Two cases of aldosterone and cortisol producing adenoma with different histopathological features
A case report
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Abstract
Rationale: Primary aldosteronism (PA), including aldosterone and cortisol producing adenoma (A/CPA), is the most common trigger of secondary hypertension. The prevalence of A/CPA may be higher than what we could recognize previously with similar studies, but only a few relevant immunohistochemical reports have confirmed this information. Collecting more clinical features and immunohistochemistry data may help us to understand A/CPA, which is very important for avoiding misdiagnosis and improving outcomes in patients with A/CPA.

Patient concerns: Both individuals included in this study had hypertension for >10 years. Computed tomography scans revealed the presence of adrenal nodules 1 year ago in patient A and 10 months ago in patient B (based on the date of the final version of this report). The relevant clinical features support PA accompanied by subclinical Cushing syndrome.

Diagnosis: Aldosterone and cortisol producing adenoma.

Interventions: The adrenal adenoma on the affected side was removed and pathological examination and immunohistochemistry were performed. Both the patients received short-term hydrocortisone treatment.

Outcomes: The blood pressure of both patients improved after surgery. Cytochrome P450 (CYP)11B1, CYP11B2, parathyroid hormone receptor 1 (PTH1R), calcium-sensing receptor (CaSR), and vitamin D3 receptor (VD3R) were all positively expressed, but the histopathological features of the expression region differed.

Lessons: The occurrence of A/CPA may be related to calcium metabolism disorders. For A/CPA, the diversity in immunohistochemistry suggests many uncertainties regarding the pathogenesis of the disease. A/CPA should be considered in new clinical and pathological classifications of PA to gain more attention from the medical community.

Abbreviations: A/CPA = aldosterone and cortisol producing adenoma, ADRR = aldosterone to direct renin concentration ratio, AVS = adrenal venous sampling, BMI = body mass index, CaSR = calcium-sensing receptor, CYP = cytochrome P450, DRC = direct renin concentration, PA = primary aldosteronism, PAC = plasma aldosterone concentration, PAC = plasma aldosterone concentration, PRA = plasma renin activity, PTH1R = parathyroid hormone receptor 1, SCS = Subclinical Cushing’s syndrome, VD3R = vitamin D3 receptor.

Keywords: adrenal tumor, cosecretion, Immunohistochemistry, primary aldosteronism, subclinical Cushing’s syndrome.

1. Introduction

According to previous population studies, about 5 to 10% of patients diagnosed with hypertension have primary aldosteronism (PA),\textsuperscript{[1]} which is the most common cause of secondary hypertension. As the understanding of such conditions deepens in the medical community, PA has been divided into 6 subtypes based on etiology.\textsuperscript{[2]} Complementarily, unilateral PA has been divided into 6 subtypes based on histopathological features.\textsuperscript{[3]} However, the prevalence of aldosterone and cortisol producing adenoma (A/CPA) is higher than what we have previously recognized, and A/CPA may also become one of the subtypes of PA in the future. To date, there are few reports addressing this issue and studies available in the community lack immunohistochemical...
results. Here, we reported 2 cases of A/CPA with different histopathological features and reviewed the literature concerning this specific condition to further understand the clinical aspects of such cases.

2. Case report

2.1. Case 1
A 53-year-old Han Chinese woman who had been suffering from hypertension for 13 years reported that a doctor found adrenal nodules in her body 1 year prior to her admission to our hospital. She was administered amlodipine besylate (5 mg QD) for blood pressure control. On admission, her body mass index (BMI) was 29.21 kg/m², and blood pressure was 145/98 mm Hg. The plasma aldosterone concentration (PAC) and direct renin concentration (DRC) were 46.1 µIU/ml. Furthermore, the patient's dynamic postural test showed that supine position PAC was 21.3 ng/dL, DRC was 0.98 µIU/ml, plasma aldosterone to direct renin concentration ratio (ADRR) was 21.73 (ng/dL)/(µIU/mL), upright position ALD-DRC-ADRR: 60.1 ng/dL to 2.33 µIU/mL to 25.79 (ng/dL)/(µIU/mL). Blood potassium was measured by the ion-selective electrode method (indirect method), blood potassium was measured by the colorimetric method, and serum parathyroid hormone and 25-OH-D3 were measured by chemiluminescence. The patient's serum potassium was 3.44 mmol/L (reference range 3.5–5.3 mmol/L), serum calcium was 2.24 mmol/L (reference range 2.11–2.52 mmol/L), serum creatinine was 96 mmol/L (reference range 3.5–5.3 mmol/L), serum calcium level 2.25 mmol/L, PTH 5.05 ng/L, and 25-OH-D3 54.8 nmol/L. Similar to the case 1, his saline infusion test and captopril inhibition test showed no inhibition, confirming a diagnosis of PA. A computed tomography scan showed a shadowed figure of an oval soft tissue density nodule in the right adrenal gland with uniform density and regular shape that was obviously enhanced. The shadow was about 2.6 × 1.9 cm with clear peripheral fat and atrophy of the left adrenal gland. The patient underwent surgery for the direct removal of the right adrenal tumor directly without AVS. Pathological examination confirmed the presence of a cortical adenoma with considerable pigment deposition. The nodule was about 2.5 cm²/2.2 cm²/cm², with grayish-black or grayish-yellow section (solid, medium), clear boundaries with the surrounding area, and adrenal cortex thickness 0.1 to 0.3 cm.

Neither patient had a family history of high blood pressure or adrenal tumors. Neither patient had full-moon face or bloody appearance. For both patients, no fat pads were identified at the back of their neck, and no hirsutism, purple striae, and centripetal obesity were observed. Plasma metanephrine and normetanephrine were normal. The test of cortisol confirmed the absence of circadian rhythm. Overnight 1 mg dexamethasone inhibition test and classic low-dose dexamethasone suppression test showed no inhibition (Table 2), thus supporting the diagnosis of SCS.

Both the patients received short-term hydrocortisone supplementation after surgery. Their blood pressure did not return to normal, but improved significantly. PAC, DRC, ADRR, serum cortisol, and serum potassium levels were all normal 3 months after surgery.

The expression of cytochrome P450 (CYP) 11B1, CYP11B2, parathyroid hormone receptor 1 (PTH1R), calcium-sensing receptor (CaSR), and vitamin D3 receptor (VD3R) were detected by immunohistochemistry and multiple fluorescence immunohistochemistry of the postoperative adrenal tissue in 2 patients. The paraffin section specimens were handled using the Opal Polaris 7-color Manual Immunohistochemistry kit (Akoya Biosciences, USA) as the manufacturer's recommendation. Multiple fluorescence immunohistochemistry images were acquired using the Vectra Polaris Automated Quantitative Pathology Imaging System (PerkinElmer, USA). Antibodies against CYP11B1 (MAB502) and CYP11B1 (MAB51251) were purchased from the Merck Group. Antibody against VD3R (12550) was purchased from Cell Signaling Technology. Antibody against CaSR (MA1-934) was purchased from Thermo Fisher Scientific. Antibody against PTH1R (MAB709) was purchased from R&D Systems. The results showed that CYP11B1 (encoding 11β-hydroxylase), CYP11B2 (encoding aldosterone synthase), and Ca(2+) metabolism-related receptors (CaSR, VD3R, and PTH1R) were positively expressed in the same region of tumor tissue. CYP11B1 and CYP11B2 were expressed in the zona glomerulosa - and zona fasciculata-like cells, respectively. Interestingly, the expression sites of CYP11B1,

### Table 1

| Vena cava | Left adrenal vein –1 | Right adrenal vein –1 |
|-----------|----------------------|----------------------|
| PAC (ng/dL) | 36.4 | 2600 | 7280 |
| Serum cortisol (nmol/L) | 613 | 1775 | 28,236 |
| PAC/serum cortisol | 0.06 | 1.46 | 0.26 |

PAC = plasma aldosterone concentration.

months ago. He was diagnosed with hypertension more than a decade ago and was treated with amiodipine besylate (5 mg QD) for blood pressure control. On admission, the patient's BMI was 29.21 kg/m², and blood pressure was 160/102 mm Hg. His dynamic postural test showed that supine position PAC was 19.48 ng/dL, plasma renin activity (PRA) 0.33 ng/ml/h, plasma aldosterone/renin activity ratio (ARR) 59.03 (ng/dL)/(ng/mL/h), and upright position PAC-PRA-ARR 32.56 ng/dL to 1.24 ng/mL/h to 26.26 (ng/dL)/(ng/mL/h). The radioimmunoassay method was used to detect PAC using a kit from Tianjin Jiuding Medical Bioengineering Co., Ltd., and to detect PRA using a kit from Beijing North Biotechnology Research Institute Co., Ltd. The upright position reference interval for PAC was 9.8 to 27.5 ng/dL, while that for PRA was 0.93 to 6.56 ng/mL/h. The serum potassium level was 3.9 mmol/L, serum calcium level 2.25 mmol/L, PTH 5.05 ng/L, and 25-OH-D3 54.8 nmol/L. Similar to the case 1, his saline infusion test and captopril inhibition test showed no inhibition, confirming a diagnosis of PA. A computed tomography scan showed a shadowed figure of an oval soft tissue density nodule in the right adrenal gland with uniform density and regular shape that was obviously enhanced. The shadow was about 2.6 × 1.9 cm with clear peripheral fat and atrophy of the left adrenal gland. The patient underwent surgery for the direct removal of the right adrenal tumor directly without AVS. Pathological examination confirmed the presence of a cortical adenoma with considerable pigment deposition. The nodule was about 2.5 cm²/2.2 cm²/cm², with grayish-black or grayish-yellow section (solid, medium), clear boundaries with the surrounding area, and adrenal cortex thickness 0.1 to 0.3 cm.

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CYP11B2, PTH1R, CaSR, and VD3R in both patients were heterogeneous (Figs. 1–5).

3. Discussion

In 1977, Hogan et al \[4\] reported a case of PA accompanied by subclinical Cushing syndrome (SCS) for the first time, and subsequent studies found this condition was not uncommon.\[5–7\] According to the literature concerning immunohistochemistry based on CYP11B1 and CYP11B2 staining, PA accompanied by SCS may occur in various forms, with A/CPA being the most common. Zhang et al have reported a case of bilateral adenomas that secrete cortisol and aldosterone, respectively,\[8\] while some researchers observed diffuse staining of CYP11B1 in adenoma lesions, and high CYP11B2 expression in the adjacent adrenal cortex.\[9\] Studies have pointed out that about 10% of PA patients have A/CPA,\[7,10,11\] and in PA patients with a single adenoma, the prevalence of A/CPA is 9.7 to 21%.\[11,12\] However, misdiagnosis often occurs because of the absence of clinical manifestations of overt cortisol levels increase in patients. Despite these diagnostic challenges, patients with A/CPA are indeed more prone to metabolic abnormalities\[13\] and present a higher risk of cardiovascular events,\[14\] a phenomenon that is worthy of attention in the medical community. A diagnosis of A/CPA may also help avoid misinterpretation of AVS\[15\] and the risk of postoperative adrenal cortical insufficiency.\[16\]

The exact pathogenesis of A/CPA is still unclear, but its histopathological features may provide important clues for further research. Xin Gao et al\[17\] confirmed that aldosterone-producing cells are sensitive to fluctuations in extracellular Ca\(^{2+}\) levels, and Ca\(^{2+}\) metabolism may directly affect hormone production, especially in the process of cosecretion of aldosterone and cortisol in the tumors of APA patients. It is noticed in this study that the expression sites of CYP11B1 and CYP11B2 in A/CPA are in the same region as those for the calcium metabolism-related

### Table 2

| Patient | ACTH, serum and urine cortisol at baseline | 1 mg DST | LDDST |
|---------|------------------------------------------|----------|--------|
|         | 8:00 STC | 0:00 STC | 8:00 ACTH | 24-h UFC | STC after test | STC after test | 24-h UFC after test |
| 1       | 409      | 137      | 8.87      | 135.9    | 134           | 125           | 77.8               |
| 2       | 478      | 351      | 1.26      | 295.4    | 99.1          | 511           | 326.6              |

Reference range: 08:00 STC, 147.3–609.3 nmol/L; 00:00 STC, <50 nmol/L; 8:00 ACTH 5.0–78 ng/L; 24-h UFC, 20.3–127.6 µg/24h.

1 mg DST = 1 mg dexamethasone inhibition test, 24-h UFC = 24-h urine free cortisol, ACTH = adrenocorticotropic hormone, LDDST = low-dose dexamethasone suppression test, STC = serum total cortisol.

![Figure 1](image_url). CYP11B1, CYP11B2, PTH1R, CaSR, and VD3R immunohistochemical expressions of irregular cord-like distribution in case 1.
receptors PTHR, CaSR, and VD3R, which is consistent with the aforementioned mechanism. In addition, different expression patterns of CYP11B1 and CYP11B2 were also identified in cases A/CPA, and it was speculated that there might be other somatic gene mutations related to calcium channels.

Furthermore, the histopathological features of A/CPA were found to exhibit zona fasciculata and zona glomerulosa-like cells. Multiple fluorescence immunohistochemical images showed that both cases 1 and 2 had coexpression of CYP11B1 and CYP11B2.

To date, in several case reports, the volume of A/CPA cases is larger than of pure APAS, and generally, the diameter of A/CPA is >2.0 or 2.5 cm.\textsuperscript{[5,16]} According to previous research on this matter, the minimum diameter in these cases is 1.1 cm, but no masses <1 cm in diameter have been reported.\textsuperscript{[18]} The diameters of A/CPA for the cases reported in this study are 2.1 cm and 2.5 cm, respectively, and it is speculated that the incidence of A/CPA in APA may be higher than that reported previously in other clinical studies. However, with the increase in tumor volume, the CYP11B1 expression level was further increased. Simultaneously, cortisol levels in the body gradually increased, confirming the diagnosis of SCS. Even in cases of confirmed A/CPA, CYP11B1 immunostaining may show low expression. However, since A/CPA is composed of many aldosterone-secreting and cortisol-secreting cells, even if the yield per cell is low,
the overall number is still significant and, because of that, SCS may still occur.[19]

Interestingly, in both cases of A/CPA reported in our study, PAC increased by >33% from baseline after the postural test, which is different from typical APA cases. This phenomenon may be related to the enhanced response to renin and decreased response to adrenocorticotropic hormone in the A/CPA patients. Whether it can be used as a diagnostic clue for A/CPA should be clarified through a relevant study involving a larger sample size. Contralateral adrenal atrophy in patients with A/CPA is a concern for the future.

The first immunohistochemical analysis of the CYP11B1, CYP11B2, and calcium metabolism-related receptors (CaSR, VDR and PTH1R) of A/CPA by multiplex immunofluorescence was performed. Unfortunately, epinephrine or norepinephrine was not used to correct adrenal venous aldosterone levels in the AVS. This is the lesson that we have learned. We also failed to genetically sequence the resected adrenal tissue, which will be our future research direction.

In conclusion, the occurrence of A/CPA may be related to calcium metabolism disorders, and the diversity of A/CPA in immunohistochemistry suggests that there are many uncertainties in the pathogenesis of the disease. Therefore, A/CPA should be considered in new clinical and pathological classifications of PA to gain more attention from the medical community.

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