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CASE REPORT

Close examination of steroidogenesis disorders in a DOC- and progesterone-producing adrenocortical carcinoma

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Abstract We report a case of hypertension, hypokalemia, and amenorrhea accompanying an adrenocortical carcinoma. A 27-year-old woman was admitted to our hospital because of a left adrenal incidentaloma. She presented with hypertension, hypokalemia, and amenorrhea; her plasma renin activity was low, but her plasma aldosterone concentration was normal, as were cortisol and androgens. By contrast, her serum concentrations of deoxycorticosterone (DOC), 18-hydroxydeoxycorticosterone, and progesterone were high, and her urinary steroid profile showed elevated secretion of 17-deoxysteroids and 11-deoxysteroids (progesterone, DOC, 11-dehydrocorticosterone, and 11-deoxycortisol), and 3β-hydroxy 5-en steroids (pregnenolone, 17-hydroxypregnenolone, and DHEA). Decreased ratios of metabolites of (1) 17-OHpregnenolone to pregnenolone and 17-OHprogesterone to progesterone, (2) corticosterone to DOC and cortisol to 11-deoxycortisol, and (3) progesterone to pregnenolone, 17-OHprogesterone to 17-OHpregnenolone and androstenedione to DHEA suggested the impairment of 17α-hydroxylase, 11β-hydroxylase, and 3β-HSD activities, respectively. After the tumor was removed, levels of all adrenal steroids were normalized. Based on the Weiss criteria, the tumor was diagnosed as an adrenocortical carcinoma, and immunohistochemical analysis of steroidogenic enzymes revealed disorganized steroidogenesis in the tumor tissue. With adrenocortical carcinomas, heterogeneity of individual steroid producing enzymes within tumor cells can lead to hypersecretion of various steroid intermediates, even when steroid end products are within the normal range.

Keywords Adrenocortical carcinoma · Hypertension · Amenorrhea · Hypokalemia · Steroid · DOC

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Introduction

The adrenal cortex produces a variety of steroid hormones, including glucocorticoids, mineralocorticoids, and androgens (Fig. 1). Approximately half of adrenocortical tumors are nonfunctioning, while the others secrete cortisol or aldosterone [1]. In addition, excess androgens are often reported in cases of adrenocortical carcinoma and hypersecretion of steroid intermediates, which is rare in adrenocortical adenomas, is sometimes reported in adrenocortical carcinoma cases. We encountered a patient with hypertension, hypokalemia, and amenorrhea accompanying an adrenal tumor that was producing steroid intermediates such as pregnenolone, progesterone, and deoxycorticosterone (DOC), among others. The first case of adrenocortical carcinoma producing mineralocorticoid intermediates (e.g., DOC or corticosterone) was reported in 1968 [2]. Despite the patient's normal aldosterone in that case, corticosterone was hypersecreted, and hypertension and hypokalemia were

observed. The first case of a DOC-producing adrenocortical carcinoma was reported in 1974 [3], and a benign DOC-producing adrenocortical adenoma was reported in 1976 [4]. Overall, however, the total number of the report of adrenal tumor producing mineralocorticoid intermediates was not so large even now. Progesterone producing adrenal tumors are even rarer [5, 6]. Only two cases with progesterone producing adrenal tumors have been reported, and both presented with amenorrhea. This may in part reflect the fact that hypersecretion of steroid intermediates can often be overlooked, as these steroid intermediates are generally not measured in daily clinical practice. In our patient, steroid end products, including biologically active steroids, were within normal ranges, despite the elevated steroid intermediates. We therefore endeavored to account for this abnormal steroidogenesis by examining the urinary steroid profile and performing an immunohistochemical analysis of the steroidogenic enzymes in the resected adrenocortical tumor tissue.

Materials and methods

Steroid hormones in plasma were measured by SRL Co., Ltd. (Tokyo, Japan). The 2- and 8-mg dexamethasone suppression test was performed using the classical Liddle method. A detailed description of urinary steroid profile analysis is available in our earlier publication [7]. Briefly, 0.2–5 ml of urine was subjected to methyloxime-trimethylsilyl derivatization after enzymatic hydrolysis and organic solvent extraction. The derivative was subjected to gas chromatography/mass spectrometry-selected ion monitoring (GC/MS-SIM) analysis, and each steroid was identified based on its retention time and the ratio of its two characteristic mass ions, and quantified using stigmasterol as an internal standard (mg/g creatinine). The steroid contents of the tumor tissue were measured by Teikoku Hormone Medical Co., Ltd. (Kawasaki, Japan) using LC-MS/MS analysis. Immunohistochemical analyses of steroidogenic enzymes were performed as previously described [8, 9].

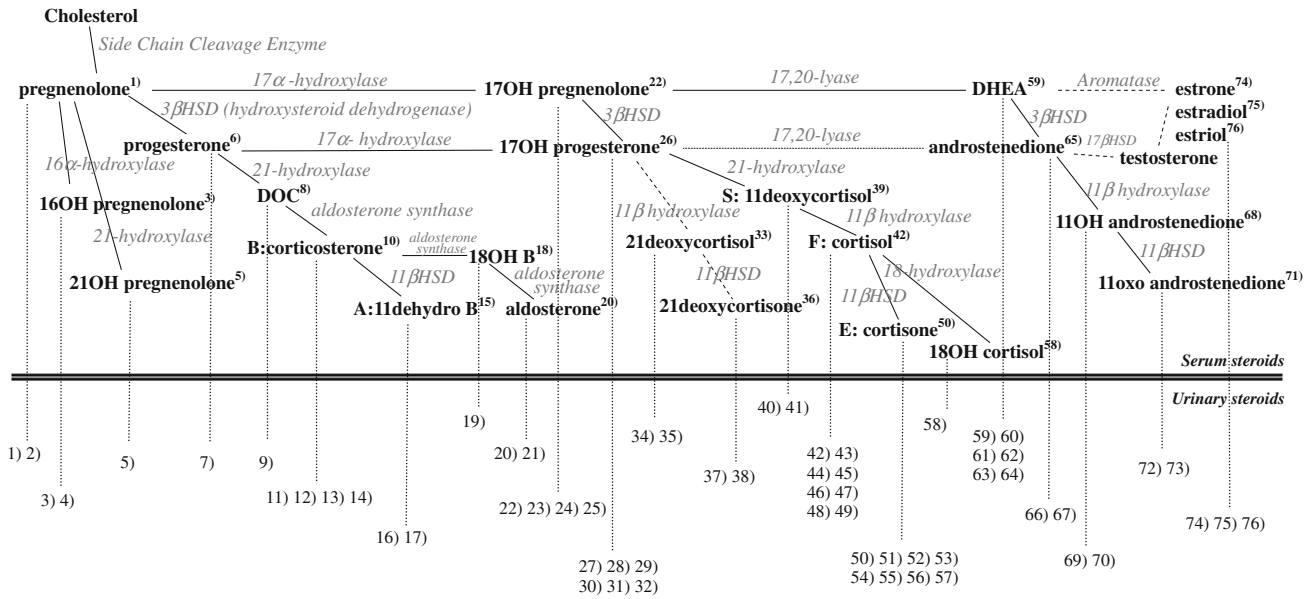

Fig. 1 Steroid metabolism map. *1* pregnenolone, *2* pregnenediol, *3* 16-hydroxypregnenolone, *4* 3,16,20-pregnenetriol, *5* 21-hydroxy-pregnenolone, *6* progesterone, *7* pregnanediol, *8* deoxycorticosterone (DOC), *9* tetrahydro-11-deoxycorticosterone, *10* corticosterone, *11* 5β-tetrahydrocorticosterone, *12* 5α-tetrahydrocorticosterone, *13* 20β-dihydrocorticosterone, *14* 6β-hydroxycorticosterone, *15* 11-dehydrocorticosterone, *16* 5β-tetrahydro-11-dehydrocorticosterone, *17* 5α-tetrahydro-11-dehydrocorticosterone, *18* 18-hydroxycorticosterone, *19* 18-hydroxy-tetrahydro-11-dehydrocorticosterone, *20* aldosterone, *21* tetrahydroaldosterone, *22* 17-hydroxypregnenolone, *23* 3,17,20-pregnenetriol, *24* 5,16-androstadiene, *25* 15,17-dihydroxypregnenolone, *26* 17-hydroxyprogesterone, *27* 5β-17-hydroxypregnanolone, *28* 5α-17-hydroxypregnanolone, *29* 20β-pregnanetriol, *30* 5β-20α-pregnanetriol, *31* 5α-20α-pregnanetriol, *32* 15,17-dihydroxypregnanolone, *33* 21deoxycortisol, *34* 11-hydroxy-17-hydroxypregnanolone, *35* pregnanetetrol, *36* 21-deoxycortisone, *37* 11-oxo-17-hydroxypregnanolone, *38* pregnanetriolone, *39* deoxycortisol, *40* 5β-tetrahydro-11-deoxycortisol, *41* 5α-tetrahydro-11-deoxycortisol, *42* cortisol, *43* 6β-hydroxycortisol, *44* 5β-tetrahydrocortisol, *45* 5α-tetrahydrocortisol, *46* 20α-cortol, *47* 20β-cortol, *48* 20α-dihydrocortisol, *49* 20β-dihydrocortisol, *50* cortisone, *51* 5β-tetrahydrocortisone, *52* 5α-tetrahydrocortisone, *53* 20α-cortolone, *54* 20β-cortolone, *55* 5α-20β-cortolone, *56* 20α-dihydrocortisone, *57* 20β-dihydrocortisone, *58* 18-hydroxycortisol, *59* DHEA, *60* androstenediol, *61* 16α-hydroxy-DHEA, *62* 16β-hydroxy-DHEA, *63* 16-oxo-androstenediol, *64* androstenetriol, *65* androstenedione, *66* androsterone, *67* etiocholanolone, *68* 11-hydroxyandrostenedion, *69* 11β-hydroxyandrosterone, *70* 11β-hydroxyetiocholanolone, *71* 11-oxoandrostenedione, *72* 11-oxoandrosterone, *73* 11-oxoetiocholanolone, *74* estrone, *75* estradiol, *76* estriol

Case report

A 27-year-old woman came to a hospital in April 2007 complaining of epigastric pain. Ultrasonography revealed a large tumor in her left adrenal region, and she was admitted to our hospital for further examination. She had been suffering from amenorrhea during the previous 2 years, though her menarche had been normal at 13 years of age. She was married but had no children. There was no family history of hypertension or endocrine tumors, and she had no past history. On physical examination, her height was 158.4 cm, body weight 45.4 kg, and body temperature 36.4°C. She denied having a recent weight change. Her blood pressure was 148/87 mmHg without postural change, and her pulse rate was 74 beats/min. Her lungs and heart were normal. The adrenal tumor was palpable under the left hypochondrium, and there were no clinical signs of virilization or hypercortisolism. Arterial blood gas analysis was nearly normal. An X-ray film of her chest was normal, and her ECG showed normal sinus rhythm but a U-wave.

The patient's laboratory results are summarized in Table 1. Serum potassium was 2.3 mEq/l, and plasma renin activity (PRA) was low (0.1 ng/ml/h), but plasma aldosterone was in the normal range (66 pg/ml). Plasma ACTH and serum cortisol were normal, and their diurnal rhythm was nearly normal (Table 2). The serum DOC level was markedly elevated and not suppressed by administration of 2- or 8-mg of dexamethasone (Table 3). Serum

Table 2 Diurnal rhythm of ACTH and cortisol

	8:00	12:00	16:00	20:00	23:00
ACTH (pg/ml)	36	24	16	9	8
Cortisol (μg/dl)	12.7	7.6	4.9	3.7	3.3

Table 1 Plasma biochemistry at 8:00 am before and after removal of the tumor

	Before	After		Plasma hormone	Before	After	
Electrolyte				ACTH	25	53	pg/ml (7–56)
Serum				LH	4.3	15.0	μU/ml (*1)
Na	143	140	mEq/l (136–144)	FSH	2.7	4.2	μU/ml (*2)
K	2.3	4.4	mEq/l (3.6–4.8)	PRL	9.1	21.8	ng/ml (6.12–30.54)
Cl	101	106	mEq/l (99–109)	Cortisol	10.4	12.6	μg/dl (5–15)
Urinary				PRA	0.1	0.8	ng/ml/h (0.2–2.7)
Na	104.6	94.5	mEq/day	Aldosterone	66	63	pg/ml (30–159)
K	35.7	24	mEq/day	DOC	8.04	0.09	ng/ml (0.03–0.33)
Cl	117.3	94.5	mEq/day	Corticosterone	5.88	–	ng/ml (0.21–8.43)
Renal function				18-OH DOC	0.13	0.06	ng/ml (0.01–0.07)
BUN	8	15	mg/dl (8–22)	Progesterone	3.53	0.52	ng/ml (*3)
Creatinine	0.5	0.9	mg/dl (0.4–0.8)	17-OH prog	3.0	0.8	ng/ml (0.2–2.8)
Urinary hormone				Pregnenolone	5.04	0.31	ng/ml (0.2–1.5)
17-OHCS	4.8	5.1	mg/day (2.4–11.8)	Estradiol	34.2	32.0	pg/ml (*4)
17-KS	6.9	6.1	mg/day (2.2–7.3)	DHEA-S	80	95	ng/ml (73–322)
Adrenaline	2.1	4.0	μg/day (3.4–26.9)	Testosterone	48.0	30.1	ng/dl (9.12–111)
Noradrenaline	48.0	235.4	μg/day (48.6–168.4)	Adrenaline	9	14	pg/ml (<100)
Dopamine	808.5	2181	μg/day (365.0–961.5)	Noradrenaline	125	117	pg/ml (100–450)
Metanephrine	0.03	0.05	mg/day (0.04–0.19)	Dopamine	5	9	pg/ml (<20)
Normetanephrine	0.10	0.14	mg/day (0.09–0.33)				

	Follicular phase	Ovulation	Luteal phase	Menopause
*1	1.76–10.24	2.19–88.33	1.13–14.22	5.72–64.31
*2	3.01–14.72	3.21–16.60	1.47–8.49	<157.79
*3	<0.92	<2.36	1.28–29.6	<0.44
*4	20–350	50–550	45–300	<21

Values in parentheses indicate normal range

BUN blood urea nitrogen, *17-OHCS* 17-hydroxycorticosteroids, *17-KS* 17-ketosteroids, *ACTH* adrenocorticotropic hormone, *LH* Lutenizing hormone, *FSH* follicle stimulating hormone, *PRL* prolactin, *PRA* plasma renin activity, *DOC* 11-deoxycorticosterone, *18-OH DOC* 18-hydroxydeoxycorticosterone, *17-OH prog* 17-hydroxyprogesterone, *DHEA-S* dehydroepiandrosterone sulfate

Table 3 Dexamethasone suppression test (Liddle method)

	Base data	2 mg day 1	2 mg day 2	8 mg day 1	8 mg day 2	Normal range
Serum						
ACTH (pg/ml)	25	<5	<5	<5	<5	7–56
Cortisol (μg/dl)	10.4	3.6	4.0	3.8	4.3	5–15
DOC (ng/ml)	8.04	6.97	6.72	6.52	5.77	0.03–0.33
18-OH DOC (ng/ml)	0.13	0.17	0.18	0.19	0.16	0.01–0.07
Progesterone (ng/ml)	3.53	3.99	3.86	4.13	4.79	<0.92
Pregnenolone (ng/ml)	5.04	4.63	4.63	4.26	5.88	0.2–1.5
Urine						
17-OHCS (mg/day)	4.8	5.3	5.0	5.9	7.0	2.2–7.3
17-KS (mg/day)	6.9	5.9	4.2	3.9	3.8	2.4–11.0
Free cortisol (μg/day)	23.1	26.7	8.9	9.2	18.1	11.2–80.3

18-hydroxydeoxycorticosterone (18-OH-DOC) was also high, though serum corticosterone (B) was within the normal range. Serum pregnenolone, progesterone, and 17-OH progesterone were also high, but testosterone and dehydro-epi-androsterone-sulfate (DHEA-S) were in the normal range. Serum and urinary concentrations of catecholamines and their metabolites were all normal (Table 1).

We also performed a complete analysis of the patient's urinary steroid metabolites (Fig. 1 and Table 4). Her urinary steroid profile showed elevated secretion of 17-deoxysteroids or 11-deoxysteroids (progesterone, DOC, 11-dehydrocorticosterone (data not shown), and 11-deoxycortisol), as well as 3β-hydroxy 5-en steroids (pregnenolone, 17-OHpregnenolone, and DHEA). Moreover, these metabolites were not suppressed by administration of dexamethasone (Table 4).

Computed tomography revealed a heterogeneous tumor in the left adrenal region (12 cm), which was pushing down on the left kidney (Fig. 2a, b). Magnetic resonance imaging (MRI) also revealed a heterogeneous tumor, which was of partially high density on T2 emphasizing phase images (data not shown). F-fluorodeoxyglucose positron emission tomography showed strong accumulation in the left adrenal mass, but no abnormal accumulation in other organs (Fig. 2c).

After a diagnosis of an adrenal tumor producing DOC and progesterone, the tumor was resected through a posterolateral skin incision. The tumor was 17 × 11 × 7.8 cm in size and weighed 480 g; the cut surface was yellowish brown in color and solid.

Based on the Weiss criteria, the resected tumor was diagnosed as an adrenocortical carcinoma. Nuclear grade was III, mitotic rate was >5 per 50 HPF, atypical mitosis was absent, character of cytoplasm was unclear, architecture of tumor was diffuse, necrosis was present, invasion of capsule was present, invasion of sinusoidal structure was present, and invasion of venous structure was absent. More than 10% of the cells were MiB 1 (Ki67)-positive (data not shown).

Table 5 shows the steroid contents in the tumor tissue. In comparison with the concentration in serum, the contents of pregnenolone, progesterone, and DOC in the tumor tissue were extremely high. This result suggests this tumor had produced these steroids.

Analysis for steroidogenic enzymes demonstrated that P450SCC immunoreactivity was diffusely positive in carcinoma cells (Fig. 3a). P450c17 immunoreactivity was not detected in the great majority of carcinoma cells (Fig. 3b). 3β-Hydroxysteroid dehydrogenase (3β-HSD) immunoreactivity was very sparsely detected (Fig. 3c). P450c21 immunoreactivity was sporadically detected (Fig. 3d). P450c11 immunoreactivity was detected in some tumor cells (data not shown).

Postoperatively, the patient's blood pressure dropped to about 100/60 mmHg, and her serum potassium level normalized without medication. Menorrhea occurred 3 months after the tumor resection. The postoperative endocrinological findings are shown in Table 1. Serum concentrations of DOC and progesterone returned to normal after the operation (Table 1), as did urinary levels of steroid metabolites (Table 4).

Discussion

We report here a patient with hypertension, hypokalemia, and amenorrhea accompanying an adrenal tumor. Because hypokalemia and low plasma renin activity were observed, despite normal plasma aldosterone levels, we presumed that the left adrenal tumor was producing mineralocorticoid intermediates such as DOC or corticosterone. Hypokalemia is most often caused by increased potassium elimination from the kidney or intestine, by insufficient oral uptake or by intracellular uptake of potassium. Our patient had taken

Table 4 Urine steroid metabolites data (mg/g creatinine)

Origin steroid	Metabolites no. in Fig. 1	Pre-Dex	Dex 2 mg	Dex 8 mg	Post-OPE	Normal range of adult female (18–49 years)			
						N	5 percentile	50 percentile	95 percentile
Pregnenolone	Σ1,2	16.800 H	12.684 H	14.925 H	0.452 H	189	0.000	0.000	0.083
Progesterone	7	11.460 H	14.994 H	18.538 H	0.484	189	0.169	0.490	3.236
DOC	9	2.467 H	2.336 H	2.386 H	0.007	187	0.000	0.000	0.040
Corticosterone	Σ11,12,16,17	1.345 H	0.904	0.964	0.285 L	189	0.307	0.600	1.067
18OHcorticosterone	19	0.023	0.009 L	0.015 L	0.017 L	63	0.020	0.043	0.117
Aldosterone	21	0.002 L	0.000 L	0.000 L	0.002 L	115	0.010	0.028	0.061
17OHpregnenolone	Σ22,23	2.757 H	1.715 H	2.301 H	0.244	189	0.121	0.250	0.554
17OHprogesterone	Σ27–32	1.263	1.176	1.320	0.459	188	0.321	0.680	1.644
11deoxycortisol	40	2.915 H	2.502 H	3.249 H	0.055	186	0.030	0.065	0.151
Cortisol	Σ44,45,51,52	6.824	1.879 L	2.051 L	4.862	189	3.552	5.430	8.178
DHEA	Σ59–64	6.026 H	1.472	1.384	1.810	189	0.434	1.390	4.076
Androstenedione	Σ66,67	1.747	0.734 L	0.772 L	2.713	189	1.372	2.500	4.292
11OH androstenedione	69	0.884	0.232 L	0.259 L	0.617	189	0.332	0.590	0.966
Estrogen	Σ74–76	0.031	0.025	0.029	0.010	189	0.000	0.025	0.081

no drug, had been eating a normal diet, and had no diarrhea. On the other hand, her urinary elimination of potassium was more than 35 mEq/day despite her low serum potassium (2.3 mEq/l), and her TTKG was 7.27. This patient's hypokalemia thus appeared to have been the result of renal potassium loss. Moreover, our finding that the patient's serum and urinary potassium normalized after resection of her tumor eliminated the possibility that a kidney disease such as renal tubular acidosis was the cause, further confirming that the hypokalemia was caused by the adrenal tumor. On the other hand, the serum and urinary levels of several adrenal steroid intermediates were elevated in this patient. The adrenal mineralocorticoids that can cause renal potassium loss are aldosterone, cortisol, corticosterone, and DOC. In this patient, basal serum aldosterone, cortisol, and corticosterone were nearly in the normal range, but DOC was extraordinarily high. Her 18-OH DOC was also slightly higher than normal, but 18-OH DOC has no effect on urinary potassium excretion, though it does stimulate hydrogen ion excretion [10]. The high serum DOC level normalized after the tumor was resected, along with the serum potassium level. In addition, the DOC content in the resected tumor tissue was high in comparison to the concentration in serum. The mineralocorticoid activity of DOC is about one twentieth that of aldosterone. This patient's serum DOC concentration was 8.04 ng/ml, which corresponds to the activity of about 400 pg/ml aldosterone, which is consistent with her serum and urinary potassium levels. Therefore, we diagnosed hypersecretion of DOC from an adrenal tumor as the major cause of our patient's hypokalemia.

In addition to her hypokalemia, this patient also exhibited amenorrhea that was cured after removal of her tumor. Amenorrhea with an adrenal tumor is most often related to hypersecretion of androgens. In this case, however, biologically active androgens were not elevated. We therefore speculated that the hypersecretion of progesterone and related hormones was the cause of the patient's amenorrhea. A connection between amenorrhea and progesterone has been reported in congenital adrenal hyperplasia characterized by 21-hydroxylase or 17-hydroxylase deficiency [11, 12]. Amenorrhea in patients with congenital adrenal hyperplasia is attributable in part to high progestogenic steroid levels exerting a "mini-pill (progestin contraceptive)" effect on the endometrium [13]. Progestin contraceptives are known to dose-dependently suppress FSH levels and reduce both the number and amplitude of LH pulses [14]. However, the minimal dose of progesterone needed to cause menstrual cycling to cease is not well established. In this case, FSH and LH were not completely suppressed, but their levels were below or near the lower limit of the normal range. This might be compatible with the patient's serum progesterone level, which was

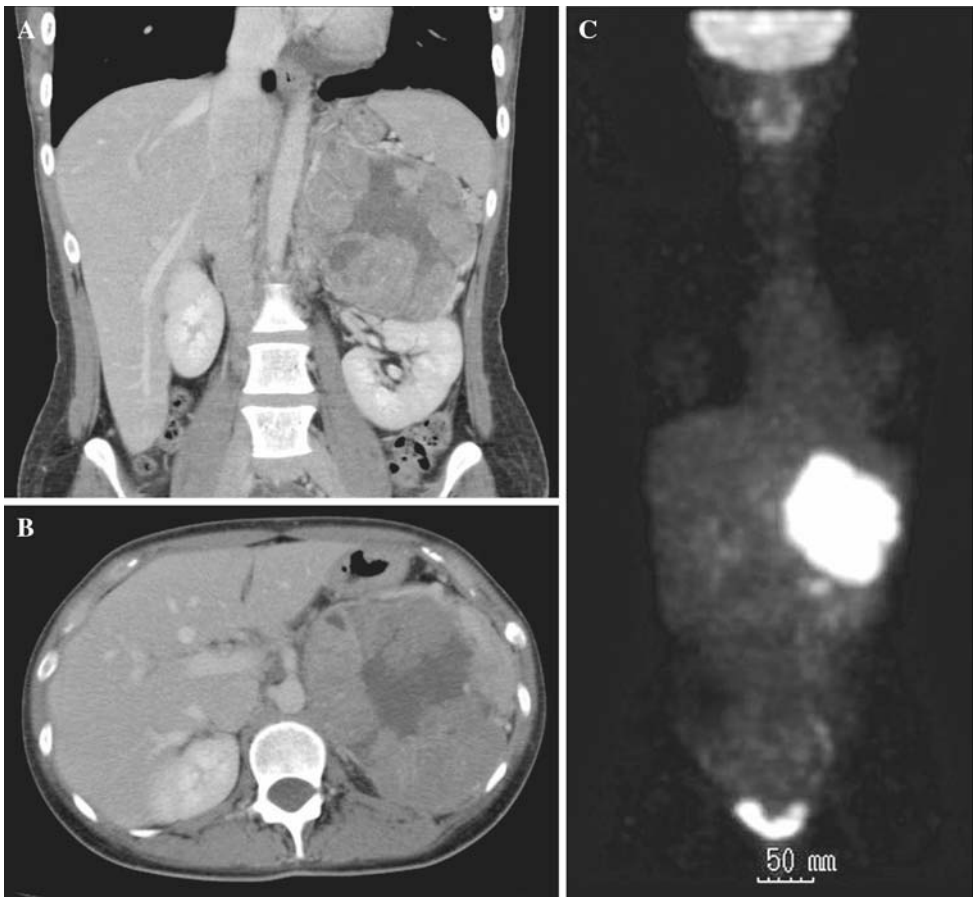

Fig. 2 **a** Contrast enhanced computed tomography, *coronal image*. **b** Contrast enhanced computed tomography, *horizontal image*. **c** F-fluorodeoxyglucose positron emission tomography

Table 5 Steroid contents in tumor tissue

Pregnenolone (ng/g)	1533.1
Progesterone (ng/g)	830.0
17OH pregnenolone (ng/g)	42.5
17OH progesterone (ng/g)	78.1
DOC (ng/g)	141.7
Cortisol (ng/g)	563.3
Aldosterone (pg/g)	83.5
DHEA (ng/g)	97.8

continuously higher than the normal range for the follicular phase, but not markedly so. Her urinary pregnanediol, which is the metabolite of progesterone, was 11.46 (mg/g creatinine) and higher than the normal range for the Luteal phase (min 1.2 to max 6.9 mg/g creatinine, $n = 26$). This suggests continuous high production of progesterone. We measured FSH and LH only once prior to the patient's surgery, but repeated measurements would have been better for determination of her LH level. Another possible explanation for the secondary amenorrhea in this patient is hypothalamic amenorrhea caused by the carcinoma. However, as the general condition of this patient was good and no weight loss was observed, we suggest it is unlikely that hypothalamic amenorrhea contributed significantly to her amenorrhea.

We also examined the ACTH dependency of this hypersecretion of steroid intermediates and found that the secretion was not suppressed during the dexamethasone suppression test (Table 3). This finding is consistent with earlier reports indicating that plasma DOC levels are under the control of ACTH in benign DOC-producing tumors, but not in malignant tumors [3, 4, 15–17]. Dexamethasone suppresses adrenal steroidogenesis by suppressing ACTH secretion from the pituitary gland. ACTH is known to regulate quantitatively the steroidogenesis at the first step, the conversion of cholesterol to pregnenolone, which is the enzymatically rate-limiting step [18]. Chronic regulation is principally at the level of transcription of the gene for cholesterol side-chain cleavage P-450 (P450SCC). Acute regulation is mediated by steroidogenic acute regulatory protein, which facilitates the rapid influx of cholesterol into mitochondria, where P450SCC resides [18]. Our result suggests that this regulatory system would be disordered in this adrenocortical carcinoma cells.

Pregnenolone, progesterone, and DOC were also not suppressed in the dexamethasone suppression test, but cortisol was suppressed (Table 3). This suggests that

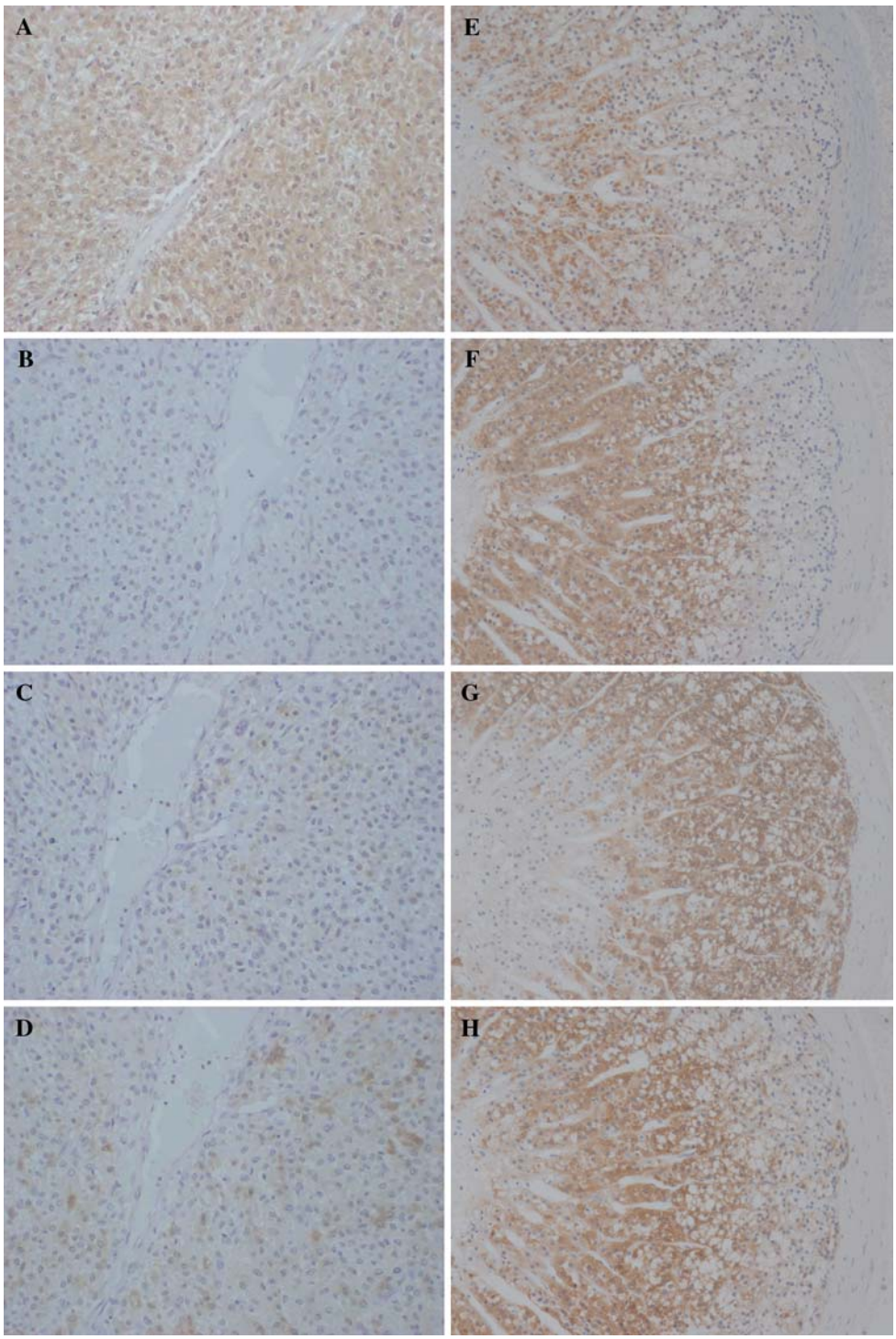

Fig. 3 Immunohistochemical analysis of steroidogenic enzymes. **a–d** The adrenocortical carcinoma, **e–h** a normal adrenal gland in another patient (*right*: zona gromerulosa). **a**, **e** P450SCC. **b**, **f** P450c17. **c**, **g** 3β-hydroxysteroid dehydrogenase (3β-HSD). **d**, **h** P450c21

pregnenolone, progesterone, and DOC were autonomously secreted from our patient's adrenal tumor, whereas cortisol was secreted from the normal adrenal gland (Fig. 1). The reason why not cortisol but pregnenolone, progesterone, and DOC were secreted from the tumor would be the deficiency of P450c17 and P450c11 in the tumor cells. It has been reported that levels of the transcriptional factor SF-1 are low, while those of nuclear receptor DAX-1 are high, in DOC-producing adrenal tumors [19]. As a consequence, expression of the steroidogenic enzyme P450c17 is weak [19]. Cytochrome P450c17 catalyzes both 17α-hydroxylation and 17,20-lyase conversion of 21-carbon steroids to 19-carbon precursors of sex steroids [20]. In the absence of P450c17, steroidogenic cells produce C21 17-deoxysteroids [18]. It also has been suggested that expression of steroidogenic enzymes plays a key causative role in the

Table 6 Ratios of urine steroid metabolites data

Enzyme activity	Origin steroid ratio	Metabolites no. in Fig. 1	Pre-Dex	Dex 2 mg	Dex 8 mg	Post-OPE	Normal range of adult female (18–49 years)			
							N	5 percentile	50 percentile	95 percentile
3βHSD	Progesterone/pregnenolone	7/2	0.06 L	0.05 L	0.05 L	2.00 L	65	2.16	5.92	15.89
	Androstenedione/DHEA	Σ66,67/Σ59–64	0.29 L	0.50 L	0.56 L	1.50	189	0.61	1.80	4.60
17α-hydroxylase	17OHpregnenolone/pregnenolone	23/2	0.46 L	0.41 L	0.45 L	5.98	65	2.03	4.72	16.82
	17OHprogesterone/progesterone	30/7	0.08 L	0.06 L	0.05 L	0.68	189	0.23	0.86	1.68
21-hydroxylase	DOC/progesterone	9/7	0.22 H	0.16 H	0.13 H	0.01	188	0.00	0.00	0.05
	11deoxycortisol/17OHprogesterone	40/30	3.33 H	2.96 H	3.48 H	0.17	189	0.04	0.16	0.44
11β-hydroxylase	Corticosterone/DOC	Σ11,12,16,17/9	0.55 L	0.39 L	0.40 L	40.71	85	10.56	51.00	155.01
	Cortisol/11deoxycortisol	Σ44,45,51,52/40	2.34 L	0.75 L	0.63 L	88.40	185	36.56	80.80	164.60

pathophysiology stemming from steroid-producing adrenal tumors [21, 22].

In the present case, the ratios of 17α-hydroxysteroids to 17-deoxysteroids (17OHpregnenolone/pregnenolone and 17OHP/progesterone), 11β-hydroxysteroids to 11-deoxysteroids (corticosterone/DOC and cortisol/11-deoxycortisol), Δ4 steroids to 3β-hydroxy 5en steroids (progesterone/pregnenolone and androstenedione/DHEA) were decreased, and the ratios of 21-hydroxysteroids to 21-deoxysteroids (DOC to progesterone and 11-deoxycortisol to 17OHP) increased, compared with control females. The abnormality of these metabolite ratios suggests impairment of 17α-hydroxylase, 11β-hydroxylase and 3β-HSD activities, and enhanced 21-hydroxylase activity in the tumor tissue (Fig. 1 and Table 6). On immunohistochemical analysis of the steroidogenic enzymes, P450SCC immunoreactivity was highly detected in the great majority of carcinoma cells, while P450c17 and 3β-HSD immunoreactivity was little detected. P450c21 and P450c11 immunoreactivity was detected in some of the carcinoma cells. These results are compatible with that of the analysis of urinary steroids.

Our analyses also revealed heterogeneity of the steroidogenesis in this adrenocortical carcinoma, which was accompanied by a corresponding heterogeneity in the expression of steroidogenic enzymes termed "disorganized steroidogenesis" in an earlier report on adrenocortical carcinomas [8]. In adrenocortical carcinomas, disordered expression of steroidogenic enzymes within tumor tissues can lead to hypersecretion of various steroid intermediates. Notably, however, this can often be overlooked because these intermediates are generally not measured in routine clinical practice. Thus, the presence of an adrenal tumor that produces steroid intermediates should not be overlooked in the differential diagnosis of hypokalemia or amenorrhea.

References

1. R.T. Kloos, M.D. Gross, I.R. Francis, M. Korobkin, B. Shapiro, Incidentally discovered adrenal masses. Endocr. Rev. **16**(4), 460–484 (1995)
2. R. Fraser, V.H. James, J. Landon, W.S. Peart, A. Rawson, C.A. Giles, A.M. McKay, Clinical and biochemical studies of a patient with a corticosterone-secreting adrenocortical tumour. Lancet **2**(7578), 1116–1120 (1968)
3. J.D. Powell-Jackson, A. Calin, R. Fraser, R. Grahame, P. Mason, G.A. Missen, P.R. Powell-Jackson, A. Wilson, Excess deoxycorticosterone secretion from adrenocortical carcinoma. Br. Med. J. **2**(5909), 32–33 (1974)
4. K. Kondo, T. Saruta, I. Saito, R. Yoshida, H. Maruyama, S. Matsuki, Benign desoxycorticosterone-producing adrenal tumor. JAMA **236**(9), 1042–1044 (1976)
5. Y. Takahashi, J. Ninomiya, J. Horiguchi, H. Shimizu, M. Sato, Y. Koibuchi, T. Yoshida, M. Yoshida, D. Takata, H. Odawara, T.

Yokoe, Y. Iino, Y. Morishita, M. Mori, Primary amenorrhea accompanied by adrenal adenoma: start of menarche soon after tumor resection. Intern. Med. **41**(11), 972–976 (2002)
6. P. van Zonneveld, H.P. Koppeschaar, T.W. de Bruin, T.M. Vroom, M.A. Blankenstein, T.J. van Vroonhoven, A patient with a progesterone-producing adrenal adenoma who presented with primary amenorrhea. Gynecol. Endocrinol. **9**(3), 189–194 (1995)
7. K. Homma, T. Hasegawa, M. Masumoto, E. Takeshita, K. Watanabe, H. Chiba, T. Kurosawa, T. Takahashi, N. Matsuo, Reference values for urinary steroids in Japanese newborn infants: gas chromatography/mass spectrometry in selected ion monitoring. Endocr. J. **50**, 783–792 (2003)
8. H. Sasano, T. Suzuki, H. Nagura, T. Nishikawa, Steroidogenesis in human adrenocortical carcinoma: biochemical activities, immunohistochemistry, and in situ hybridization of steroidogenic enzymes and histopathologic study in nine cases. Hum. Pathol. **24**(4), 397–404 (1993)
9. A. Sugawara, K. Takeuchi, T. Suzuki, K. Itoi, H. Sasano, S. Ito, A case of aldosterone-producing adrenocortical adenoma associated with a probable post-operative adrenal crisis: histopathological analyses of the adrenal gland. Hypertens. Res. **26**(8), 663–668 (2003)
10. M.C. Damasco, F. Diaz, J.P. Anal, C.P. Lantos, Acute effects of three natural corticosteroids on the acid-base and electrolyte composition of urine in adrenalectomized rats. Acta Physiol. Lat. Am. **29**, 305 (1979)
11. R.L. Rosenfield, S. Bickel, A.K. Razdan, Amenorrhea related to progestin excess in congenital adrenal hyperplasia. Obstet. Gynecol. **56**(2), 208–215 (1980)
12. D.J. Holmes-Walker, G.S. Conway, J.W. Honour, G. Rumsby, H.S. Jacobs, Menstrual disturbance and hypersecretion of progesterone in women with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Clin. Endocrinol. (Oxf) **43**(3), 291–296 (1995)
13. J. Helleday, B. Siwers, E.M. Ritzén, K. Carlström, Subnormal androgen and elevated progesterone levels in women treated for congenital virilizing 21-hydroxylase deficiency. J. Clin. Endocrinol. Metab. **76**(4), 933–936 (1993)
14. D.J. Hemrika, E.H. Slaats, J.C. Kennedy, T.J. de Vriess Robles-Korsen, J. Schoemaker, Pulsatile luteinizing hormone patterns in long term oral contraceptive users. J. Clin. Endocrinol. Metab. **77**(2), 420–426 (1993)
15. Y. Toyoda, M. Mizukoshi, M. Umemoto, M. Kuchii, K. Ueyama, S. Tomimoto, A. Baba, H. Shima, I. Nishio, Y. Masuyama, Adrenal tumor producing 11-deoxycorticosterone, 18-hydroxy-11-deoxycorticosterone and aldosterone. Intern. Med. **35**(2), 123–128 (1996)
16. K. Makino, K. Yasuda, M. Okuyama, M. Ojima, N. Sasano, K. Miura, An adrenocortical tumor secreting weak mineralocorticoids. Endocrinol. Jpn. **34**(1), 65–72 (1987)
17. S.E. Ishikawa, T. Saito, K. Kaneko, K. Okada, S. Fukuda, T. Kuzuya, Hypermineralocorticism without elevation of plasma aldosterone: deoxycorticosterone-producing adrenal adenoma and hyperplasia. Clin. Endocrinol. (Oxf) **29**(4), 367–375 (1988)
18. W.L. Miller, Steroidogenic enzymes. Review. Endocr. Dev. **13**, 1–18 (2008)
19. H. Shibata, Y. Ikeda, T. Mukai, K. Morohashi, I. Kurihara, T. Ando, T. Suzuki, S. Kobayashi, M. Murai, I. Saito, T. Saruta, Expression profiles of COUP-TF, DAX-1 and SF-1 in human adrenal gland and adrenocortical tumors: possible implications in steroidogenesis. Mol. Genet. Metab. **74**, 206–216 (2001)
20. R.J. Auchus, The genetics, pathophysiology, and management of human deficiencies of P450c17. Review. Endocrinol. Metab. Clin. North Am. **30**(1), 101–119, vii (2001)
21. T. Ogishima, H. Shibata, H. Shimada, F. Mitani, H. Suzuki, T. Saruta, Y. Ishimura, Aldosterone synthase cytochrome P-450 expressed in the adrenals of patients with primary aldosteronism. J. Biol. Chem. **266**, 10731–10734 (1991)
22. H. Shibata, H. Suzuki, T. Ogishima, Y. Ishimura, T. Saruta, Significance of steroidogenic enzymes in the pathogenesis of adrenal tumor. Acta Endocrinol. **128**, 235–242 (1993)