Endocrine Journal 2011, **58** (7), 543-551

ORIGINAL

Clinicopathological features of primary aldosteronism associated with subclinical Cushing's syndrome

Kiichiro Hiraishi[1)], Takanobu Yoshimoto[1)], Kyoichiro Tsuchiya[1)], Isao Minami[1)], Masaru Doi[1)], Hajime Izumiyama[1)], Hironobu Sasano[2)] and Yukio Hirata[1)]

[1)] *Department of Clinical and Molecular Endocrinology, Tokyo Medical and Dental University Graduate School, Tokyo 113-8519, Japan*

[2)] *Department of Pathology, Tohoku University Graduate School of Medicine, Sendai 980-8575, Japan*

Abstract. Primary aldosteronism (PA), an autonomous aldosterone hypersecretion from adrenal adenoma and/or hyperplasia, and subclinical Cushing syndrome (SCS), a mild but autonomous cortisol hypersecretion from adrenal adenoma without signs or symptoms of Cuhing's syndrome, are now well-recognized clinical entities of adrenal incidentaloma. However, the clinicopathological features of PA associated with SCS (PA/SCS) remain unknown. The present study was undertaken to study the prevalence of PA/SCS among PA patients diagnosed at our institute, and characterize their clinicopathlogical features. The prevalence of PA/SCS was 8 of 38 PA patients (21%) studied. These 8 PA/SCS patients were significantly older and had larger tumor, higher serum potassium levels, lower basal plasma levels of aldosterone, ACTH and DHEA-S as well as lower response of aldosterone after ACTH stimulation than those in 12 patients with aldosterone-producing adenoma without hypercortisolism. All 8 PA/SCS patients showed unilateral uptake by adrenal scintigraphy at the ipsilateral side, whereas the laterality of aldosterone hypersecretion as determined by adrenal venous sampling varied from ipsilateral (3), contralateral (2), and bilateral side (2). 6 PA/SCS patinets who underwent adrenalectomy required hydrocortisone replacement postoperatively. Histopathological analysis of the resected adrenal tumors from 5 PA/SCS patients revealed a single adenoma in 3, and double adenomas in 2, with varying degrees of positive immunoreactivities for steroidgenic enzymes (3β-HSD, $P450_{C17}$) by immunohistochemical study as well as *CYP11B2* mRNA expression as measured by real-time RT-PCR. In conclusion, PA/SCS consists of a variety of adrenal pathologies so that therapeutic approach differs depending on the disease subtype.

Key words: Primary aldosteronism, Subclinical Cushing's syndrome, Adrenal venous sampling, Cortisol replacement

PRIMARY aldosteronism (PA) is characterized by autonomous aldosterone hypersecretion from adrenal gland, resulting in low-renin hypertension often accompanied by hypokalemia and metabolic alkarosis. Recent epidemiologic studies have revealed that PA is more common cause of secondary hypertension than previously thought with its higher prevalence (5-15 %) in patients with hypertension [1-5].

Subclinical Cushing's syndrome (SCS) is characterized as subtle cortisol hypersecretion from adrenal tumor but lack of specific signs or symptoms of overt Cushing syndrome (CS) [6]. SCS has been described in approximately 5-20 % in incidentally discovered adrenal mass (adrenal incidentaloma) [7, 8]. Given about 4 % of subjects older than 60-year-old harboring such adrenal tumors, SCS is considered not rare as previously thought [9].

Since Hogan *et al*. [10] first reported a patient who had a single adrenal adenoma concurrently producing cortisol and aldosterone, several PA/SCS patients have been described in the literature, suggesting the higher prevalence of PA/SCS than previously thought. Recently, Piaditis *et al*. have reported that PA associated with hypercortisolism occurs 12.1% in 83 adrenal incidentalomas [11]. However, the exact prevalence and clinical and pathological features of PA/SCS have not been fully understood.

To address these issues, the present retrospective

Received Dec. 22, 2010; Accepted Apr. 1, 2011 as K10E-402
Released online in J-STAGE as advance publication Apr. 27, 2011

Correspondence to: Yukio Hirata, M.D., Ph.D., Department of Clinical and Molecular Endocrinology, Tokyo Medical and Dental University Graduate School, 1-5-45 Yushima, Bunkyo-ku, Tokyo, 113-8519 Japan. E-mail: yhirata.cme@tmd.ac.jp

study was undertaken to evaluate the prevalence of PA/SCS among 38 PA patients diagnosed at our institute, and characterize their clinicopathological features.

Patients and Methods

Patients

We retrospectively studied 38 PA patients diagnosed and followed up at Tokyo Medical and Dental University Hospital from 2005 to 2008. The diagnosis of PA was made on the basis of suppressed plasma renin activity (PRA) (≤1.0 ng/mL/hr) and elevated plasma aldosterone concentration (PAC) (≥15 ng/dL) and/or PAC to PRA ratio (ARR) (>20), and subsequent confirmation by suppressed PRA (≤1.0 ng/mL/hr) after furosemide-upright posture [12]. A rapid ACTH test (Cortrosyn® 250 μg, iv bolus) was also performed [13]. Prior to the confirmatory tests, the patients were withdrawn of antihypertensive drugs for at least 2 weeks except for those with severe hypertension who had been treated with Ca-channel blockers and/or α-blockers. All patients underwent 3 mm-thin slice adrenal CT scan, and ^{131}I-adosterol adrenal scintigraphy in 33 patients.

The diagnosis of SCS was made on the basis of the diagnostic criteria proposed by the Research Committee for Adrenal Diseases supported by Japanese Ministry of Health, Labor and Welfare [14]; presence of adrenal incidentaloma, lack of Cushingoid features, and normal basal but autonomous cortisol secretion with no suppression of cortisol by low-dose (1 mg) and high-dose (8 mg) dexamethasone suppression test (DST) (>3 μg/dL and > 1μg/dL, respectively), and at least one of the following additional endocrine data: 1) suppressed plasma ACTH (<10 pg/mL) and/or decreased response of ACTH after CRH stimulation, 2) loss of cortisol diurnal rhythm, 3) decreased serum DHEA-S levels, 4) unilateral uptake of ^{131}I-adosterol by adrenal scintigraphy. Among 8 PA/SCS patients, six underwent unilateral adrenalectomy.

Adrenal venous sampling (AVS)

35 PA patients including 6 PA/SCS patients underwent AVS to differentiate between unilateral or bilateral aldosterone hypersecretion [12]. To determine the source of aldosterone hypersecretion by AVS, the following diagnostic criteria were used; 1) PAC more than 1400 ng/dL [15], 2) lateralized ratio (LR) defined as the ratio of PAC corrected by cortisol (PAC/F) in the dominant adrenal vein over that in the non-dominant adrenal vein greater than 4, 3) contralateral ratio (CR) defined as PAC/F in the non-dominant adrenal vein over that in inferior vena cava less than 1 [16]. Since cortisol levels considerably differed in adrenal veins in PA/SCS patients, the absolute value of PAC rather than LR or CR was employed for diagnostic criteria for laterality of aldosterone hypersecretion in PA/SCS patients.

Immunohistochemistry

Immunohistochemical analysis of steroidogenic enzymes, including 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17α-hydroxylase ($P450_{c17}$), were performed on the processed formalin-fixed, paraffin-embedded serial sections by the biotin-streptavidin amplified method (Nichirei, Tokyo, Japan) as described [17].

Real-time quantitative RT-PCR for CYP11B2

Tumor samples were collected during surgery at our institute with informed consent from each patient. The experimental protocol was approved by the Ethical Committees of our institute. RNA extraction, first-strand cDNA synthesis, and real-time quantitative RT-PCR were carried out using TaqMan fluorescence methods (Bio-Rad Laboratories, Hercules, USA) as described [18]. PCR primers and Taqman probe for *CYP11B2* were designed as described previously [19]; forward primer, 5'-CTCTACCCTGTGGGTCTGTT TT-3' ; reverse primer, 5'-GGATTATACCGCTCAGG CC-3' ; Taqmanprobe, 5'-TACAGGTTTTCCTCTAC TCG-3' (PCR products size: 153 bp). The human *CYP11B2* cDNA (sequenced from +1126 to +1277 based on GenBank ID NM 000498) were cloned by RT-PCR using RNA sample from one of aldosterone-producing adenoma (APA) tissue, which was then subcloned into the pCR2.1-TOPO vector (Invitrogen, Carlsbad, CA, USA). The identity of clone was confirmed by sequence analysis. Serial dilution of the cDNA plasmid was used to generate standard curves for quantification of the expression of *CYP11B2* mRNA, and mRNA levels were expressed as attomoles per μg of total RNA.

Cardiovascular risk factors

Hypertension is defined as diastolic pressure (DBP) (> 90 mmHg) and/or the systolic pressure (SBP) (> 140 mmHg) [20] ; impaired glucose tolerance (IGT) as fasting plasma glucose (110-125 mg/dL) and/or 2-h plasma glucose (140-199 mg/dL) on 75g OGTT;

diabetes mellitus (DM) as fasting plasma glucose (≥ 126) and/or 2-h plasma glucose (≥ 200 mg/dL) on 75g OGTT or elevated HbA1c level (≥ 6.1 %) [21] ; dyslipidemia as triglyceride (TG) (≥ 150 mg/dL), HDL-cholesterol (< 40 mg/dL), or LDL-cholesterol (≥ 140 mg/dL) [22]. Patients treated with medications for hypertension, DM/IGT, dyslipidemia were included as having the respective risk factors.

Evaluation of clinical outcome

The postoperative follow-up periods of PA/SCS and APA patients were 26.2±20.1 months and 28.8±22.5 months, respectively. Changes of blood pressure, glycemic and lipid profiles during the follow-up periods were evaluated as improvement and unchanged. Improvement of hypertension is defined as normalized blood pressure (SBP<140 mmHg and/or DBP<90 mmHg) and/or anti-hypertensive drugs discontinued or decreased in number or dose. Improvement of DM/IGT is defined as normoglycemia/IGT on OGTT, decreased HbA1c levels by more than 0.3% and/or insulin/oral hypoglycemic agents discontinued or decreased in number or dose. Improvemend of dyslipidemia is defined as normalized plasma lipids levels (TG<150 mg/dL, HDL-cholesterol≥40 mg/dL, LDL-cholesterol<140 mg/dL) or anti-dyslipidemic drugs discontinued or decreased in number or dose.

Statistical analysis

All clinical data are expressed as mean ± SD and *CYP11B2* mRNA data are expressed as mean ± S.E.M. The χ^2 test was used for comparing proportions of subjects in two groups. Comparison between groups was made using non-paired *t* test. All statistical analyses were performed using Windows software Prism 5.0 (GraphPad Software, La Jolla, CA, USA).

Results

By screening 38 PA patients diagnosed at our hospital, we found 8 PA patients associated with SCS (21 %). The clinical and biochemical characteristics of these 8 PA/SCS patients compared to those of 12 PA patients without SCS are shown in Table 1. There were no statistical differences between PA/SCS and APA patients of sex, BMI, blood pressure (systolic, diastolic), prevalences of hypertension and DM/IGT, basal PRA or cortisol levels. By contrast, PA/SCS patients were significantly (P<0.05) older and had higher prevalence of dyslipidemia than APA patients. PA/SCS patients had significantly (P<0.05) higher serum potassium levels and lower urinary potassium excretion than APA patients, while PAC, ARR, plasma ACTH and DHEA-S levels were significantly (P<0.05) lower, and post-DST (1mg) cortisol levels were significantly (P<0.01) higher in PA/SCS patients than those in APA patients. The responses of PAC and PAC/cortisol after ACTH stimulation were significantly (P<0.01) lower in PA/SCS patients than those in APA patients. The tumor size (21.8±4.0 mm) of PA/SCS patients were significantly (P<0.01) larger than that (12.4±3.5 mm) of APA patients. All 8 PA/SCS patients showed unilateral single adrenal tumor by CT scan consistent with the ipsilateral uptake of ^{131}I-adosterol scintigraphy. Selective AVS with ACTH stimulation performed in 6 patients showed aldosterone hypersecretion identical to ipsilateral side in 2 (Cases 1, 8), contralateral side in 2 (Cases 3, 6), and bilateral side in 2 (Cases 2, 5) (Table 2).

Six (Cases 1, 2, 4, 5, 7, 8) of 8 PA/SCS patients who underwent unilateral adrenalectomy required hydrocortisone replacement postoperatively. Histopathological and immnohistochemical results of the resected specimens from 5 patients except for Case 4 (not available) are summarized in Table 3. Three (Cases 1, 2, 7) showed a single adenoma with positive immunoreactivities for both 3β-HSD and $P450_C17$, while 2 (Cases 5, 8) showed double adenomas, being the larger one with positive immunoreactivities for both 3β-HSD and $P450_C17$, and the smaller one with positive immunoreactivity for 3β-HSD, but not for $P450_C17$. In the adjacent adrenal cortex of all 5 operated patients, both the zona fasciculata and the zona reticularis were atrophic, whereas the zona glomerulosa was hyperplastic with negative 3β-HSD immunoreactivity (“paradoxical hyperplasia”).

After adrenalectomy, hypertension improved in 5 of 6 PA/SCS patients (83%), and all 12 APA patients (100%) (Table 4). DM/IGT improved in 2 of 4 PA/SCS patients (50%), and 2 of 6 APA patients (33%). Dyslipidemia improved in 2 of 5 PA/SCS patients (40%), but unchanged in 3 APA patients. There were no statistical differences between PA/SCS and APA patients of postoperative PAC, PRA or ARR, whereas postoperative plasma ACTH levels were normalized in all 6 PA/SCS patients (Table 1).

CYP11B2 mRNA levels were measured by a quantitative real-time RT-PCR in the resected tumor specimens available from 3 cases with PA/SCS, consisting

Table 1 Clinical, biochemical, endocrine and imaging characteristics of 8 PA/SCS patients in comparison with those of 12 PA patients

	PA/SCS (n=8)	APA (n=12)
Clinical		
Men/Women	1/7	5/7
Age (years)	62.0±9.5*****	49.3±12.4
Body mass index (kg/m^2)	23.4±2.8	24.5±2.5
Systolic blood pressure (mmHg)	146.0±13.6	146.3±28.1
Diastolic blood pressure (mmHg)	88.3±17.4	92.6±27.6
Hypertension (%)	7 (88%)	12 (100%)
DM / IGT (%)	5 (63%)	6 (50%)
Dyslipidemia (%)	7 (88%)******	3 (25%)
Biochemical		
Serum potassium (mEq/L)	3.8±0.2*****	3.1±0.6
Urinary sodium excretion (mEq/day)	130.2±29.4	102.3±25.8
Urinary potassium excretion (mEq/day)	39.3±7.1*	49.8±11.47
Total cholesterol (mg/dL)	207.0±43.2	183.8±25.5
Low-density lipoprotein cholesterol (mg/dL)	122.0±37.2	107.3±19.2
High-density lipoprotein cholesterol (mg/dL)	65.4±16.5	57.8±12.3
Triglyceride (mg/dL)	133.1±62.0	135.1±80.1
Fasting plasma glucose (mg/dL)	96.6±20.0	89.7±7.4
Insulin (μU/mL)	4.5±2.0	4.0±2.0
HOMA-R	1.1±0.6	0.9±0.5
Hemoglobin A1c (%)	5.6±0.9	5.1±0.5
Endocrine	Preoperative (Postoperative) value	
PAC (ng/dL)	19.3±15.4*** (**11.7±4.2**)**	37.4±14.7 (11.3±5.2)
PRA (ng/mL/h)	0.19±0.10 (2.70±2.69)	0.18±0.11 (1.74±1.33)
ARR	122.3±81.4*** (**14.2±14.1**)**	254.0±124.5 (9.4±7.4)
ACTH (pg/mL)	6.6±1.8**** (**36.1±17.9**)**	30.1±9.9 (32.6±8.4)
Cortisol (μg/dL)	13.1±1.4 (11.0±3.9)	11.4±3.9 (12.9±3.3)
DST (1mg / 8mg)	8.2±2.1****** / 8.5±2.1	1.1±0.2 / -
DHEA-S (ng/mL)	215.4±272.1*****	1088.7±1007.0
Post-ACTH PAC/cortisol	1.2±0.2******	2.4±0.4
ΔPAC	14.9±2.1******	40.6±11.3
Imaging		
Tumor size (mm) by CT scan	21.8±4.0******	12.4±3.5

PA: primary aldosteronism, SCS: subclinical Cushing's syndrome, APA: aldosterone producing adenoma, DM: diabetes, IGT: impaired glucose tolerance, HOMA-R: Homeostasis model assessment insulin resistance index, PAC: plasma aldosterone concentration, PRA: plasma rennin activity, ARR: aldosterone-renin ratio, ACTH: adrenocorticotropic hormone, DST: dexamethasone suppression test, ΔPAC: increment of peak PAC value from the basal level after ACTH stimulation
**P*<0.05 ***P*<0.01

Table 2 Laterality of adrenal lesion by CT scan and adrenal venous sampling (AVS)

Case #	Laterality		PAC (ng/dL) / F (μg/dL) before and after ACTH stimulation	
	CT	AVS	Right adrenal vein	Left adrenal vein
1	L	L	19 / 7.5 → 103 / 218.6	1432 / 58.1 → 2406 / 346.1
2	L	Bi	868 / 30.5 → 1289 / 209.6	44 / 245.6 → 1701 / 1369.8
3	L	R	79 / 6.5 → 2301 / 106	23 / 39.4 → 984 / 823.3
4	L	ND	ND	ND
5	R	Bi	267 / 50.6 → 2120 / 1384.6	43 / 65 → 1740 / 465.7
6	L	R	240 / 35.9 → 3750 / 232.1	66 / 182.6 → 765 / 1044.6
7	L	ND	ND	ND
8	R	R	83 / 111.9 → 1900 / 1337.1	14 / 26.1 → 823 / 129.3

PAC: plasma aldosterone concentration, F: cortisol, L: left, R: right, Bi: bilateral, ND: not determined

Table 3 Immunohistochemical staining

Case #			$P450_{c17}$	3β-HSD
1	single adenoma		+	+
2	single adenoma		+	+
4	single adenoma		NA	NA
5	double adenomas	large adenoma	+	+
		small adenoma	-	+
7	single adenoma		+	+
8	double adenomas	large adenoma	+	+
		small adenoma	-	+

$P450_{c17}$: 17α-hydroxylase, 3β-HSD: 3β-hydroxysteroid dehydrogenase, NA: not available

Table 4 Comparison of clinical outcome of cardiovascular risk factors between 6 PA/SCS patients and 12 APA patients after adrenalectomy

	Prevalence	Clinical outcome	
		Improvement	No change
PA/SCS			
Hypertension	6/6 (100%)	5/6 (83%)	1/6 (17%)
DM/IGT	4/6 (67%)	2/4 (50%)	2/4 (50%)
Dyslipidemia	5/6 (83%)	2/5 (40%)	3/5 (60%)
APA			
Hypertension	12/12 (100%)	12/12 (100%)	0/12 (0%)
DM/IGT	6/12 (50%)	2/6 (33%)	4/6 (67%)
Dyslipidemia	3/12 (25%)	0/3 (0%)	3/3 (100%)

PA: primary aldosteronism, SCS: subclinical Cushing's syndrome, APA: aldosterone-producing adenoma, DM: diabetes mellitus, IGT: impaired glucose tolerance

of a single adenoma (Case 1), larger one of double adenomas (Cases 5, 8), 3 cases with APA alone and 3 cases with SCS alone for comparison; smaller adenomas in Cases 5 and 8 were not available. As shown in Fig. 1, *CYP11B2* mRNA levels in Case 1 were comparable to those of APA, whereas those in Cases 5 and 8 were low almost comparable to those of SCS.

Discussion

In the present study, we found 8 PA/SCS among 38 PA patients (21 %) screened and diagnosed at our institute. Although few epidemiologic studies on the prevalence of PA associated with SCS in adrenal tumors have been reported thus far, Piaditis *et al*. have recently reported that the prevalence of PA associated with SCS was 12.1% in 83 adrenal incidentalomas. Taken together, it is suggested that the coincidence of SCS is not rare among PA patients.

In the present study, unilateral radioactivity uptake by adrenal scintigraphy was indentical to the tumor

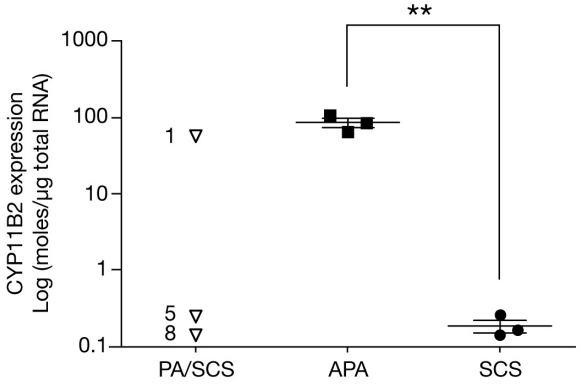

Fig. 1 *CYP11B2* mRNA expression by various adrenocortical adenomas.
CYP11B2 mRNA levels determined by real-time quantitative RT-PCR in primary aldosteronism (PA)/subclinical Cushing's syndrome (SCS) (Cases 1, 5, 8: ▽), aldosterone-producing adenoma (APA) without SCS (n=3: ●), SCS without APA (n=3: ■), are shown by scattergrams; each point with bar shows mean ± S.E.M. ** $P<0.01$.

side detected by CT imaging in all 8 PA/SCS patients, which is compatible with concomitant cortisol- and/or aldosterone-producing tumors. By contrast, localization of aldosterone hypersecretion by AVS was discordant with the tumor side by imaging tests; 2 (Cases 1, 8) at the ipsilateral side, 2 (Cases 3, 6) at the contralateral side, and 2 (Cases 2, 5) at the bilateral side. These findings lend a support to the contention that localization of aldosterone hypersecretion should be confirmed by AVS even in a patient whose adrenal tumor is evident by CT imaging and/or adrenal scintigraphy.

The clinical features between PA/SCS and APA revealed significant differences of age, prevalence of dyslipidemia, serum and urinary potassium levels, tumor size, and certain endocrine data. Older age and higher prevalence of dyslipidemia in our PA/SCS patients were compatible with those of SCS patients [23, 24]. Despite autonomous, but subtle cortisol hypersecretion in PA/SCS, the present study revealed that PA/SCS patients had higher serum potassium levels and lower urinary potassium excretion as well as lower PAC and ARR than did APA patients. This could be mainly attributable to lower basal secretion of aldosterone in PA/SCS compared to that in APA. It is well recognized that ACTH acutely stimulates aldosterone secretion [25], whereas ACTH chronically reduces aldosterone secretion [26]. Thus, the suppressed ACTH secretion due to mild cortisol excess in PA/SCS could not explain the lower PAC and higher serum potassium levels in our PA/SCS patients. However, the lower response of PAC after ACTH stimulation in PA/SCS than in APA as demonstrated in this study, could partly account for the relatively reduced aldosterone secretion in PA/SCS, although its exact mechanism(s) remains to be determined. Nevertheless, the present results suggest that the possible association of PA should be taken into account in SCS patients with moderate PAC and normokalemia.

It should be noted that Cases 2 and 5 showed bilateral aldosterone hypersecretion by AVS, who underwent unilateral adrenalectomy at the side of cortisol hypersecretion by their will. Postoperatively, Case 2 could reduce the dose of Ca-channel blocker (improvement), while Case 5 continued to take the same number and dose of anti-hypertensive drugs (unchanged). Although the exact reason for the improvement of hypertension in Case 2 remains unclear, removal of the single adenoma secreting preferential cortisol to aldosterone could partly account for the improvement.

The tumor size in our 8 PA/SCS patients was larger than that in APA patients, which is compatible with the epidemiologic study that cortisol-producing adenomas appear to be larger than in APA [27]. Collectively, concomitant production of cortisol should be considered in PA patients with relatively larger adrenal tumors and/or low plasma ACTH levels. In agreement with a previous report [28], hydrocortisone replacement after adrenalectomy was needed in our PA/SCS patients. Thus, one should rule out the possible association of SCS among PA patients in order to prevent postoperative adrenal insufficiency.

The histopathological and immunohistochemical study combined with the measurement of *CYP11B2* mRNA expression in the resected specimen proves to be useful for localizing the adrenal lesion(s) responsible for aldosterone and cortisol hypersecretion. In our three patients (Cases 1, 2, 7), a solitary adenoma with positive 3β-HSD and $P450_C17$ immunoreactivities associated with paradoxical hyperplasia of zona glomerulosa in the adjacent tissue, is compatible with concomitant production of cortisol and aldosterone. This is also consistent with the abundant expression of *CYP11B2* mRNA in the tumor tissue from Case 1, comparable to that in APA. However, the other two patients (Cases 5, 8) with double adenomas showed different patterns; the larger adenoma showed positive 3β-HSD and P450c17 immunoreactivities, but barely detectale *CYP11B2* mRNA expression comparable to those in the adenoma from SCS without PA, suggesting that these adenomas predominantly produce cortisol, but not aldosterone. Smaller adenomas, on the other hand, showed positive immunoreactivity for 3β-HSD, but not for $P450_C17$, we assume that these smaller adenomas are highly likely to be the source of aldosterone hypersecretion, although *CYP11B2* mRNA expression could not be measured. Adrenal CT imagings of Cases 5 and 8 showed a single mass lesion in the right adrenal, 22 mm (Case 5) and 20 mm (Case 8), respectively, without any other recognizable mass lesions. Postoperative histopathological study of the resected adrenal specimens of both cases revealed another small adenoma (< 6mm) undetectable on CT imaging.

Many cases of PA with hypercortisolism reported thus far [29-33] are due to a solitary adenoma co-secreting aldosterone and cortisol, as in Cases 1 and 7. However, aldosterone hypersecretion as confirmed by AVS in our 4 patients (Cases 2, 3, 5, 6) was from the contralateral side of the adrenal lesions by imaging

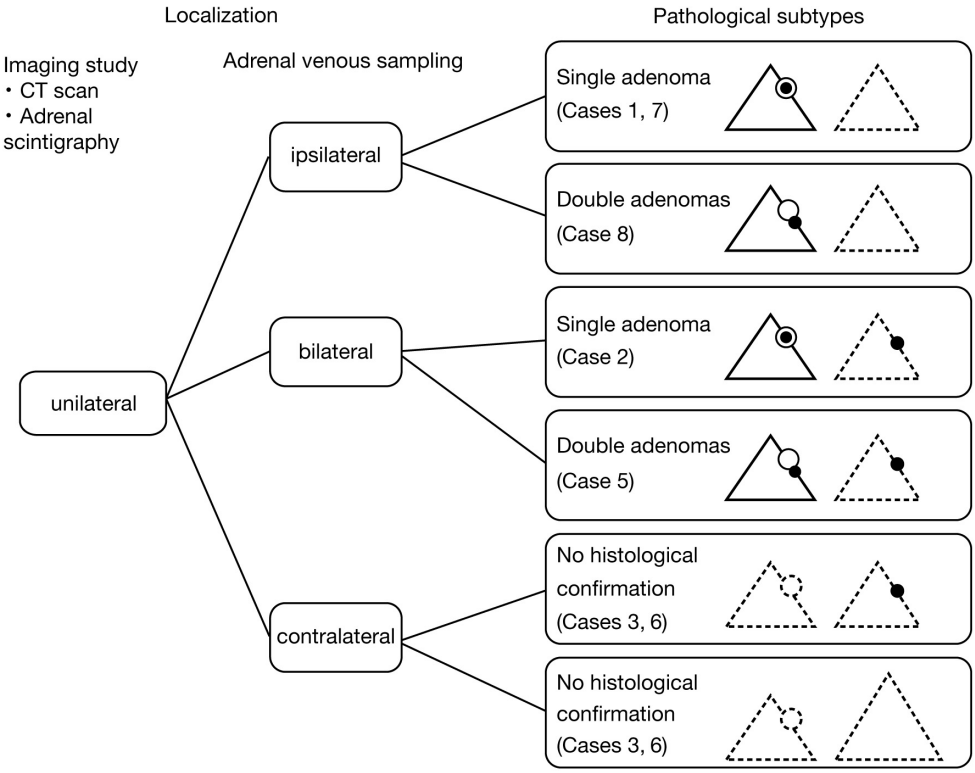

Fig. 2 Schematic adrenal subtype patterns in our primary aldosteronism/subclinical Cushing's syndrome (PA/SCS) patients. Given the unilateral localization of cortisol-producing adenoma, aldosterone hypersecretion determined by AVS could be localized in ipsilateral, bilateral and contralateral side; aldosterone-producing adenoma (●), cortisol-producing adenoma (○), aldosterone- and cortisol-producing adenoma (◉). The solid and the dotted lines illustrate histologically proven cases and non-operated cases, respectively.

studies. It has been reported that 27 of 93 PA patients (29 %) are microadenoma (micro-APA) only detectable by AVS [34]. Thus, micro-APA could be present at the contralateral side in such cases. Taken together, our PA/SCS patients could be classified into various subtypes (Fig. 2). While cortisol hypersecretion is usually concordant with imaging studies, aldosterone hypersecretion as determined by AVS is frequently discordant with imaging studies, in which micro-APA may coexist at ipsilateral, bilateral, or contralateral side. In our 2 patients (Cases 3, 6) without surgery, it is possible to speculate, although histologically not proven, that cortisol-producing adenoma could be associated concomitantly with PA caused by contralateral micro-APA, hyperplasia and/or bilateral hyperplasia (Fig. 2). Thus, the present study raised the question that PA/SCS could be caused by various adrenal pathologies, so that therapeutic approach may differ depending on the disease subtype.

In summary, the prevalence of PA accompanied by subtle cortisol hypersecretion (21 %) appears to be more often than previously thought. Among older PA patients, lower PAC, ARR, normal potassium level and suppressed plasma ACTH in the presence of larger adrenal tumor are the common clinical and biochemical features to suspect coexisting SCS. AVS should be performed in such suspected PA/SCS patients because of a variety of adrenal pathologies, and therapeutic approach may differ depending on the disease subtype. It should also be noted that hydrocortisone replacement is necessary during and after adrenalectomy to prevent postoperative adrenal insufficiency.

Acknowledgments

We thank Dr. K. Kihara and his colleagues, Department of Urology, for adrenal surgery, and Dr. T. Akashi, Department of Pathology, for pathological diagnosis. This study was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, and the Ministry of Health, Welfare, and Labor of Japan.

References

1. Gordon RD, Stowasser M, Tunny TJ, Klemm SA, Rutherford JC (1994) High incidence of primary aldosteronism in 199 patients referred with hypertension. *Clin Exp Pharmacol Physiol* 21: 315-318.
2. Fardella CE, Mosso L, Gomez-Sanchez C, Cortes P, Soto J, Gomez L, Pinto M, Huete A, Oestreicher E, Foradori A, Montero J (2000) Primary hyperaldosteronism in essential hypertensives: prevalence, biochemical profile, and molecular biology. *J Clin Endocrinol Metab* 85: 1863-1867.
3. Lim PO, Dow E, Brennan G, Jung RT, MacDonald TM (2000) High prevalence of primary aldosteronism in the Tayside hypertension clinic population. *J Hum Hypertens* 14: 311-315.
4. Mulatero P, Stowasser M, Loh KC, Fardella CE, Gordon RD, Mosso L, Gomez-Sanchez CE, Veglio F, Young WF Jr (2004) Increased diagnosis of primary aldosteronism, including surgically correctable forms, in centers from five continents. *J Clin Endocrinol Metab* 89: 1045-1050.
5. Rossi GP, Sacchetto A, Chiesura-Corona M, De Toni R, Gallina M, Feltrin GP, Pessina AC (2001) Identification of the etiology of primary aldosteronism with adrenal vein sampling in patients with equivocal computed tomography and magnetic resonance findings: results in 104 consecutive cases. *J Clin Endocrinol Metab* 86: 1083-1090.
6. Reincke M (2000) Subclinical Cushing's syndrome. *Endocrinol Metab Clin North Am* 29: 43-56.
7. Terzolo M, Osella G, Ali A, Borretta G, Cesario F, Paccotti P, Angeli A (1998) Subclinical Cushing's syndrome in adrenal incidentaloma. *Clin Endocrinol (Oxf)* 48: 89-97.
8. Terzolo M, Bovio S, Pia A, Reimondo G, Angeli A (2009) Management of adrenal incidentaloma. *Best Pract Res Clin Endocrinol Metab* 23: 233-243.
9. Bovio S, Cataldi A, Reimondo G, Sperone P, Novello S, Berruti A, Borasio P, Fava C, Dogliotti L, Scagliotti GV, Angeli A, Terzolo M (2006) Prevalence of adrenal incidentaloma in a contemporary computerized tomography series. *J Endocrinol Invest* 29: 298-302.
10. Hogan MJ, Schambelan M, Biglieri EG (1977) Concurrent hypercortisolism and hypermineralocorticoidism. *Am J Med* 62: 777-782.
11. Piaditis GP, Kaltsas GA, Androulakis II, Gouli A, Makras P, Papadogias D, Dimitriou K, Ragkou D, Markou A, Vamvakidis K, Zografos G, Chrousos G (2009) High prevalence of autonomous cortisol and aldosterone secretion from adrenal adenomas. *Clin Endocrinol (Oxf)* 71: 772-778.
12. Minami I, Yoshimoto T, Hirono Y, Izumiyama H, Doi M, Hirata Y (2008) Diagnostic accuracy of adrenal venous sampling in comparison with other parameters in primary aldosteronism. *Endocr J* 55: 839-846.
13. Dorin RI, Qualls CR, Crapo LM (2003) Diagnosis of adrenal insufficiency. *Ann Intern Med* 139: 194-204.
14. Nawata H, Demura T, Suda T, Takayanagi R (1996) Adrenal preclinical Cushing's syndrome, in Annual Report of the Ministry of Health and Welfare "Disorders of Adrenal Hormones" Research Committee, Japan *223-226* : (In Japanese).
15. Omura M, Saito J, Yamaguchi K, Kakuta Y, Nishikawa T (2004) Prospective study on the prevalence of secondary hypertension among hypertensive patients visiting a general outpatient clinic in Japan. *Hypertens Res* 27: 193-202.
16. Funder JW, Carey RM, Fardella C, Gomez-Sanchez CE, Mantero F, Stowasser M, Young WF Jr, Montori VM (2008) Case detection, diagnosis, and treatment of patients with primary aldosteronism: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab* 93: 3266-3281.
17. Sasano H, Miyazaki S, Sawai T, Sasano N, Nagura H, Funahashi H, Aiba M, Demura H (1992) Primary pigmented nodular adrenocortical disease (PPNAD): immunohistochemical and in situ hybridization analysis of steroidogenic enzymes in eight cases. *Mod Pathol* 5: 23-29.
18. Yoshimoto T, Fukai N, Sato R, Sugiyama T, Ozawa N, Shichiri M, Hirata Y (2004) Antioxidant effect of adrenomedullin on angiotensin II-induced reactive oxygen species generation in vascular smooth muscle cells. *Endocrinology* 145: 3331-3337.
19. Satoh M, Nakamura M, Saitoh H, Satoh H, Akatsu T, Iwasaka J, Masuda T, Hiramori K (2002) Aldosterone synthase (CYP11B2) expression and myocardial fibrosis in the failing human heart. *Clin Sci (Lond)* 102: 381-386.
20. Ogihara T, Saruta T, Rakugi H, Fujimoto A, Ueshima K, Yasuno S, Oba K, Takeda K, Higaki J, Nakao K (2009) Relationship between the achieved blood pressure and the incidence of cardiovascular events in Japanese hypertensive patients with complications: a sub-analysis of the CASE-J trial. *Hypertens Res* 32: 248-254.
21. Seino Y, Nanjo K, Tajima N, Kadowaki T, Kashiwagi A, Araki E, Ito C, Inagaki N, Iwamoto Y, Kasuga M, Hanafusa T, Haneda M, Ueki K (2010) Report of the committee on the classification and diagnostic criteria of diabetes mellitus. *J Jpn Diabe Soc* 53: 450-467.
22. Teramoto T, Sasaki J, Ueshima H, Egusa G, Kinoshita M, Shimamoto K, Daida H, Biro S, Hirobe K, Funahashi T, Yokote K, Yokode M (2007) Diagnostic criteria for dyslipidemia. Executive summary of Japan Atherosclerosis Society (JAS) guideline for diagnosis and prevention of atherosclerotic cardiovascular diseases for Japanese. *J Atheroscler Thromb* 14: 155-158.

23. Rossi R, Tauchmanova L, Luciano A, Di Martino M, Battista C, Del Viscovo L, Nuzzo V, Lombardi G (2000) Subclinical Cushing's syndrome in patients with adrenal incidentaloma: clinical and biochemical features. *J Clin Endocrinol Metab* 85: 1440-1448.
24. Tauchmanova L, Rossi R, Biondi B, Pulcrano M, Nuzzo V, Palmieri EA, Fazio S, Lombardi G (2002) Patients with subclinical Cushing's syndrome due to adrenal adenoma have increased cardiovascular risk. *J Clin Endocrinol Metab* 87: 4872-4878.
25. Gordon RD, Stowasser M, Klemm SA, Tunny TJ (1995) Primary aldosteronism--some genetic, morphological, and biochemical aspects of subtypes. *Steroids* 60: 35-41.
26. Holland OB, Carr B (1993) Modulation of aldosterone synthase messenger ribonucleic acid levels by dietary sodium and potassium and by adrenocorticotropin. *Endocrinology* 132: 2666-2673.
27. Rockall AG, Babar SA, Sohaib SA, Isidori AM, Diaz-Cano S, Monson JP, Grossman AB, Reznek RH (2004) CT and MR imaging of the adrenal glands in ACTH-independent cushing syndrome. *Radiographics* 24: 435-452.
28. Sugawara A, Takeuchi K, Suzuki T, Itoi K, Sasano H, Ito S (2003) A case of aldosterone-producing adrenocortical adenoma associated with a probable post-operative adrenal crisis: histopathological analyses of the adrenal gland. *Hypertens Res* 26: 663-668.
29. Rossi E, Foroni M, Regolisti G, Perazzoli F, Negro A, Santi R, Grasselli C, Galli P, Gardini G (2008) Combined Conn's syndrome and subclinical hypercortisolism from an adrenal adenoma associated with homolateral renal carcinoma. *Am J Hypertens* 21: 1269-1272.
30. Suzuki J, Otsuka F, Inagaki K, Otani H, Miyoshi T, Terasaka T, Ogura T, Omori M, Nasu Y, Makino H (2007) Primary aldosteronism caused by a unilateral adrenal adenoma accompanied by autonomous cortisol secretion. *Hypertens Res* 30: 367-373.
31. Sasaki N, Iwase M, Arima H, Nohara S, Bandai S, Yao T, Fujii K, Iida M (2006) Overt diabetes mellitus in a patient with combined primary aldosteronism and Cushing's syndrome. *Intern Med* 45: 1237-1242.
32. Willenberg HS, Spath M, Maser-Gluth C, Engers R, Anlauf M, Dekomien G, Schott M, Schinner S, Cupisti K, Scherbaum WA (2010) Sporadic solitary aldosterone- and cortisol-co-secreting adenomas: endocrine, histological and genetic findings in a subtype of primary aldosteronism. *Hypertens Res* 33: 467-472.
33. Adachi J, Hirai Y, Terui K, Nakano T, Fukuda Y, Suda T, Sasano H (2003) A report of 7 cases of adrenal tumors secreting both cortisol and aldosterone. *Intern Med* 42: 714-718.
34. Omura M, Sasano H, Saito J, Yamaguchi K, Kakuta Y, Nishikawa T (2006) Clinical characteristics of aldosterone-producing microadenoma, macroadenoma, and idiopathic hyperaldosteronism in 93 patients with primary aldosteronism. *Hypertens Res* 29: 883-889.