The pathophysiology of aldosterone-producing adenomas associated with their tumor size

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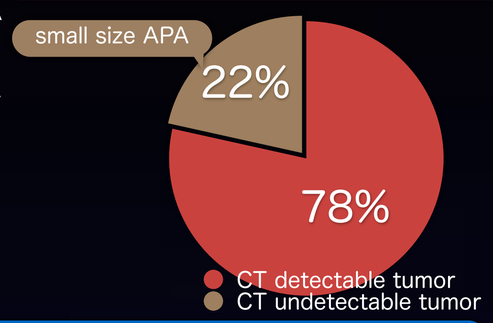
Background

- Primary aldosteronism (PA) is the most common form of secondary hypertension. The prevalence of PA is reported to be approximately 5-10% in hypertensive patients and approximately 20% in the patients with resistant hypertension.
- The prevalence of CT-undetectable APA (aldosterone-producing adenoma) among all APA patients is currently estimated to be 13–30%.
- Hypertension was cured or markedly improved after adrenalectomy in almost all reported cases.

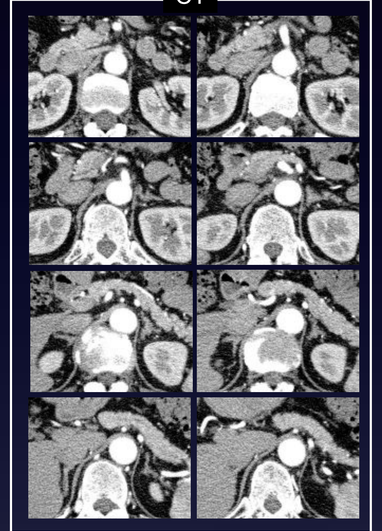
Young WF, et al 2004 *Surgery* 136:1227-1235
Omura M, et al 2006 *Hypertens Res* 29:883-889
Karashima S, et al 2011 *Steroids* 76:1363-1366
Ishidoya S, et al 2011 *Urology* 78:68-73
Satoh F et al 2007 *Hypertension Res* 30:1083-1095

Detection rate of CT(N=144)



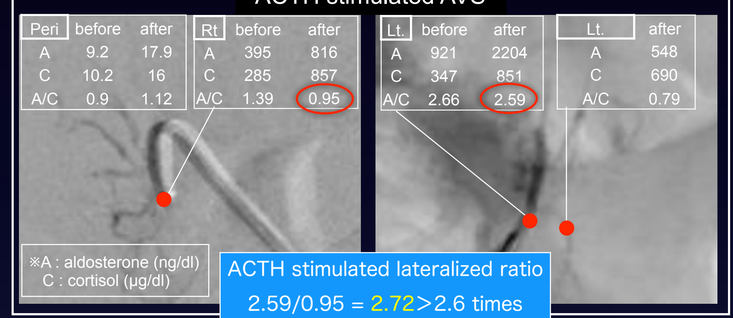
CASE : 51y.o. Male

CT



No adrenal tumor

ACTH stimulated AVS



ACTH stimulated lateralized ratio
2.59/0.95 = 2.72>2.6 times

Clinical Course

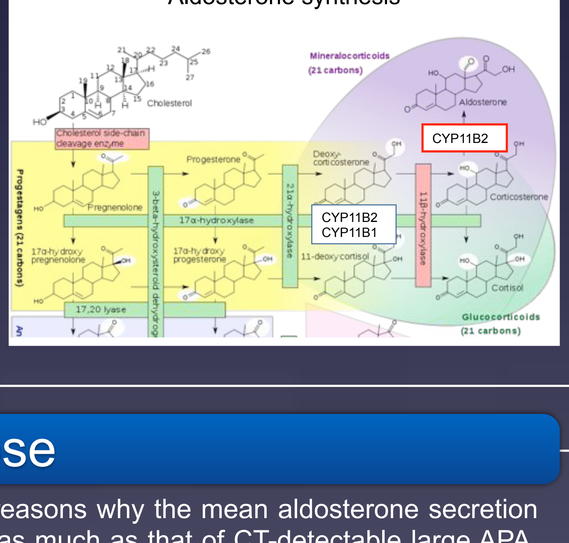
	First visit	Before ADX	POD 7	POD 72
Plasma Renin Activity (ng/ml/h)	0.3	0.7	0.2	0.3
Plasma Aldosterone (ng/dl)	11.8	26.9	4.9	4.6
Urinary Aldo (μg/day)	10.1	14.8	1	
ACTH challenge Aldo	27.1			7.1
ACTH challenge Cortisol (μg/dl)	16			15.2
ACTH challenge Aldo/Cortisol	1.7			0.47
Cr (mg/dl)	0.7	0.7	0.7	0.63
eGFR(ml/min/1.73m	93.2	93.2	93.2	104.1
K (mEq/l)	3.6	4.5	4.5	4
BP (mmHg)	167/113	128/74	129/87	126/90
Anti hypertension drugs	No drugs	SPL 100mg Amlodipine 10mg	No drugs	No drugs

PA was cured

- Small size APAs undetectable by CT have been histopathologically analyzed and the reasons why aldosterone hypersecretion from CT-undetectable small adenomas is sufficient to cause clinically overt PA have remained unknown.
- Recently, Gomez-Sanchez C.E. et al developed the monoclonal antibodies which can distinguish CYP11B1 and CYP11B2.

Gomez-Sanchez C.E. et al *Mol Cell Endocrinol*. 2014 Mar 5;383(1-2):111-7

Aldosterone synthesis



Purpose

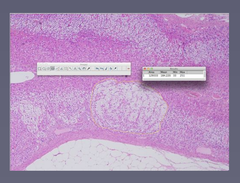
The main purpose of this study was to explore the reasons why the mean aldosterone secretion capacity of CT-undetectable small APA could reach as much as that of CT-detectable large APA, and the reasons why the clinical improvement after surgical treatment in both APA could be similar. Therefore, we evaluated the correlation between tumor size and the status of steroidogenic enzymes including HSD3B, CYP17A1, CYP11B1, and CYP11B2, which are all related to aldosterone production, using immunohistochemistry in order to clarify the status of aldosterone biosynthesis in small APAs.

Methods

- From May 2010 to October 2012, we experienced 100 APA cases which consisted of 20 CT-undetectable cases and 80 CT-detectable cases. We then selected 1 every 4 cases continually among CT-detectable cases to be able to compare the same number of the cases. Therefore, we could study forty patients with APAs in this study. All patients were diagnosed with PA on the basis of our previously published protocols.※

※ The lateralization index was defined as the aldosterone/cortisol ratio in the adrenal vein divided by that in the contralateral adrenal vein. The lateralization index after cosyntropin stimulation exceeded 4.0 in 33 patients and was between 2.6 and 4 in 7 patients (mean: 9.5, range: 2.6–36). All patients underwent laparoscopic unilateral adrenalectomy on the basis of AVS findings and subsequent pathological examination to confirm the existence of APA in the resected adrenal gland. Satoh F et.al 2007 *Hypertension Res* 30:1083-1095

- The maximum diameter and area of each tumor were determined on hematoxylin–eosin-stained tissue slides by ImageJ (Ver. 1.47, NIH).
- We tentatively classified 40 APA cases into the following two groups: the smaller and larger groups determined at 60mm^2 which represents the median of the area of APAs which we could obtain using ImageJ.



- Immunohistochemical staining was performed with antibodies against CYP11B1, CYP11B2, HSD3B and CYP17A1.

Name	Animal	Clone	Dilution	Antigen processing
HSD3B	rabbit	polyclonal	X2500	no process
CYP17A	rabitt	polyclonal	x500	autocrave
CYP11B2	mouse	monoclonal	X750	autocrave
CYP11B1	rat	monoclonal	x200	autocrave

For immunohistochemical staining, 5-μm-thick sections were cut on a microtome and deparaffinized with xylene and ethanol. To detect CYP17A, sections were antigen-retrieved with an autoclave (5 min in citric acid buffer, pH 6.0). To detect HSD3B and CYP17A, sections were treated with a blocking reagent (Histofine, Nichirei, Tokyo, Japan) for 30 min at room temperature. Sections were incubated with either αHSD3B (1:2,500) or αCYP17A1 (1:500) overnight at 4°C. Immunoreactivity was visualized with 3,3′-diaminobenzidine (DAB; brown staining) with a peroxidase-based Histofine Simple Stain Kit (Nichirei, Tokyo, Japan) and counterstained with hematoxylin. Immunostaining for CYP11B1 and CYP11B2 was performed using the streptavidin-biotin amplification method using ImmPRESS reagent (Vector, Burlingame, CA, USA). Antigens were retrieved by heating the glue-coated slides in EDTA (pH 9.0) in an autoclave for 5 min. Blocking was performed for 1 hour using blocking buffer (normal horse serum 5% with SDS 0.5%) at room temperature. Antigen-antibody complexes were visualized with DAB solution (1 mmol/L DAB, 50 mmol/L Tris-HCl buffer [pH 7.6], and 0.006% H_2O_2) and counterstained with hematoxylin.

- Immunoreactivity was assessed semiquantitatively according to McCarty's H-score.

500 cells were evaluated

H-score calculation formula

$$\text{H-score} = \frac{\{(3 \times X)+(2 \times Y)+(1 \times Z)+0x(500-X-Y-Z)\}x100}{500}$$

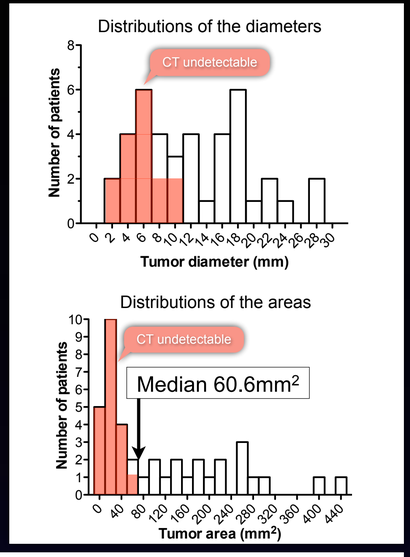
The percentage of stained cells is multiplied by a number from 0 to 3, reflecting the intensity of their immunopositivity.

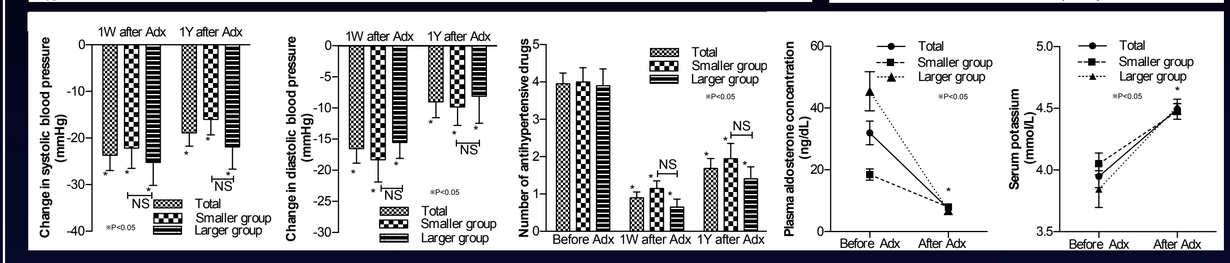
Results

Comparison of clinical characteristics between the smaller APA group and larger APA group

	Total N=40	smaller (<60mm2) N=20	larger (≥60mm2) N=20	P value
Gender (M, F)	28, 12	17, 3	11, 9	*< 0.05
Age (years)	52.2 ± 1.9	57.7 ± 2.8	46.7 ± 2.1	*< 0.05
Duration of hypertension (years)	10.6 ± 1.3	13.6 ± 2.2	7.6 ± 1.4	*< 0.05
Number of anti-hypertensive drugs	3.95 ± 0.29	4.00 ± 0.39	3.90 ± 0.45	NS
Body mass index (kg/m2)	25.4 ± 0.5	25.9 ± 0.7	24.8 ± 0.8	NS
Systolic blood pressure (mmHg)	149.2 ± 3.0	144.9 ± 4.0	153.6 ± 4.6	NS
Diastolic blood pressure (mmHg)	94.1 ± 2.0	93.5 ± 3.2	94.6 ± 2.4	NS
Serum creatinine (mg/dl)	0.79 ± 0.04	0.81 ± 0.04	0.77 ± 0.06	NS
Serum pottasium (mmol/l)	3.55 ± 0.08	3.75 ± 0.10	3.37 ± 0.12	*< 0.05
Prevalence of hypokalemia (%)	42.5	20	65	*< 0.0001
Plasma aldosterone concentration (ng/dl)	31.9 ± 3.8	18.5 ± 1.9	45.4 ± 6.2	*< 0.0001
Serum cortisol (μg/dl)	7.99 ± 0.68	8.24 ± 1.16	7.72 ± 0.73	NS
Plasma renin activity (ng/ml/hr)	0.20 ± 0.02	0.16 ± 0.10	0.24 ± 0.04	NS
Aldosterone/renin activity (ng/dl per ng/ml/hr)	220.5 ± 29.5	156.4 ± 23.2	284.5 ± 51.1	NS
Urinary aldosterone excretion (μg/day)	20.3 ± 2.7	13.6 ± 1.8	27.0 ± 4.7	*< 0.05
Urinary free cortisol excretion (μg/day)	46.4 ± 5.1	48.3 ± 8.2	44.4 ± 6.2	NS
Captopril challenge ARR (ng/dl per ng/ml/hr)	217.9 ± 61.6	93.2 ± 9.7	342.6 ± 117.7	NS
Cortisol after DST (μg/dl)	1.30 ± 0.11	1.27 ± 0.16	1.33 ± 0.16	NS
Lateralization index in AVS before cosyntropin loading	18.19 ± 4.04	11.18 ± 3.3	26.00 ± 7.53	*< 0.05
Lateralization index in AVS after cosyntropin loading	9.47 ± 1.05	7.59 ± 0.97	11.46 ± 1.83	NS
Diameter of adrenal tumor (mm)	11.9 ± 1.1	5.9 ± 0.6	17.8 ± 1.0	*< 0.0001
Area of adrenal tumor (mm^2)	113.8 ± 18.5	22.2 ± 3.5	205.5 ± 23.0	*< 0.0001

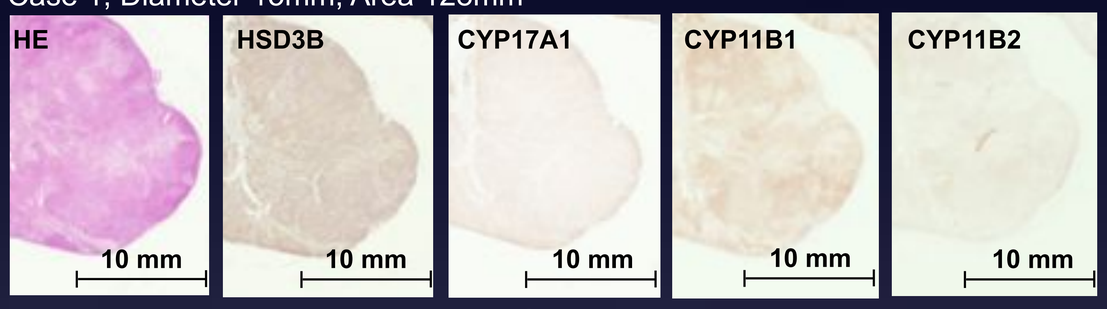
APA: aldosterone producing adenoma, DST:1mg of dexamethasone suppression test, ARR: plasma aldosterone concentration per plasma renin activity
AVS:Adrenal venous sampring, Lateralization index : the value of aldosterone/cortisol of the higher side divided by that of lower side
hypokalemia is defined as below 3.5 mmol/l



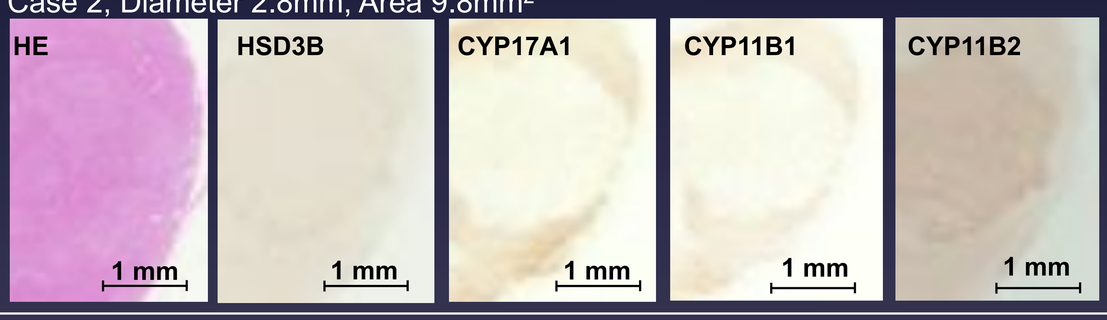


Immunohistochemistry

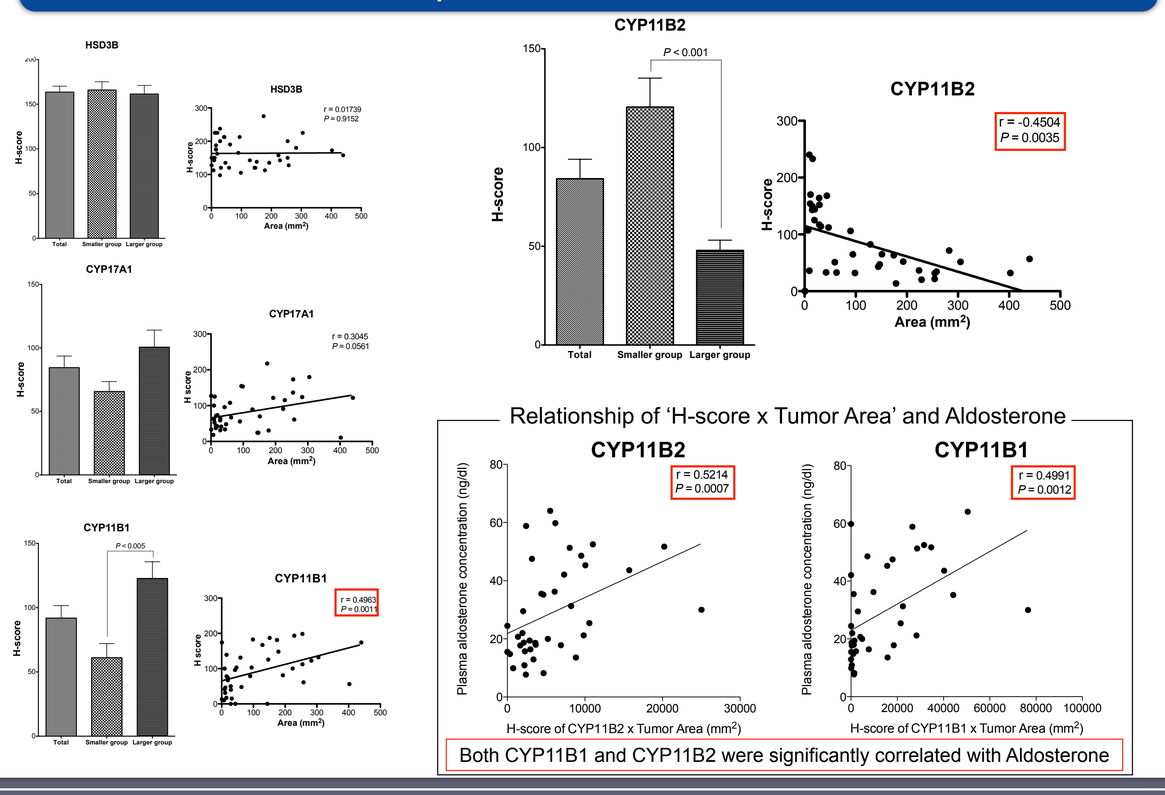
Case 1, Diameter 15mm, Area 128mm^2



Case 2, Diameter 2.8mm, Area 9.8mm^2



Relationship of H-score and Tumor Area



Relationship of 'H-score x Tumor Area' and Aldosterone

Both CYP11B1 and CYP11B2 were significantly correlated with Aldosterone

Discussion

- The total production of steroids including aldosterone was generally considered higher in the larger APA group than the smaller APA group. However, tumor area was inversely correlated with the H-score of CYP11B2, which is the rate-limiting step of aldosterone biosynthesis, and positively correlated with the H-score of CYP11B1. These findings did demonstrate that the smaller tumors had higher CYP11B2 expression per area and cell.
- CYP11B2 levels alone do not necessarily represent abundant aldosterone production, because several other factors (e.g., the levels of steroidogenic enzymes upstream of CYP11B2) also play pivotal roles in overall aldosterone production. However, this marked expression of CYP11B2 per area and cell in the tumors may at least explain why small APAs below the detection limit of CT can result in clinically overt hyperaldosteronism.

Conclusion

In both smaller and larger groups, laparoscopic adrenalectomy based on the results of AVS significantly improved blood pressure, plasma aldosterone concentration, urinary aldosterone excretion, and the number of antihypertensive drugs. The present study demonstrated that small adenomas could produce sufficient aldosterone to cause clinically overt primary aldosteronism because of the significantly higher CYP11B2 expression per tumor area.

Ono Y et al *Hypertension* 2014 Aug. In press