiles in peripheral and adrenal venous samples indicated that 11-oxygenated androgens were mainly secreted in the nontumoral adrenal cortex, although tumor cells partially showed CYP11B1 expression. 11-β-hydroxy-androstenedione; 11-OHAD, 11-OHT, 11-hydroxytestosterone; 11-ketoAD, 11-ketoandrostenedione;11-ketoT, 11-ketotestosterone; A4, androstenedione; A5, 5-androstene-3β,17β-diol; DHEA, dehydroepiandrosterone; DHEA-S, dehydroepiandrosterone sulfate; DHT, dihydrotestosterone; E1, estrone; E2, estradiol; T, testosterone.

adrenal glands, which have a negligible influence on those peripheral levels. Therefore, the loss of responsiveness to ACTH might be one of the characteristic features of tumorigenesis in sex steroid-producing adrenal cells.

To the best of our knowledge, this is the first reported case of EPAT and concomitant PA. The patient's right adrenal gland was at first clinically considered to harbor an aldosterone-producing adenoma. However, AVS demonstrated similar aldosterone-to-cortisol ratios both in the RAV and the LAV, confirming bilateral hyperaldosteronism. In addition, the subsequent evaluation of CYP11B2 in the resected specimen indicated that aldosterone was produced in the adjacent nonneoplastic adrenal gland tissue, not in the tumor. MNs are characterized by the presence of CYP11B2-positive and both 11β-hydroxylase- and 17α-hydroxylase-negative cortical micronodules located in the CYP11B2-negative zona glomerulosa, which could occur bilaterally [12]. The postoperative biochemical changes that occurred in our patient suggested that the remaining left adrenal gland could harbor some of the causes of hyperaldosteronism, such as MNs. These findings indicated that the localization of hyperaldosteronism and hyperestrogenemia obtained by immunohistochemical analysis of steroidogenic enzymes was consistent with AVS results, whereas the association between EPAT and MNs is still virtually unknown. Goto et al first reported the case of a patient with an estrogen-producing adrenocortical adenoma in whom they performed AVS to determine the localization of hyperestrogenemia, and subsequently confirmed it by immunohistochemistry and reverse transcriptase-polymerase chain reaction analysis of aromatase [3]. Sequential steroid profiling analysis using LC-MS/MS in combination with AVS could contribute to the diagnosis and further exploration of various adrenal disorders.

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