18-Oxocortisol Synthesis in Aldosterone-Producing Adrenocortical Adenoma and Significance of *KCNJ5* Mutation Status

Yuta Tezuka, **Yuto Yamazaki**, **Masaaki Kitada**, **Ryo Morimoto**, **Masataka Kudo**, **Kazumasa Seiji**, **Kei Takase**, **Yoshihide Kawasaki**, **Koji Mitsuzuka**, **Akihiro Ito**, **Jun Nishikawa**, **Noriko Asai**, **Yasuhiro Nakamura**, **Celso E. Gomez-Sanchez**, **Sadayoshi Ito**, **Mari Dezawa**, **Hironobu Sasano**, **Fumitoshi Satoh**

Division of Nephrology, Endocrinology and Vascular Medicine, Department of Medicine (Y.T., R.M., M.K., S.I., F.S.), Department of Pathology (Y.Y., H.S.), Department of Diagnostic Radiology (K.S., K.T.), and Department of Urology (Y.K., K.M., A.I.), Tohoku University Hospital, Sendai, Miyagi, Japan; Division of Clinical Hypertension, Endocrinology and Metabolism (Y.T., F.S.) and Department of Stem Cell Biology and Histology (M.K., M.D.), Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan; Aska Pharma Medical Co Ltd, Kawasaki, Japan (J. Nishikawa, N.A.); Division of Pathology, Faculty of Medicine, Tohoku Medical and Pharmaceutical University, Sendai, Miyagi, Japan (Y. Nakamura); and Division of Endocrinology, G.V. (Sonny) Montgomery VA Medical Center and University of Mississippi Medical Center, Jackson (C.E.G.-S.).

Abstract

Peripheral 18-oxocortisol (18oxoF) level could contribute to the detection of aldosterone-producing adenoma (APA) in patients with primary aldosteronism. However, peripheral 18oxoF varies among such patients, which is a big drawback concerning its clinical application. We studied 48 cases of APA, 35 harboring *KCNJ5* mutation, to clarify the significance of clinical and pathological parameters about peripheral 18oxoF. Peripheral 18oxoF concentration ranged widely from 0.50 to 183.13 ng/dL and correlated positively with intratumoral areas stained positively for steroidogenic enzymes ($P < 0.0001$). The peripheral 18oxoF level also correlated significantly with that of circulating aldosterone ($P < 0.0001$) but not with that of cortisol, a precursor of 18oxoF. However, a significant correlation was detected between peripheral 18oxoF and intratumoral glucocorticoids ($P < 0.05$). In addition, peripheral 18oxoF correlated positively with the number of hybrid cells double positive for 11β-hydroxylase and aldosterone synthase ($P < 0.0001$). Comparing between the cases with and those without *KCNJ5* mutation, the KCNJ5-mutated group demonstrated a significantly higher concentration of peripheral 18oxoF (28.4 ± 5.6 versus 3.0 ± 0.9 ng/dL, $P < 0.0001$) and a larger intratumoral environment including the hybrid cells ($P < 0.001$), possibly representing a deviation from normal aldosterone biosynthesis. After multivariate

analysis, *KCNJ5* mutation status turned out to be the most associated factor involved in 18oxoF synthesis in APA (*P*<0.0001). Results of our present study first revealed that enhanced 18oxoF synthesis in APA could come from a functional deviation of aldosterone biosynthesis from the normal zona glomerulosa and the utility of peripheral 18oxoF measurement could be influenced by the prevalence of *KCNJ5* mutation in an APA.

Keywords

adrenocortical adenoma; 18-oxocortisol; hybrid cells; hyperaldosteronism; *KCNJ5* mutation

Primary aldosteronism (PA) is a hypertensive disorder resulting in more severe organ damage than essential hypertension because of both direct and indirect effects of autonomous aldosterone secretion.[1,2] PA is also the most common cause of secondary hypertension, and its prevalence is considered to be as high as 5% to 10% of all patients with hypertension.[3,4] Early diagnosis and appropriate management for PA are, therefore, clinically required to decrease the elevated risk of developing hypertensive complications, such as cardiovascular and cerebral events.[5] However, confirmatory tests of PA and adrenal venous sampling for determining PA subtypes are generally time-consuming, invasive and labor intensive.[6,7]

18-oxocortisol (18oxoF) has been recently reported as a potential clinical biomarker for differentiating subtypes of PA.[8] 18oxoF has been also termed hybrid steroid because of its structural features of both glucocorticoid and mineralocorticoid.[9] In healthy human subjects, 18oxoF is converted from circulating cortisol by aldosterone synthase, CYP11B2,[9] and its clinical significance is scarce because of its extremely low concentration in plasma. However, it is also true that patients with PA have higher plasma and urinary concentrations of 18oxoF than patients with essential hypertension.[10,11] In addition, we have previously demonstrated using segmental adrenal venous sampling that aldosterone-producing adenoma (APA) secretes more 18oxoF into adrenal veins than the contralateral adrenal gland and in case of bilateral hyperaldosteronism (BHA).[12] Therefore, as peripheral plasma concentration of 18oxoF (p18oxoF) is significantly elevated in patients with APA than in those with BHA, this parameter could be useful to differentiate APA from BHA among patients with PA.[8] Although not totally accurate, measurement of p18oxoF is expected to be a useful and low-invasive biomarker for clinical subtyping of PA.

However, the status of 18oxoF synthesis in APA, as well as its clinical and biological significance, has remained largely unknown. For instance, the concentration of p18oxoF enormously differs among APA cases. In particular, one-fourth of patients with APA had concentrations of p18oxoF as low as those found in patients with BHA.[8] The concentration of p18oxoF has been known to be influenced by the intratumoral status of CYP11B2, a rate-limiting enzyme of 18oxoF. In addition to that, 11β-hydroxylase (CYP11B1) is also a candidate influential factor because of converts 11-deoxycortisol to cortisol and the status of glucocorticoid synthesis in APA.[13,14] In familial hyperaldosteronism (FH) type 1, a chimeric gene of *CYP11B1* and *CYP11B2* has been well known as a pivotal factor for enhanced 18oxoF synthesis.[15] Both CYP11B1 and CYP11B2 double-positive hybrid cells (B1+B2

cells) have been also reported to exist in APAs,[16] which could contribute to the high concentration of p18oxoF.

In addition, the utility of p18oxoF concentration level in determining subtypes of PA appears to differ between whites and Asian patients.[8,17] This difference could be possibly because of the status of a somatic gene mutation in APA,[18] especially *KCNJ5* mutation, because the prevalence of *KCNJ5* mutation in APAs among Europeans is significantly lower than that among Japanese.[19,20] The influence of *KCNJ5* mutation on 18oxoF synthesis has remained unclear but results of several previously reported studies demonstrated that *KCNJ5* mutation-induced adrenocortical cells and APA had a potential to produce abundant 18oxoF. [18,21]

We, therefore, hypothesized that 18oxoF synthesis in APA could be influenced by the intratumoral status of the precursor steroids synthesis and CYP11B1 and CYP11B2 double-positive hybrid tumor cells. Thus, in this study, we examined the potential impacts of these pathological factors on 18oxoF synthesis in APA and evaluated how *KCNJ5* mutation could influence 18oxoF synthesis.

Methods

The present study was approved by the ethics committee of Tohoku University School of Medicine (numbers: 2011–236, 2014–1-404, and 2015–1-278). We obtained informed consents from all PA cases examined. The data that support the findings of this study are available from the corresponding author on reasonable request.

Diagnosis of PA and Eligibility of APA

The APA cases were retrospectively retrieved from a cohort of the PA Sendai Study in which individual patients were registered once they provided their informed consent in writing. All the cases were diagnosed with PA and underwent unilateral adrenalectomy in Tohoku University Hospital (Sendai, Japan), from 2011 after the Great Earthquake that affected our area, until 2015. The diagnosis of PA was confirmed by the captopril challenge test (50 mg of captopril), and the laterality of hyperaldosteronism was determined by cosyntropin-stimulation adrenal venous sampling as previously reported.[22] In this procedure, we used commercially available kits to measure plasma renin concentration and plasma aldosterone concentration; namely, Renin Riabead Kit (Dainabot, Tokyo, Japan) and SPAC-S Aldosterone Radioimmunoassay Kit (TFB, Inc, Tokyo), respectively.[22] Blood pressure was measured using Omron Hem 907 (Omron Healthcare Co Ltd, Kyoto, Japan) as shown in our previous study.[8] After unilateral adrenalectomy, the removed adrenal glands were analyzed and confirmed as APA by histopathologic examination and based on postoperative biochemical data. Exclusion criteria were as follows: the patients with other concurrent adrenal tumor(s) revealed by computed tomography; with a cortisol concentration of >3.0 μg/dL after an overnight 1-mg dexamethasone suppression test[23]; with multiple APA; and those showing intratumoral heterogeneity of CYP11B2 expression.[24] In total, 48 cases were examined in this study.

Measurement of p18oxoF and Intratumoral Steroid Hormones

Using fusaric acid, p18oxoF was measured by liquid chromatography-tandem mass spectrometry as previously reported.[8] Peripheral blood samples for p18oxoF measurement were obtained in the morning after a 30-minute rest. In this study, the lower limit of quantification for 18oxoF was 0.50 ng/dL because of the amount of plasma sample used. Besides, we measured tissue concentrations of 18oxoF, aldosterone, corticosterone, cortisol, and 11-deoxycortisol by liquid chromatography-tandem mass spectrometry. Sample preparation for the measurement of each steroid hormone by liquid chromatography-tandem mass spectrometry is described in the online-only Data Supplement. The steroid concentration in frozen tissue was adjusted by estimated tumor volume based on the length, width, and height of the removed APA.

Histopathologic Analysis for Expression of Steroidogenic Enzymes

All resected adrenal specimens were embedded in paraffin for immunohistochemical staining of CYP11B1, CYP11B2, and C17 (17α-hydroxylase), and for immunofluorescence staining of B1+B2 cells.[16,25,26] The immunohistochemical staining and immunofluorescence staining methods are described in the online-only Data Supplement. All immunohistochemical staining sections were digitally scanned and captured by Image Scope AT2 (Leica, Wetzlar, Germany) for digital image analysis. We measured the cross-sectional area (CSA) of APA, and the immunoreactivity of each of these enzymes above was quantitated using HALO digital image software Area Quantification version 1.0 (Indica Laboratories, Corrales, NM) as previously described.[25] The status of individual steroidogenic enzymes was evaluated by the following 2 quantitative approaches. One was the ratio of positive area to CSA of APA referred as immu-noreactivity, and the other the area of positive staining in APA referred as positive area.

As for the manual counting of the number of B1+B2 cells, the immunofluorescence images were obtained as square pictures of 1024 pixels (512 μm) using a confocal laser-scanning microscope system (A1R, Nikon, Tokyo). The number of B1+B2 cells was calculated as the average of the counts obtained from 3 pictures from other lesions in each APA cases and adjusted for CSA. As controls, 7 cases of non-functional adrenocortical adenoma were analyzed.

Detection of *KCNJ5* Mutation in APA

We prepared 5 other tissue sections of those adrenal specimens at 10 μm thickness for the detection of *KCNJ5* mutation in APA and micro-dissected APA regions in the sections.[25] Genomic DNA was isolated from those tissues using the Qiagen AllPrep DNA/RNA FFPE kit and submitted it to polymerase chain reaction using a *KCNJ5* primer (forward 5'-CGA CCA AGA GTG GAT TCC TT-'3 and reverse 5'-AGG GTC TCC GCT CTC TTC TT-'3) as already described by our group.[27] Detection of *KCNJ5* mutation was performed with the Abi Prism 310 genetic analyzer (Applied Biosystems, Foster City, CA). In this study, we did not evaluate other somatic mutations such as *CACNA1D* and *ATPase* because of its extremely lower frequency compared with that of *KCNJ5* mutation or wild type in Japanese patients with APA.[20]

Statistical Analysis

In terms of p18oxoF and plasma renin activity, the values below lower limits of detection were assigned to analytical sensitivity values for subsequent statistical comparison. All data were presented as the mean±SD when in normal distribution and as the median (interquartile range) when in log-normal distribution. For analysis, data on p18oxoF, plasma aldosterone concentration, presence of intratumoral steroid, CSA, expression of enzymes, and number of B1+B2 cells were log-transformed because of their distributions. Spearman correlation analysis and Mann-Whitney *U* test were used for measuring the strength of the relationship between 2 variables and comparison between different groups, respectively. To investigate independent correlates, we also performed multivariate linear regression analysis with the backward stepwise procedure. Statistical significance was set at *P* 0.05, and all analyses were performed with Stat Flex version 6.0 (Artech Co Ltd, Osaka, Japan).

Results

Baseline Parameters of 48 APA Cases

Baseline characteristics of all APA cases (n=48) were summarized in Table 1. The great majority of the cases examined harbored computed tomography-detectable adrenal tumors which were pathologically diagnosed as APA, while only 3 cases had no computed tomography-detectable adrenal tumors. These 3 cases were confirmed as aldosterone-producing microad-enomas. The concentration of p18oxoF ranged from 0.50 to 183.13 ng/dL with a median of 11.92 ng/dL (Figure S1 in the online-only Data Supplement). Spearman correlation analysis revealed that the concentration of p18oxoF was significantly correlated with age (Spearman *r*=–0.471, *P*=0.0007) and duration of hypertension (Spearman *r*=–0.328, *P*=0.02).

The associations between p18oxoF and other steroid hormones in plasma and urine were then evaluated (Figure 1). The concentration of p18oxoF was significantly correlated with plasma aldosterone concentration, possibly because of their common rate-limiting enzyme CYP11B2.[9] However, the concentration of p18oxoF was by no means correlated with that of circulating cortisol, a precursor of 18oxoF. Similar trends were detected between p18oxoF and urinary concentration of aldosterone and cortisol. 18oxoF is a derivative of cortisol, but p18oxoF did not demonstrate any significant correlation with the concentration of either circulating or urinary cortisol.

Peripheral 18oxoF and Immunoreactivities of Steroidogenic Enzymes in Tumor Tissues

The mean maximum diameter (MD) and CSA of APAs were 13.96 mm and 63.93 mm^2, respectively. p18oxoF was significantly correlated with MD and CSA of APAs (Figure 2). The ratio of CYP11B1 correlated negatively with that of MD, whereas the ratio of CYP11B2 was positively correlated with that of MD; however, no significant correlation was observed with the ratio of C17 (Figure S2). However, all areas that stained positively for the enzymes examined were positively and significantly correlated with MD. In addition, p18oxoF was significantly correlated with the areas showing positive staining for steroidogenic enzymes in these cases. The results indicated that APA cases with a high concentration of p18oxoF

possibly expressed substantial amounts of steroidogenic enzymes to produce glucocorticoids as well as aldosterone.

Tissue Concentration of 18oxoF and Other Steroid Hormones

We then measured 18oxoF and other steroid hormones in frozen tissue of 15 available cases of APA to identify possible influences of intratumoral synthesis of its precursors on the concentration of 18oxoF. Both p18oxoF and tissue concentration of 18oxoF were significantly and positively correlated (Figure 1). Tissue concentrations of 18oxoF and aldosterone were also significantly correlated with each other (Spearman r=0.843, P<0.01). Of particular interest, tissue concentration of 18oxoF and that of cortisol were also positively correlated in contrast to their concentrations in peripheral blood (Spearman r=0.675, P<0.01). 18oxoF was also significantly correlated with the concentration of 11-deoxycortisol, a precursor of cortisol (Spearman r=0.871, P<0.01) but not with that of corticosterone, a precursor of aldosterone in the tissues examined. p18oxoF was also correlated with the tissue concentration of aldosterone, cortisol, and 11-deoxycortisol, indicating that not circulating cortisol but an intratumoral synthesis of its precursors significantly regulated 18oxoF synthesis in APA.

Association of p18oxoF and CYP11B1 and CYP11B2 Double-Positive Hybrid Cells in APA

We then evaluated B1+B2 cells in APA (Figure S3). The number of these hybrid cells was 48.8 (21.7–101.7)/mm^2 in APA and significantly larger than that in nonfunctional adrenocortical adenoma (3.8 [1.8–6.1]/mm^2, P=0.0007). This tendency remained the same before and after adjustment with CSA. B1+B2 cells in APA were also positively and significantly correlated with MD (Spearman r=0.308, P=0.03) and CSA (Spearman r=0.536, P=0.0001), while in nonfunctional adrenocortical adenoma no correlation was observed with any clinicopathological factor. In addition, the status of B1+B2 cells in APA was significantly correlated with p18oxoF (Spearman r=0.617, P<0.0001) and plasma aldosterone concentration (Spearman r=0.312, P=0.03) after adjustment for CSA (Figure S3). Serum cortisol concentration, however, did not correlate with the status of B1+B2 cells in APA. These results as a whole indicated that a large number of B1+B2 cells also could lead to enhanced 18oxoF synthesis in APA, as reported in FH type I.

Influence of *KCNJ5* Mutation Status on Intratumoral Synthesis of Steroid Hormones in APA

We subsequently examined the association between clinico-pathological factors and *KCNJ5* mutation status about their influence on p18oxoF synthesis in APA. Thirty-five of the 48 APA cases (72.9%, including 1 case of microadenoma) harbored the *KCNJ5* mutation (Table 1). The concentration of p18oxoF in *KCNJ5*-mutated APA was significantly higher than that in APA without this mutation (Table 1). Between 2 major *KCNJ5* mutations, G151R and L168R, the L168R group showed a significantly higher concentration of p18oxoF than the G151R group (P=0.03, Tables S1 and S2). In addition, interestingly, the areas that stained positive for steroidogenic enzymes and B1+B2 cells were significantly more abundant in *KCNJ5*-mutated APA than in APA without the *KCNJ5* mutation. The *KCNJ5* mutation status could account for the differences in p18oxoF concentration among patients of different age. *KCNJ5* mutation could be a key driver which accelerates 18oxoF synthesis by promoting synthesis of its precursors and direct synthesis from B1+B2 cells.

All the factors related to the enhanced 18oxoF synthesis in APA correlated with tumor size. By incorporating age, sex, the status of immunoreactivity of each steroidogenic enzyme, MD, number of B1+B2 cells and *KCNJ5* mutation status, multivariate linear regression analysis with the backward stepwise procedure revealed independent associated factors for 18oxoF synthesis in APA (Table 2). Immunoreactivity of CYP11B2, MD, and *KCNJ5* mutation status turned out as an independent associated factor. Finally, we confirmed that *KCNJ5* mutation status was the most significantly associated factor about enhanced 18oxoF synthesis in APA.

Discussion

The results of the present study demonstrated that 18oxoF synthesis in APA was regulated by both the status of intratumoral synthesis of its precursors and the number of B1+B2 cells. This is the first study which clearly demonstrated an intratumoral unique environment where rate-limiting enzymes of both of aldosterone and cortisol was crucial for the synthesis of p18oxoF. It was also notable that the environment could be reflected in the tumor size and that the presence of *KCNJ5* mutation in APA was an independent associated factor for enhanced 18oxoF synthesis mediated by both of the factors above.

In normal adrenals, 18oxoF is mainly converted from circulating cortisol by CYP11B2 in the zona glomerulosa because of centripetal blood flow.[9,28] 18oxoF is, therefore, recognized as a by-product of CYP11B2, in general. Our previous studies confirmed that not only normal adrenal tissue but also adrenal hyperplasia showed preserved zonation of the adrenal cortex and did not express both CYP11B1 and CYP11B2 in the same cortical cells as FH.[16,29,30] In contrast with those previous findings, our present study showed that APA harbors an environment of mixture of several steroidogenic enzymes. The environment could lead to 18oxoF synthesis by cell to cell interaction because of easier contact between CYP11B2 and the precursors of 18oxoF because of expression of CYP11B1 and C17. Contrary to circulating cortisol, the amount of intra-tumoral glucocorticoids demonstrated a significant correlation with p18oxoF, indicating that those steroids were mainly converted to 18oxoF in APA. However, we could by no means rule out the possibility of the influence of circulating cortisol on 18oxoF synthesis because of the influence of several related factors, such as its binding protein and different half-life.

Additionally, our study revealed that B1+B2 cells in APA could be a cause of direct 18oxoF synthesis. In FH type 1, the expression of the chimeric gene of *CYP11B1* and *CYP11B2* in adrenocortical cells was reported to cause 18oxoF overproduction.[31] An in vitro study also confirmed that adrenocortical cells expressing both cortisol and aldosterone synthetases could produce 18oxoF directly.[32] Therefore, the existence of these hybrid cells in APA could be thought as an associated factor for 18oxoF synthesis. Moreover, the acquisition of these hybrid cells in APA might represent the sequence of neo-plastic or abnormal development. The correlation of p18oxoF with tumor size was considered plausible because the size of a tumor is generally considered to be in parallel with the degree of deviation of normal cells in which tumor arise. These findings were also consistent with the fact that p18oxoF could clinically detect larger APAs, not microadenomas, with more accuracy.[8]

Moreover, the present results also revealed that the presence of the *KCNJ5* somatic mutation was also significantly correlated with 18oxoF synthesis in APA via an intratumoral unique environment. The mutated *KCNJ5* gene increases sodium influx and chronic depolarization in aldosterone-producing cells, resulting in autonomous overproduction of aldosterone by increased CYP11B2 expression.[33]

Results of previously reported meta-analysis study clearly demonstrated clinical significance of *KCNJ5* mutation in APA on severe hyperaldosteronism.[34] However, HAC15 cells harboring increased expression of the *KCNJ5* mutation gene were also reported to increase mRNA expression of CYP11B1 as well as of CYP11B2 in vitro.[35] Therefore, the production of cortisol and 18oxoF was also more pronounced in HAC cells harboring the *KCNJ5* mutation than in those with *KCNJ5* wild-type gene in vitro.[21] In addition, results of previously reported study did demonstrate the impacts of somatic mutations of APA on the plasma concentration of 18oxoF.[18] Williams et al[18] reported that *KCNJ5*-mutated APA group had significantly higher levels of 18oxoF and 18-hydroxycortisol than other genotypes, such as *CACNA1D* and ATPase, although the cause of these difference has remained unclear. Results of our present study did reveal that the difference could be derived from intratumoral tissue microenvironment, that is, the degree of expression of steroidogenic enzymes per cells and the hybrid cells. However, the association between these hybrid cells and *KCNJ5* mutation in the human body has remained unclear in a mechanistic sense, but in FH type 3 which is caused by genetic mutation *KCNJ5,* adrenal glands harbors abundant hybrid cells.[36] In our study, *KCNJ5* mutation status was similarly correlated with not only the area that stained positive for CYP11B2 but also for both aldosterone and p18oxoF, which also indicated that these changes in *KCNJ5*-mutated cells of APA could occur via the same mechanisms. However, serum cortisol concentration was by no means correlated with 18oxoF or *KCNJ5* mutation status in our cohort. This is because circulating cortisol in APA cases largely consist of cortisol secretion from adjacent and contralateral adrenal glands. The presence of somatic *KCNJ5* mutations might reflect the biological deviation from normal aldosterone-producing adrenocortical cells, but further investigations are required for clarification.

These results also demonstrated that the clinical utility of measuring p18oxoF for subclassification of PA could depend on the prevalence of the *KCNJ5* mutation in APA. In this study, 29 out of 35 APAs harboring the *KCNJ5* mutation (82.9%) had significantly high concentrations of p18oxoF of >6.1 ng/dL, which was significantly high and corresponded to the maximum value found in BHA in our previous study.[8] However, only 2 out of 13 cases (15.4%) with *KCNJ5* wild type had a concentration of p18oxoF higher than this particular value. In addition, there were 6 cases (12.5%) with a p18oxoF concentration lower than 1.2 ng/dL corresponding to the minimum value found in BHA.[8] In all of these 6 cases, the tumors turned out to harbor the wild-type *KCNJ5* gene and were smaller than 10 mm in their greatest dimension. As it is well known, the prevalence of the *KCNJ5* mutation in APA is markedly different among different races.[34] Therefore, measurement of p18oxoF could have potential for subtyping the patients with PA in a cohort with a known high prevalence of *KCNJ5*-mutated APA, such as Asian patients.[20,34,37, 38] However, further studies are needed to determine the detailed methods of measuring p18oxoF and the cutoff value of p18oxoF for PA subtyping.

It is also entirely true that our present study had some limitations. First, we excluded APA cases with nonfunctional adenomas or cortisol cosecretion to exclude their possible influences. Second, the analysis of intratumoral steroid concentration was performed only in 15 APA cases because of tissue availability. Third, we could not evaluate the difference of p18oxoF between *KCNJ5* mutation and other reported relatively rare somatic mutations of APA because of the rarity of these mutated APA among Japanese. Therefore, further investigations are required to clarify these points.

Perspectives

We first revealed that *KCNJ5* mutation resulted in more enhanced 18oxoF synthesis in APA by increasing the status of intratumoral glucocorticoid synthesis and because of the high number of CYP11B1 and CYP11B2 double-positive hybrid cells compared with APAs harboring the wild-type *KCNJ5* gene. This result indicated that the presence of *KCNJ5* mutation, which is rarely detected in normal and hyperplastic adrenocortical cells, might reflect the degree of functional deviation from normal aldosterone-producing cells. Results of our previous study demonstrated that the measurement of p18oxoF could contribute to the differentiation of patients with PA but its utility was indeed influenced by the prevalence of *KCNJ5* mutations among patients with APA. This could be considered one reason why measurement of p18oxoF was less effective among European patients with PA.[17] Therefore, among the cohort of APA patients with high prevalence of the *KCNJ5* mutation, measurement of p18oxoF is considered clinically useful by selecting the patients requiring adrenal venous sampling and possibly shortening the time from initial diagnosis to proper management.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Novelty and Significance

What Is New?

- We revealed that intratumoral environment of aldosterone-producing adenoma could cause the differences in peripheral 18-oxocortisol concentration in aldosterone-producing adenoma and the significance of *KCNJ5* mutation.

What Is Relevant?

- Peripheral 18-oxocortisol concentration is an expected biomarker for easier and less invasive differentiation of aldosterone-producing adenoma among patients with primary aldosteronism but its utility could depend on the prevalence of the *KCNJ5* mutation in aldosterone-producing adenoma.

Summary

Our present study first revealed that enhanced 18-oxocortisol synthesis in aldosterone-producing adenoma could come from intratumoral glucocorticoid synthesis and, 11β-hydroxylase and aldosterone synthase double-positive hybrid cells, which could reflect the functional deviation of aldosterone biosynthesis from the normal zona glomerulosa. The *KCNJ5* somatic mutation status is the most associated factor involved in 18-oxocortisol synthesis.
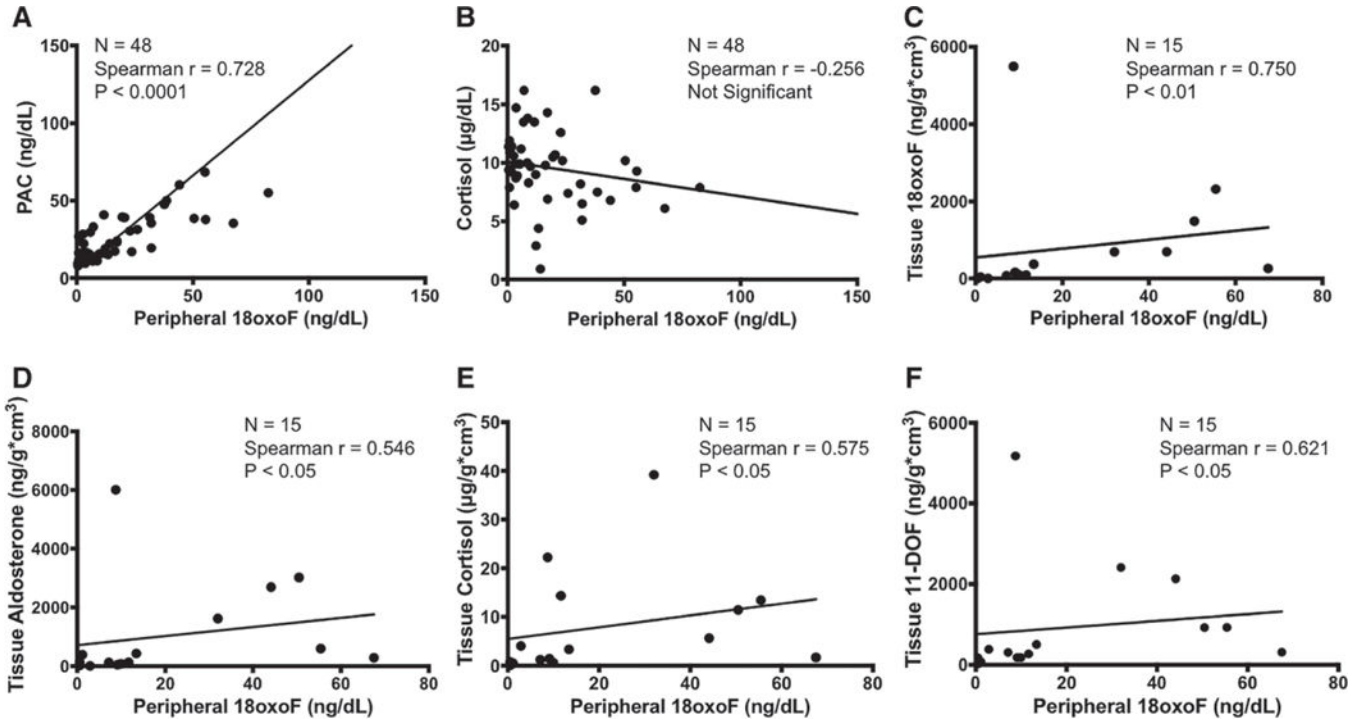
Figure 1.
Correlations between peripheral 18-oxocortisol (18oxoF) and circulating (**A** and **B**) or
intratumoral steroid (**C–F**). Peripheral 18oxoF was significantly correlated with plasma
aldosterone concentration (PAC) but not with circulating cortisol, a precursor of 18oxoF.
However, intratumoral steroid evaluation revealed a significant correlation between
peripheral 18oxoF and intratumoral glucocorticoids, cortisol, and 11-deoxycortisol (11-
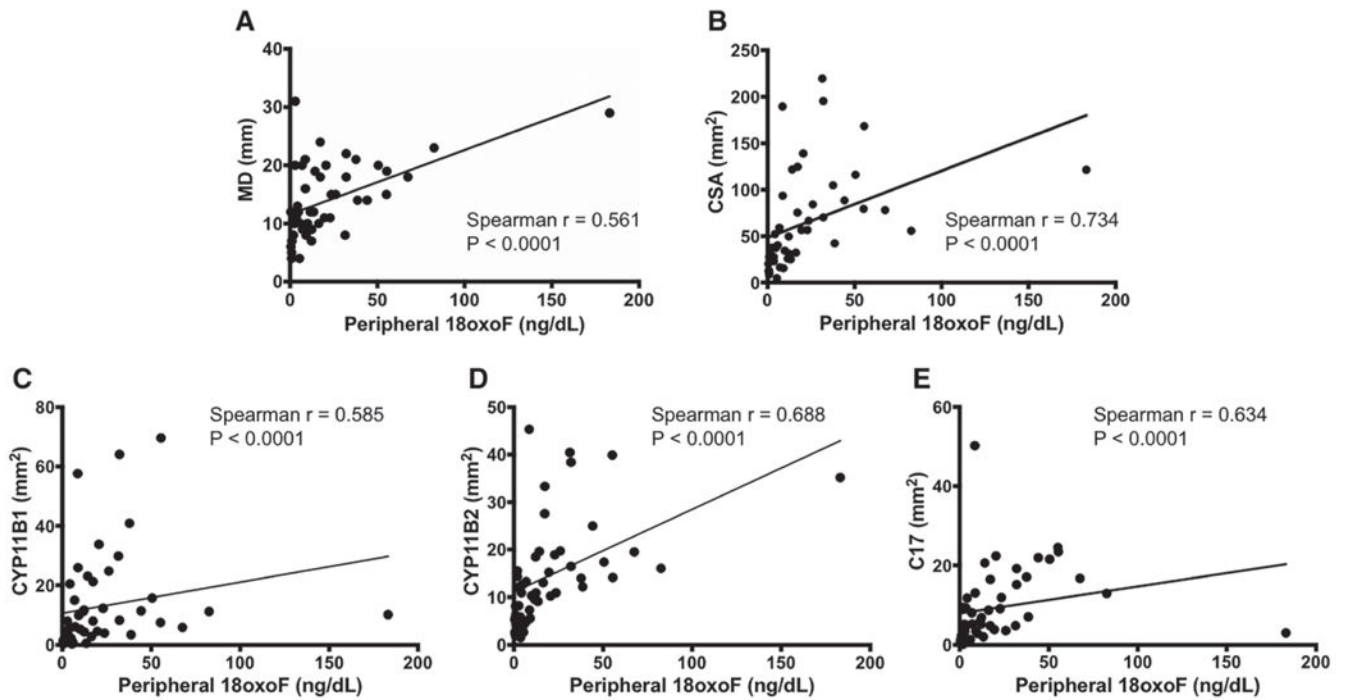DOF).

Figure 2.
Comparison between peripheral 18-oxocortisol (18oxoF) and pathologic parameters. **A** and
B, Peripheral 18-oxocortisol (18oxoF) was significantly correlated with the tumor size. **C–E**,
Similarly, peripheral 18oxoF showed positive correlations with the expression of all the
steroidogenic enzymes examined. C17 indicates 17α-hydroxylase; CSA, cross-sectional
area; CYP11B1, 11β-hydroxylase; CYP11B2, aldosterone synthase; and MD, maximum
diameter.

Table 1.

Clinical and Pathological Baseline Parameters of APA

Parameters	All	KCNJ5 Positive	KCNJ5 Negative	P Value
N	48	35	13	…
Age, y *	51.7±11.8	49.0±11.5	59.0±9.5	<0.01
Sex, M and F *	26, 22	14, 21	12, 1	<0.01
Body mass index, kg/m²	24.7±3.4	24.0±2.8	26.5±4.1	NS
PAC, ng/dL †	40.8 [31.7–57.6]	48.6 [35.2–62.2]	32.6 [24.8–41.3]	<0.05
PRA, ng/mL per hour	0.20 [0.10–0.45]	0.30 [0.10–0.50]	0.20 [0.10–0.23]	NS
ARR, ng/dL per ng/mL per hour	200.4 [95.4–404.8]	198.3 [95.5–409.6]	202.5 [92.1–348.3]	NS
18-oxocortisol, ng/dL †	11.9 [3.7–28.7]	17.3 [8.9–36.2]	1.9 [0.92–3.6]	<0.0001
Duration of hypertension, y †	9.0 [2.0–16.0]	5.0 [2.0–11.8]	17.0 [8.8–23.5]	<0.05
Blood pressure, mm Hg	151.0±21.2/95.3±15.3	151.4±22.8/94.9±22.8	149.9±16.8/96.4±13.0	NS
The number of AHT, n †	2.7±1.7	2.3±1.4	3.5±2.0	<0.05
Decrease in AHT after surgery, y †	1.7±1.5	1.5±1.5	2.2±1.1	NS
Serum potassium, mmol/L	3.84±0.53	3.85±0.54	3.83±0.53	NS
Usage of potassium replacement, %	81.3	82.9	76.9	NS
MD of APA, mm §	14.0±6.3	15.6±5.3	9.6±7.0	<0.001
CSA of APA, mm² †	46.0 [25.8–86.4]	66.6 [38.3–113.4]	20.0 [11.8–26.2]	<0.0001
Immunoreactivity, %				
CYP11B1 †	13.3 [7.9–24.0]	15.3 [8.4–25.0]	7.8 [5.4–15.8]	<0.05
CYP11B2 *	27.7±12.2	24.2±10.8	37.3±11.0	<0.01
C17	15.2±8.8	15.6±8.6	14.3±9.8	NS
Positive area, mm²				
CYP11B1 §	6 8 [2 5–15 3]	10 2 [4 7–22 6]	1.3 [0.6–4.6]	<0.001
CYP11B2 §	12.4 [6.7–18.7]	14.1 [10.5–19.7]	6.2 [3.9–10.2]	<0.001
C17 §	5.6 [2.9–14.1]	9.1 [4.0–17.0]	1.8 [0.7–5.2]	<0.001

Parameters	All	*KCNJ5* Positive	*KCNJ5* Negative	*P* Value
B1+B2 cells, n/mm²§	48.8 [21.7–101.7]	67.5 [33.4–126.5]	21.7 [3.5–33.5]	<0.001
CSA-adjusted B1+B2 cells, n§	3402.7 [578.0–7836.7]	4559.7 [1928.0–10797.2]	278.3 [72.8–602.7]	<0.001
KCNJ5 mutation, %	72.9	100	0	…

Data are described as mean±SD in normal distribution and median [interquartile range] in log-normal distribution. *P* values are calculated based on comparisons between *KCNJ5* mutation positive and negative APA cases. AHT indicates antihypertensive agent; APA, aldosterone-producing adenoma; ARR, aldosterone-to-renin ratio; B1+B2 cells, the number of CYP11B1 and CYP11B2 coexpressed cells; C17, 17α-hydroxylase; CSA, cross-sectional area; CYP11B1, 11β-hydroxylase; CYP11B2, aldosterone synthase; C17, 17α-hydroxylase; MD, maximum diameter; NS, not significant; PAC, plasma aldosterone concentration; and PRA, plasma renin activity.

*
P<0.01.

†
P<0.05.

‡
P<0.0001.

§
P<0.001.

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Table 2.

Factors Associated With 18oxoF Synthesis in APA

Variable	β	SE	Standardized β	95% CI	P Value
Immunoreactivity of CYP11B2, aldosterone synthase, % [*]	0.029	0.012	0.25	0.040–0.46	0.02
Maximum diameter of APA, mm [*]	0.057	0.023	0.26	0.047–0.47	0.02
KCNJ5 mutation (+, -) [†]	1.93	0.37	0.62	0.381–0.86	<0.0001

Adjusted R^2=0.619, F=16.32, and $P<0.0001$. 18oxoF indicates 18-oxocortisol; and APA, aldosterone-producing adenoma.

[*] $P<0.05$.

[†] $P<0.0001$.