Circulating Plasma Concentrations of ACE2 in Primary Aldosteronism and Cardiovascular Outcomes

Running title: pACE2 vs. clinical outcomes in PA after Adrenalectomy

Vin-Cent Wu, MD, PhD¹, Kang-Yung Peng, PhD¹, Ya-Hui Hu, MD², Chin-Chen Chang³, MD, PhD, Chieh-Kai Chan, MD⁴, Tai-Shuan Lai, MD, PhD¹, Yen-Hung Lin, MD, PhD¹, Shuo-Meng Wang, MD, PhD⁵, Ching-Chu Lu, MD, PhD⁶, Yu-Chun Liu, MD⁷, Yao-Chou Tsai², Jeff S Chueh, MD, PhD⁵.

ORCID ID for Jeff S Chueh: 0000-0002-0713-4904

¹ Department of Internal Medicine, Department of Imaging Medicine³, Department of Nuclear Medicine⁶, National Taiwan University Hospital and College of Medicine, National Taiwan University, Taipei, Taiwan

² Taipei Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Taipei, Taiwan

⁴ Department of Internal Medicine, National Taiwan University Hospital, Hsin-Chu branch, Hsin-Chu County, Taiwan.

⁵ Department of Urology, National Taiwan University Hospital and College of Medicine, National Taiwan University, Taipei, Taiwan
Disclosure Statement: The authors have nothing to disclose.

Key words: pACE2, ADAM17, TMPRSS2, primary aldosteronism, adrenalectomy, MRA

Address for correspondence: Jeff S Chueh, MD, PhD. Email: jeffchueh@gmail.com Phone: +886 2 23123456 ext. 63098, and fax: +886 2 23952333.

Acknowledgements: The authors thank the staff of the Second Core Lab in the Department of Medical Research of National Taiwan University Hospital for technical assistance. This study was supported by Taiwan National Science Council (grants NSC 101-2314-B-002-132-MY3, NSC100-2314-B-002-119, NSC 101-2314-B-002-085-MY3, MOST 104-2314-B-002-125-MY3) and NTUH 100-N1776, 101-M1953, 102-S2097.

Word counts and number of tables/figures:

Abstract: 318 words
Main text: 3024 words

Figures: 3
Tables: 2

Authors’ Email lists

Kang-Yung Peng; pengky68@gmail.com
Ya-Hui Hu; huyahuihuyahui@hotmail.com
Chieh-Kai Chan; ck881267@gmail.com
Jeff S Chueh; jeffchueh@gmail.com
Vin-Cent Wu; q91421028@ntu.edu.tw
Shuo-Meng Wang, dturo62@yahoo.com.tw
Chin-Chen Chang, macotoc@gmail.com
Tai-Shuan Lai, taishuanster@gmail.com
Yen-Hung Lin, austinar34@gmail.com
Ching-Chu Lu, kelvinlu@ntu.edu.tw
Yu-Chun Liu, MD, ycliu_ntuh@yahoo.com
Yao-Chou Tsai, tzuhan.tsai@msa.hinet.net

Abbreviations:
ACEi, ACE inhibitor;
ACE2, angiotensin-converting enzyme 2;
ADAM17, metalloprotease 17;
Af, atrial fibrillation;
Ang II, angiotensin II;
APA, aldosterone-producing adenoma;
ARR, aldosterone-renin ratio;
AT1R, angiotensin I receptor;
biPA, bilateral PA;
BP, blood pressure;
CABG, coronary artery bypass graft;
CKD, chronic kidney disease.
CHF, congestive heart failure;
CVD, cardiovascular diseases;
EH, essential hypertension;
GAM, generalized additive model;
GEO, Gene Expression Omnibus;
NCBI, National Center for Biotechnology Information;
PA, primary aldosteronism;
PAC, plasma aldosterone concentration;
PASO, Primary Aldosteronism Surgery Outcome;
PBMCS, peripheral blood mononuclear cells,
PCR, polymerase chain reaction;
PRA, plasma renin activity;
MACE, major cardiovascular events;
MRA, minorcorticoid receptor antagonist;
MI, myocardial infarction;
RAAS, renin-angiotensin-aldosterone system;
uPA, unilateral PA;
The plasma concentrations of angiotensin-converting enzyme 2 ([pACE2]) have been independently associated with cardiovascular diseases.

**Objective:** Higher [pACE2] concentrations could be found in patients with primary aldosteronism (PA) and might lead to increased cardiovascular events.

**Methods:** Using an inception observational cohort, we examined [pACE2] among 168 incident patients with PA. The expression of ACE2, serine protease 2 (TMPRSS2), and metalloprotease 17 (ADAM17) were assessed in peripheral blood mononuclear cells (PBMCs).

**Results:** Incident PA and EH patients had similarly elevated [pACE2] (47.04±22.06 vs. 46.73±21.06 ng/ml, p = 0.937). Age was negatively (β, -2.15, p = 0.033) and higher serum potassium level (β, 2.29, p = 0.024) was positively correlated with higher [pACE2] in PA patients.

Clinical complete hypertension-remission after adrenalectomy (PASO criteria) was achieved in 36 (50%) of the 72 surgically-treated uPA patients. At follow-up, the [pACE2] decreased in surgically-treated patients who had (p<0.001) or had no (p = 0.006) hypertension-remission, but the [pACE2] attenuation was not significant in uPA (p = 0.085) and biPA (p = 0.409) administered with minercorticoid receptor antagonist (MRA). Persistently elevated [pACE2] (> 23ng/ml) after targeted treatments was related to all-cause mortality and cardiovascular events among PA patients (HR, 8.8, p=0.04); with a mean followed up of 3.29 years. TMPRSS2 mRNA expression was higher in uPA (p= 0.018) and EH (p= 0.038) patients than that in normotensive controls; it was also decreased after adrenalectomy (p< 0.001).

**Conclusions:** PA and EH patients had elevated [pACE2] and higher expression of TMPRSS2 mRNA compared to those of normotensive population. Persistently elevated [pACE2] (> 23ng/ml) after targeted treatments was associated the risk of mortality and incident
cardiovascular events.

Keywords: angiotensin-converting enzyme 2 (ACE2), aldosterone, hyperaldosteronism, primary aldosteronism, COVID-19

Introduction

Primary aldosteronism (PA) is the most common cause of secondary hypertension and is reportedly present in 5%–20% or more of hypertensive patients (1,2). PA occurs independent of the physiological autoregulation of renin-angiotensin-aldosterone system (RAAS) and is not suppressed by sodium loading. Despite similar severity of hypertension, PA patients have higher cardiovascular morbidities and mortality than those without it (2).

Angiotensin-converting enzyme 2 (ACE2) is present on endothelial cells and has been identified as an important RAAS counter-regulator, capable of mitigating deleterious actions mediated by angiotensin II (Ang II) and angiotensin I receptor (AT1R) (3). The plasma form of ACE2 (pACE2) is derived via proteolytic shedding of membrane-bound ACE2. However, elevated plasma ACE2 concentrations [pACE2] could play a pivotal detrimental role in the regulation of blood pressure (BP), diabetes, heart failure, coronary heart disease, and chronic kidney disease (4-6). Higher [pACE2] has been associated with an increased risk of greater disease severity (7), all-cause mortality, cardiovascular and even non-cardiovascular deaths (8). Elevated [pACE2] is the highest-ranked predictor of mortality compared with other established risk factors for cardiovascular diseases (CVD) (7-8), long-term cardiac death (9) and has been identified as a
circulating indicator of diabetes, biological ageing, coagulopathy, and mortality(7).

As a novel negative regulator of the RAAS and as the SARS-CoV-2 targeted receptor for the virus to infect humans, ACE2 provides a fundamental connection between viral infection, CVD and immunity(10). Additionally, plasma ACE2-SARS-CoV-2 fusion particles could also be threatening since they can interact with endothelial membrane-bound ACE2, resulting in endothelial damage(11). The imbalance of ACE2 was shed by desintegrin and metalloproteinase domain 17 (ADAM17), along with specific genetic factors that are mainly associated with type II transmembrane serine protease (TMPRSS2) expression(12). The interactions of ACE2, ADAM17 and TMPRSS2 in concert facilitate SARS-CoV-2 viral entry(12) setting the stage for the development of COVID-19 infection.

Despite overwhelming evidence that MRA or adrenalectomy treatment reduces morbidity and mortality in PA patients, the potential impact on the susceptibility for ACE2 expression has encouraged further investigations into the effect of respective targeted treatments, especially during the COVID-19 pandemic period. Given that the over-activity of aldosterone and dysregulation of the RAAS are implicated in the pathophysiology of PA(13) and the data on [pACE2] before and/or after targeted treatments with adrenalectomy or MRA is lacking, this study aimed to understand whether [pACE2] could be differentially expressed or regulated in PA patients and be associated with the outcomes by various treatments. We further identified the expressions of ACE2, ADAM17 and TMPRSS2 in patients’ peripheral blood mononuclear cells (PBMCs) to gain insight into the hypertensive aldosterone- phenotype of PA patients.

Materials and Methods
Data sources and study population

Patients aged >18 years who had been diagnosed with PA were prospectively recruited from January 2017 to January 2020 and were monitored until January 2021. We matched essential hypertension (EH) patients as control group.

Screening, confirmation, and the subtype identification of incident patients with PA were performed in patients with hypertension according to the standard TAIPAI protocol and aldosteronism consensus in Taiwan(14). All original anti-hypertensive medications were discontinued for at least 21 days before PA screening and confirmatory tests. Doxazosin and/or diltiazem were administered to control markedly high BP during the work-up stage when required. The diagnosis of PA in hypertensive patients was based on the inappropriate hypersecretion of aldosterone and according to the fulfillment of the standard criteria(15) (methods detailed in the Supplementary Files, sfigure 1(16)).

Confirmation tests

Fulfillment of the following three criteria confirmed a diagnosis of PA: (1) autonomous excess aldosterone production evidenced with an aldosterone-renin ratio (plasma aldosterone concentration (PAC)/plasma renin activity (PRA); ARR) > 35(ng/dL)/(ng/mL/h); (2) a TAIPAI score larger than 60%; or (3) post-saline loading PAC > 16 ng/dL, or PAC/PRA > 35 (ng/dL)/(ng/mL/h) shown in a post-captopril/losartan test(14).
Peripheral blood mononuclear cells (PBMC) isolation

Whole blood samples were collected from PA, EH patients and normotensive controls and subjected to PBMC isolation using the Ficoll density-gradient separation approach, as previously reported (17).

Ethical approval of the study protocol

The study complied with the Declaration of Helsinki and was approved by the National Taiwan University Hospital Research Ethics Committee (No. 200611031R, 201901114RIND). All participants received comprehensive written information and signed a consent form before their inclusion in the study.

Measurement of plasma ACE2 concentrations

The plasma concentrations of ACE2 ([pACE2]) were measured using a commercially available sandwich enzymatic immunoassay via following the manufacturer's recommendations (Wuhan Fine Biotech Co., Ltd., Wuhan, China; FineTest Cat# EH0027, RRID:AB_2920799, https://scicrunch.org/resolver/AB_2920799) (18,19). The range of the kit is 0.391–25ng/ml, and the sensitivity is 0.234ng/ml (Supplementary Methods).

Outcome measurements

Primary composite outcome were all-cause mortality and cardiovascular events included de-novo (incident) major cardiovascular events (MACE), atrial fibrillation (Af) and/or congestive heart failure (CHF) after the index date of PA confirmation. MACE was defined as the incidence of major cardiovascular events that include non-fatal myocardial infarction (MI), coronary artery bypass graft (CABG), nonfatal stroke, positive findings in coronary angiography (20,21). To corroborate long-term outcome events, we have further validated TAIPAI records with the
Taiwan National Health Insurance Research Database (referring to Supplement).

Secondary outcome was according to the Primary Aldosteronism Surgery Outcome (PASO) consensus on clinical and biochemical outcomes (stable 1) (16,22). Patients were evaluated monthly for the first 3 months postoperatively and every 3 months thereafter. PA patients treated with MRA were monitored every 3 months.

**Determination of ACE2 / ADAM17/ TMPRSS2 expression**

Total RNA was extracted from PBMCs using a column-based method with Direct-zol RNA MiniPrep (Zymo Research, Irvine, CA, USA). RNA quality was assessed by the Nanodrop ((Thermo Fisher Scientific, Waltham, MA, USA) to ensure that the yield of RNA was sufficient for polymerase chain reaction (PCR) sequencing analysis. Reverse transcription was performed using 2 ug of total RNA, and real-time PCR of ACE2/ADAM17/TMPRSS2 was performed with a Fast SYBR™ Green Master Mix (Thermo Fisher Scientific) by using a CFX96 Real-Time PCR Detection System and CFX Manager Software (Bio-Rad, Hercules, CA, USA) (sTable 2)(16). Relative transcript levels were obtained with normalization to GAPDH transcript levels.

**Sample size calculation**

The study was paired sample designed to have a type I error level of 0.01 and type II error level of 0.01. We showed the minimum required number of pairs was 154 and the power was 90%. (supplementary methods)

**Statistical analysis**

A two-tailed p value <0.05 was considered statistically significant. Cox regression models with time-varying covariates accounted for their influences on risk of outcome of interesting. Time-
varying covariates took the value 0 before the start of surgery treatment and could switch to 1 at the start of treatment. Date of censoring was defined as the earliest of the date of death or cardiovascular events of study subjects during follow-up, date of follow-up termination whichever comes the earliest. We also calculated E-values to assess how strong an unmeasured confounder would be necessary to disregard an observed treatment outcome relationship (23).

Continuous variables are expressed as mean ± standard deviation or median (interquartile interval) as appropriate. A normal distribution was attained by appropriate transformations of skewed variables as PRA and ARR.

Statistical analyses were performed using Stata 14.2 MP (Stata Corporation, College Station, TX, USA) and R software, version 3.4.4 (Free Software Foundation, Inc., Boston, MA, USA).

Results
Baseline characteristics
We enrolled 168 consecutive patients of newly identified PA (56.0% women; mean 54.5 years), 40 patients of EH (52.5% women; mean 50.2 years), and 24 normotensive patients (54.2% women; mean 52 years) during the study period (Table 1). PA patients had higher BP, higher PAC, higher ARR, lower PRA and lower serum potassium concentration ([K]) than those of the EH patients. Before enrollment, more PA patients were administered with β-blocker, and fewer with ACEi than EH patients. EH patients had lower LogARR, lower systolic BP (SBP), higher [K], and higher PRA than either unilateral PA (uPA) or bilateral PA (biPA) (Figure 1). Furthermore, 38 uPA, 29 EH and 24 normotensive patients agreed to have their mRNA expressions in PBMCs evaluated (stable 3)(16). Patients of PA and EH had higher expression of ACE2 than
normotensive individuals.

Factors related to pACE2

uPA, biPA and EH patients had similar [pACE2] that were significantly higher than that of the normotensive controls (54.7± 20.8, 49.7± 21.7, 53.9± 16.6, vs. 30.7± 13.9 ng/ml, respectively; all p< 0.05; Figure 1). EH patients had similar K levels, but had higher PAC, SBP and lower PRA as well as ARR than normotensive controls (Figure 1).

Table 4 (16) summarizes the approaches used to model the relationships between [pACE2] and one or more underlying clinical and biochemical parameters. Age was negatively (β, -2.15, p= 0.033), while potassium levels (β, 2.29, p= 0.024) were positively associated with [pACE2] in the multivariate linear regression model.

A correlation matrix showed limited biomarkers directly associated (Figure 1a), and the linear correlation network depicted very few baseline characteristics that were closely correlated with pACE2 (Figure 1b, sFigure 2)(16). A generalized additive model (GAM) plot showed that plasma potassium levels were positively yet non-linearly correlated to pACE2 among patients with PA (p< 0.005) (sFigure 3)(16).

Characteristics of PA patients before and after targeted treatments

In those who underwent adrenalectomy, regardless of their complete clinical success (hypertension-remission, n= 36) or not (n= 36), their [pACE2] and PAC were significantly attenuated, while their potassium level and PRA levels were increased in comparison to their pre-operative data (Table 2, Figure 2a). The [pACE2] after adrenalectomy, in both the hypertension-remission and hypertension-uncured groups, were significantly reduced and
similar to those of normotensive controls (34.6±23.7, 36.7±19.5, vs. 30.7±13.9 ng/ml, respectively, both P> 0.05). Intriguingly, in multivariate regression modules, pACE2 were neither related to clinical hypertension-remission (p= 0.891) or biochemical remission (p= 0.555) according to the PASO criteria.

There were 96 incident patients with PA (50 uPA; 46 biPA) treated with MRA for at least one year during the study period. After MRA treatment, the PRA and potassium levels were increased, while BPs were decreased. However, the [pACE2] did not significantly change in uPA (p= 0.085) or biPA (p= 0.409) patients after MRA treatment (Table 2) (Figure 2b).

Post treatment [pACE2] associated with all-cause mortality and incident cardiovascular events

After a follow-up of 3.29±0.57 years among PA patients, 5 (3.0%) expired, 14 (8.3%) had MACE, 5 (3.0%) had Af, while 7 (4.2%) had CHF. Since the median concentration of pACE2 in healthy volunteers is 23ng/mL (22, 23), we validated if [pACE2] > 23ng/ml is a risk predictor. In the Cox proportional hazard model, [pACE2] > 23ng/ml after targeted treatments was a risk factor associated with all-cause mortality and cardiovascular events. (HR, 8.8, p=0.004).

The relative risk for all-cause mortality and cardiovascular events was 4.2, while E-value for the point estimates was 7.9. This analysis indicated no substantial unmeasured confounding.

Subgroup analysis: factors affecting [pACE2]

To delineate the [pACE2] in PA versus EH patients, we further performed subgroup analysis. The forest plot showed that the use of anti-hypertensive medications, even RAAS inhibitors, the duration of hypertension, diabetic status, the statuses of hypokalemia, larger adenomas, chronic kidney disease etc. did not confound the [pACE2] (Figure 2c).
The expression of cellular ACE2/ADAM17/TMPRSS2 among PBMCs

We showed that the expression of cellular ACE2 and ADAM17 mRNA in PBMCs was not different among uPA, EH patients and normotensive controls. However, TMPRSS2 expression was lower in the normotensive controls (0.66±0.42 mRNA folds change (FC)) than that in uPA (1.66 ±1.80 FC, p= 0.018) and EH (1.45±1.61FC, p= 0.038) patients. Of note, there was no difference in the expression of ACE2 and TMPRSS2 between PA and EH patients (p= 0.696). The chronological changes of TMPRSS2 mRNA in PBMCs decreased after adrenalectomy (n=10, 2.41± 2.52 to 1.60 ±1.69 FC, p< 0.001), but even though the cellular TMPRSS2 mRNA expression at one year in uPA patients who was treated with MRA showed a trend of decrease, there was no statistically significant change (n=10, 1.91±1.90 to 1.57±1.75 FC, p= 0.054). (Figure 3)

To further explore the interplay of ACE2/ADAM17/TMPRSS2 and RAAS, we used expression profile available at the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) (From GEO Query DataSets for GSE71994). The expression of cellular TMPRSS2 is positively correlated with that of cellular ACE2 but negatively correlated with that of ADAM17 in PBMCs. (supplementary methods, sFigure 4)(16)

Discussion

We found that PA patients, including both uPA and BiPA, had elevated [pACE2] similar to that of EH patients; their [pACE2] were higher than that of the normotensive controls. We also revealed that adrenalectomy attenuated [pACE2] in uPA patients, regardless of hypertension-remission or not; similar observation was not found in uPA or biPA patients who underwent MRA treatment. The [pACE2] were positively, yet non-linearly, correlated with the younger patients and serum potassium levels in PA patients. After targeted treatments, higher level of
[pACE2] was associated with a greater risk of long-term mortality and incident cardiovascular events. We further showed that there was higher TMPRSS2 expression in the PBMCs of uPA and EH patients than that of the normotensive population, but their ADAM17 and ACE2 mRNA expression in PBMCs were all similar. Arguably, we speculate that elevated [pACE2] and cellular TMPRSS2 expression might be associated with increased risk and severity to SARS-CoV-2 infection in PA patients due to its disease mechanism involving the binding of its spike protein to ACE2(24,25). (sFigure 5)(16) 

**High [pACE2] in PA and EH patients and its clinical relevance**

In a previous observational study, [pACE2] was positively associated with biomarkers reflecting myocardial injury and neurohormonal activation, cardiac injury, heat failure(26), stroke(27) and all-cause mortality. Importantly, [pACE2] are usually low in healthy population(28), and higher in patients with CVD,(29) which could correlate with the extent of tissue damages or CVD progression. In our patients who underwent targeted treatments, their post-treatment [pACE2] could predict their cardiovascular outcomes. High [pACE2] was correlated with makers of inflammation as well as endothelial dysfunction(30).

The association between SARS-CoV-2 and ACE2 points to the frequent involvement of hypertension during COVID-19 pathogenesis (31) and mechanisms that directly link to COVID-19 features in the lung, including inflammation, oxidative stress and fibrosis(32). A recent paper confirmed that small molecules halofuginone and homoharringtonine could block TMPRSS2 activity and lead to marked resistance to SARS-CoV-2 infection—a piece of direct evidence showing attenuating TMPRSS2 expression/activity maybe the key to prevent or treat the infection(33); our finding of diminished TMPRSS2 expression after adrenalectomy in uPA.
patients could hypothetically achieve similar benefit.

Our study found that unilateral PA patients had attenuated [pACE2] and TMPRSS2 levels after adrenalectomy, regardless of their hypertension-remission. Thus, theoretically when these uPA patients have mitigated TMPRSS2 expression and lower [pACE2] in the circulation and tissue fluid after adrenalectomy, their risks of SARS-CoV-2 infection would also be decreased.

**Factors related to [pACE2]**

Our study showed that [pACE2] had a nonlinear association with younger PA patients and those PA patients with relatively higher serum [K] is noteworthy, as it may be due to the lower potassium concentration and younger population of PA patients in nature. ACE2 was reported to decrease the RAAS activity and thus related to attenuated potassium excretion from renal collecting tubules(34). Interestingly, hypokalemia appears to be a prominent biological marker of ACE2 down-regulation in patients with Covid-19 infection(35).

**[pACE2] decreased in PA patients treated with adrenalectomy, but not with MRA**

MRAs increases ACE2 mRNA expression and activity in patients with chronic heart failure as well as in a hypertensive rat model(36-38). In heart failure patients, the ACE2 activity and ACE mRNA are increased after short-term MRA treatment(36); however, the long-term expression of ACE2, including [pACE2] changes, after MRA treatment has been lacking. Decrease in pACE2 during a weight loss diet intervention is associated with amelioration in metabolic health, fat mass, and markers of angiotensin peptide. In this study, we showed unprecedentedly that [pACE2] did not significantly change after MRA treatment in patients with uPA and biPA. In recent studies, adrenalectomy in patients with uPA was found to decrease glucocorticoid
secretion, restore osteoporosis, attenuate adverse metabolic risks and improve the quality of life (39,40), attributed to decreased glucocorticoid levels in addition to mineralocorticoid excess(41). Thus, we propose that adrenalectomy should be the treatment of choice for feasible uPA patients, and every effort should be made for early diagnosis of PA, especially uPA, in order to prevent its associated long-term morbidities, and to benefit from the high possibility of hypertension-remission and biochemical advantages like the decrease of [pACE2] in uPA patients after ipsilateral adrenalectomy.

[pACE2] and ACE2 activity

A previous study measured ACE2 catalytic activity, by way of a quenched fluorescent substitute assay, showed that it could be related to CVD(36,42). Most importantly, augmented [pACE2] is associated with adverse cardiac risks(43) and outcomes(44). However, the pathophysiological mechanisms to explain the apparent discrepancy between the negative prognostic impact of [pACE2] versus the protective effects of member-bound ACE2 remains unexplored. It is likely that there could be complex interactions between cellular expressions, enzymatic shedding, and impaired pACE2 plasma clearance and therefore changes of truncated pACE2 concentrations(8).

Subgroup analysis

ARBs have been reported to alter ACE2 expression more consistently in several studies, both at the mRNA and protein levels(45). Yet, in heart failure patients, [pACE2] was not associated with ACE inhibitor (ACEi) or ARBs use(24). In line with this, our finding suggested that PA patients who were treated with ARBs, ACEi or MRA before the PA confirmation period did not interfere
with our conclusion in parallel subgroup analyses. [pACE2] has been demonstrated to decrease in men without chronic kidney disease (CKD) and that it is independently associated with other classical CV risk factors, such as advanced age and diabetes(46). Our forest plots showed that increased [pACE2] in PA patients was independent of their comorbidities of DM, CVD or CKD.

**The expression of ACE2, ADAM17 and TMPRSS2 in PBMCs**

The decreased expression of ACE2 and TMPRSS2 in circulating PBMCs have implications for lower risk of SARS-CoV-2 infection and/or the severity of COVID-19(33,47). As pACE2 holds the binding site for SARS-CoV-2, it is possible that sequestration of SARS-CoV-2 by pACE2 may enable cell entry of the virus into tissues where membrane-bound ACE2 is poorly expressed. The attachment of the S protein of SARS-CoV-2 to ACE2 triggers ADAM17 activation, and leads to increasing membrane ACE2 down-modulation, and reducing surface ACE2 expression. The cytoplasmic tail cleavage of membranous ACE2 was achieved by synergistic action of TMPRSS2 and ADAM17(47). There were reports indicating increased expression of TMPRSS2 induced by hormones or the coexistent specific genetic variants(48,49), which may lead to exacerbation of membranous ACE2 cleavage—possibly enabling SARS-CoV-2 to enter the cells. This finding was supported by the positive correlation between TMPRSS2 and ACE2, while negative correlation between TMPRSS2 and ADAM17 from the GEO expression profile in hypertension patients from our results. As TMPRSS2 is expressed outside of the lung and can therefore contribute to the extrapulmonary spread of viruses, our results raised a suspicion that PA patients could be more likely to suffer from SARS-CoV-2 infection because of their higher level of TMPRSS2 expression.

This is further attested by the recent paper that small molecules halofuginone and homoharringtonine blocked TMPRSS2 activity and led to marked resistance to SARS-CoV-2
infection; such direct evidence of attenuating TMPRSS2 expression and lowering risks of infection could be achieved among the uPA patients who undergo ipsilateral adrenalectomy\(^\text{(33)}\). Thus, we speculated that the enriched TMPRSS2 in PBMCs is correlated with the pACE2 expression.

**Limitation**

This study has several limitations. First, while ACE2 is the main cellular port of entry for SARS-CoV-2, it is imperative to note that we have not directly measured the susceptibility for a SARS-CoV-2 infection by way of ACE2 in vitro or for a deleterious disease progression in patients of PA. Some risk factors, e.g. male gender, diabetes, were reported to be independent risks correlating to [pACE2]\(^\text{(7)}\), but it was not found in our analysis. This was partly due to specific characteristics of our patients with PA. Second, although we held all anti-hypertensive medications that could interfere with the RAAS during the screening and confirmatory stages, there are some physiological states that could also disconcert the [pACE2]. Third, [pACE2] may not necessarily be parallel to membrane-bound ACE2 expression because ACE2 shedding is mediated by disintegrin and ADAM-17 and may not be relevant to the total extent of tissue-bound ACE2 activity. The equilibrium between circulating pACE2 and membrane-bound ACE2 remains, to the best of our knowledge, incompletely understood\(^\text{(51)}\). Assays using fluorogenic substrates demonstrate that pACE2 can hydrolyze angiotensin-I and angiotensin-II analogues. The truncated plasma form of ACE2 could further indicate the production of angiotensin-(1–9) and angiotensin-(1–7), by mass spectrometry\(^\text{(52)}\). Fourth, we hypothesized that the increased expression of TMPRSS2 mRNA from PBMCs could lead to the susceptibility to SARS-CoV-2.
infection. It warrants further exploration of TMPRSS2 as a potential target for viral spread and infectivity; even recent article has shown attenuating TMPRSS2 expression by small molecules lowered the risks of SARS-CoV-2 infection. Epidemiologic studies in large cohorts of COVID-19 cases, even with patients of PA and appropriate controls, are required to confirm this hypothesis. Fifth, short follow-up time and the low number of subjects with MACE makes the study susceptible to an alpha error. However, the E-values of our analysis are greater than known relative risk, unmeasured confounding cannot explain away the ACE2 level and composite outcomes. Importantly, the power analysis showed our enrolled number of PA patients could achieve a power of 90%. Sixth, we did not acknowledge the status of somatic mutation and pACE2 level. Seventh, limited number of EH as well as normotensive were enrolled in this study. Finally, the different comorbidities in the patient’s cohorts may substantially confound our results. However, we used the paired t analysis comparing patients’ biochemistry data at baseline with their post-treatment data. We also performed multiple univariate logistic and linear regression analysis of [pACE2] to determine potential confounders. Further studies are warranted to evaluate the direct relationship and mechanism of [pACE2] and the susceptibility or severity of COVID-19 disease in PA patients.

**Perspectives**

MAS is a high affinity functional receptor for angiotensin 1-7 (Ang 1-7) (53). The question of whether MAS, like ACE2, is also differentially up-regulated in PA patients, will be an interesting subject for future studies to address. In hypertensive patients with concomitant COVID-19 infection, ACE2 levels at presentation could not be used in prognosis and mortality of COVID-19
Whether up-regulation of [pACE2] has important functional consequences in PA patients in regard to cardiovascular diseases and metabolic abnormalities remains to be determined. However, our emerging data raised the intriguing possibility that targeted therapeutic approaches to PA by way of modulating the arm of RAAS may be achieved through changes to [pACE2]. We demonstrated specific augmented [pACE2] via mononuclear TMPRSS2 in uPA and hypertensive patients. Given the detrimental role of pACE2 in SARS-CoV-2 entry and/or subsequent disease severity at the site of infection and distant cells, monitoring [pACE2] in SARS-CoV-2 comorbid patients could be crucial. Recently, viral entry through angiotensin II type 1 receptor and arginine vasopressin receptor 1B with ADAM17-mediated cleavage of ACE2 has recently been reported as a novel mechanism of SARS-CoV-2 infection(25). These pre-clinical findings suggest that patients with aldosterone-enhanced hypertension may have increased binding affinity to SARS-CoV-2 which might explain why hypertension is a risk factor for higher susceptibility to develop COVID-19. Our report showed that it could be very worthwhile to measure [pACE2] in PA patients, not only as the counter regulator of RAAS, a marker of targeted treatments, but also as an important therapeutic frontier related to cardiovascular events for PA patients.

Conclusions

Our findings suggest that pACE2 concentrations were significantly elevated in EH and various PA patients. The post-treatment persistently elevated pACE2 level > 23 ng/mL was associated with the risk of long-term mortality and incident cardiovascular events. Adrenalectomy in uPA patients, regardless of hypertension-remission, attenuates pACE2 levels; such changes are not
found in uPA or biPA patients who underwent MRA treatment. We further demonstrated the higher expression of *TMPRSS2* in EH and uPA patients compared to that of normotensive controls; such expression was attenuated after adrenalectomy in uPA patients.

Data Availability

Some or all data sets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Conflict of Interest declaration: The authors declare that they have NO affiliations with or involvement in any organization or entity with any financial interest in the subject matter or materials discussed in this manuscript.

Acknowledgements

The authors greatly appreciate the Taiwan Primary Aldosteronism Investigation (TAIPAI) Study Group study and Second Core Lab in National Taiwan University Hospital for technical assistance. This study was supported by Taiwan National Science Council [107-2314-B-002-026-MY3, 108-2314-B-002-058, 109-2314-B-002-174-MY3], National Health Research Institutes [PH-102-SP-09)], National Taiwan University Hospital [109-S4634, PC-1264, PC-1309, VN109-09, UN109-041, UN110-030] and Mrs. Hsiu-Chin Lee Kidney Research Fund.
1. Hannemann A, Wallaschofski H. Prevalence of primary aldosteronism in patient's cohorts and in population-based studies—a review of the current literature. *Horm Metab Res*. 2012;44(3):157-162.

2. Milliez P, Girerd X, Plouin PF, Blacher J, Safar ME, Mourad JJ. Evidence for an increased rate of cardiovascular events in patients with primary aldosteronism. *J Am Coll Cardiol*. 2005;48(8):1243-1248.

3. Patel VB, Zhong JC, Grant MB, Oudit GY. Role of the ACE2/Angiotensin 1-7 Axis of the Renin-Angiotensin System in Heart Failure. *Circ Res*. 2016;118(8):1313-1326.

4. Goulter AB, Goddard MJ, Allen JC, Clark KL. ACE2 gene expression is up-regulated in the human failing heart. *BMC Med*. 2004;2:19.

5. Gheblawi M, Wang K, Viveiros A, Nguyen Q, Zhong JC, Turner AJ, Raizada MK, Grant MB, Oudit GY. Response by Gheblawi et al to Letter Regarding Article, "Angiotensin-Converting Enzyme 2: SARS-CoV-2 Receptor and Regulator of the Renin-Angiotensin System: Celebrating the 20th Anniversary of the Discovery of ACE2". *Circ Res*. 2020;127(2):e46-e47.

6. Zhou X, Zhang P, Liang T, Chen Y, Liu D, Yu H. Relationship between circulating levels of angiotensin-converting enzyme 2-angiotensin-(1-7)-MAS axis and coronary heart disease. *Heart Vessels*. 2020;35(2):153-161.

7. Wallentin L, Lindback J, Eriksson N, Hijazi Z, Eikelboom JW, Ezekowitz MD, Granger CB, Lopes RD, Yusuf S, Oldgren J, Siegbahn A. Angiotensin-converting enzyme 2 (ACE2) levels in relation to risk factors for COVID-19 in two large cohorts of patients with atrial fibrillation. *European heart journal*. 2020;41(41):4037-4046.

8. Narula S, Yusuf S, Chong M, Ramasundarahettige C, Rangarajan S, Bangdiwala SI, van Eikels M, Leineweber K, Wu A, Pigeyre M, Pare G. Plasma ACE2 and risk of death or cardiometabolic diseases: a case-cohort analysis. *Lancet*. 2020;396(10256):968-976.

9. Almenglo C, Couselo-Seijas M, Agra RM, Peteiro M, Gonzalez-Juanatey JR, Eiras S, Alvarez E. Soluble angiotensin-converting enzyme levels in heart failure or acute coronary syndrome: revisiting its modulation and prognosis value. *J Mol Med (Berl)*. 2021.

10. Wang K, Gheblawi M, Oudit GY. Angiotensin Converting Enzyme 2: A Double-Edged Sword. *Circulation*. 2020;142(5):426-428.

11. Driggin E, Madhavan MV, Bikdeli B, Chuich T, Laracy J, Biondi-Zoccai G, Brown TS, Der Nigoghossian C, Zidar DA, Haythe J, Brodie D, Beckman JA, Kirtane AJ, Stone GW, Krumholz HM, Parikh SA. Cardiovascular Considerations for Patients, Health Care Workers, and Health Systems During the COVID-19 Pandemic. *J Am Coll Cardiol*. 2020;75(18):2352-2371.

12. Zumla A, Chan JF, Azhar EI, Hui DS, Yuen KY. Coronaviruses - drug discovery and therapeutic options. *Nat Rev Drug Discov*. 2016;15(5):327-347.

13. Carocchia B, Vanderriele PE, Seccia TM, Piazza M, Lenzini L, Prisco S, Torresan F, Domenig
O, Iacobone M, Poglitsch M, Rossi GP. Aldosterone and cortisol synthesis regulation by angiotensin-(1-7) and angiotensin-converting enzyme 2 in the human adrenal cortex. *J Hypertens.* 2021.

14. Wu VC, Hu YH, Er LK, Yen RF, Chang CH, Chang YL, Lu CC, Chang CC, Lin JH, Lin YH, Wang TD, Wang CY, Tu ST, Jeff Chueh SC, Chang CC, Tseng FY, Wu KD, group T. Case detection and diagnosis of primary aldosteronism - The consensus of Taiwan Society of Aldosteronism. *J Formos Med Assoc.* 2017;116(12):993-1005.

15. Chan CK, Kim JH, Chueh E, Chang CC, Lin YF, Lai TS, Huang KH, Lin YH, Wu VC. Aldosterone level after saline infusion test could predict clinical outcome in primary aldosteronism after adrenalectomy. *Surgery.* 2019;166(3):362-368.

16. Wu VC. Data from: Circulating Plasma Concentrations of ACE2 in Primary Aldosteronism and Cardiovascular Outcomes https://figshare.com/s/381c6ab9067cac50d6a5, 2022.

17. Samuel J, Jayne S, Chen Y, Majid A, Wignall A, Wormull T, Najeeb H, Luo JL, Jones GD, Macip S, Dyer MJ. Posttranscriptional Upregulation of p53 by Reactive Oxygen Species in Chronic Lymphocytic Leukemia. *Cancer Res.* 2016;76(21):6311-6319.

18. Zhang K, Meng X, Li D, Yang J, Kong J, Hao P, Guo T, Zhang M, Zhang Y, Zhang C. Angiotensin(1-7) attenuates the progression of streptozotocin-induced diabetic renal injury better than angiotensin receptor blockade. *Kidney Int.* 2015;87(2):359-369.

19. Malek V, Sharma N, Gaikwad AB. Simultaneous inhibition of neprilysin and activation of ACE2 prevented diabetic cardiomyopathy. *Pharmacol Rep.* 2019;71(5):958-967.

20. Wu VC, Wu CH, Huang TM, Wang CY, Lai CF, Shiao CC, Chang CH, Lin SL, Chen YY, Chen YM, Chu TS, Chiang WC, Wu KD, Tsai PR, Chen L, Ko WJ, Group N. Long-term risk of coronary events after AKI. *J Am Soc Nephrol.* 2014;25(3):595-605.

21. Wu VC, Wang SM, Huang KH, Tsai YC, Chan CK, Yang SY, Lin LY, Chang CC, Lu CC, Lin YH, Chen YM, Chueh JS. Long-term mortality and cardiovascular events in patients with unilateral primary aldosteronism after targeted treatments. *Eur J Endocrinol.* 2021;186(2):195-205.

22. Williams TA, Lenders JWM, Mulatero P, Burrello J, Rottenkolber M, Adolf C, Satoh F, Amar L, Quinkler M, Deinum J, Beuschlein F, Kitamoto KK, Pham U, Morimoto R, Umakoshi H, Prejibisz A, Kocjan T, Naruse M, Stowasser M, Nishikawa T, Young WF, Jr., Gomez-Sanchez CE, Funder JW, Reincze M, Primary Aldosteronism Surgery Outcome i. Outcomes after adrenalectomy for unilateral primary aldosteronism: an international consensus on outcome measures and analysis of remission rates in an international cohort. *Lancet Diabetes Endocrinol.* 2017;5(9):689-699.

23. VanderWeele TJ, Ding P. Sensitivity analysis in observational research: introducing the E-value. *Ann Intern Med.* 2017;167(4):268-274.

24. Chirinos JA, Cohen JB, Zhao L, Hanff T, Sweitzer N, Fang J, Corrales-Medina V, Anmar R, Morley M, Zamani P, Bhattacharya P, Brandimarto J, Jia Y, Basso MD, Wang Z, Ebert C, Ramirez-Valle F, Schafer PH, Seiffert D, Gordon DA, Cappola T. Clinical and Proteomic Correlates of Plasma ACE2 (Angiotensin-Converting Enzyme 2) in Human Heart Failure. *Hypertension.* 2020;76(5):1526-1536.

25. Yeung ML, Teng JLL, Jia L, Zhang C, Huang C, Cai JP, Zhou R, Chan KH, Zhao H, Zhu L, Siu KL, Fung SY, Yung S, Chan TM, To KK, Chan JF, Cai Z, Lau SKP, Chen Z, Jin DY, Woo PCY, Yuen KY. Soluble ACE2-mediated cell entry of SARS-CoV-2 via interaction with proteins.
related to the renin-angiotensin system. *Cell*. 2021;184(8):2212-2228 e2212.

26. Hisatake S, Kiuchi S, Kabuki T, Oka T, Dobashi S, Ikeda T. Serum angiotensin-converting enzyme 2 concentration and angiotensin-(1-7) concentration in patients with acute heart failure patients requiring emergency hospitalization. *Heart Vessels*. 2017;32(3):303-308.

27. Mogi M, Kawajiri M, Tsukuda K, Matsumoto S, Yamada T, Horiuchi M. Serum levels of renin-angiotensin system components in acute stroke patients. *Geriatr Gerontol Int*. 2014;14(4):793-798.

28. Uri K, Fagyas M, Kertesz A, Borbely A, Jenei C, Bene O, Csanadi Z, Paulus WJ, Edes I, Papp Z, Toth A, Lizanecz E. Circulating ACE2 activity correlates with cardiovascular disease development. *J Renin Angiotensin Aldosterone Syst*. 2016;17(4).

29. Epelman S, Tang WH, Chen SY, Van Lente F, Francis GS, Sen S. Detection of soluble angiotensin-converting enzyme 2 in heart failure: insights into the endogenous counter-regulatory pathway of the renin-angiotensin-aldosterone system. *Journal of the American College of Cardiology*. 2008;52(9):750-754.

30. Lundstrom A, Ziegler L, Havervall S, Rudberg AS, von Meijenfeldt F, Lisman T, Mackman N, Sanden P, Thalin C. Soluble angiotensin-converting enzyme 2 is transiently elevated in COVID-19 and correlates with specific inflammatory and endothelial markers. *J Med Virol*. 2021;93(10):5908-5916.

31. Teigell Munoz FJ, Garcia-Guijarro E, Garcia-Domingo P, Perez-Nieto G, Roque Rojas F, Garcia-Pena M, Nieto Gallo MA, Melero Bermejo JA, de Guzman Garcia-Monge MT, Granizo JJ. A safe protocol to identify low-risk patients with COVID-19 pneumonia for outpatient management. *Intern Emerg Med*. 2021.

32. Benigni A, Cassis P, Remuzzi G. Angiotensin II revisited: new roles in inflammation, immunology and aging. *EMBO Mol Med*. 2010;2(7):247-257.

33. Chen Y, Lear TB, Evankovich JW, Larsen MB, Lin B, Alfaras I, Kennerdell JR, Salminen L, Camarco DP, Lockwood KC, Tuncer F, Liu J, Myerburg MM, McDyer JF, Liu Y, Finkel T, Chen BB. A high-throughput screen for TMPRSS2 expression identifies FDA-approved compounds that can limit SARS-CoV-2 entry. *Nat Commun*. 2021;12(1):3907.

34. Chen D, Li X, Song Q, Hu C, Su F, Dai J, Ye Y, Huang J, Zhang X. Assessment of Hypokalemia and Clinical Characteristics in Patients With Coronavirus Disease 2019 in Wenzhou, China. *JAMA Netw Open*. 2020;3(6):e2011122.

35. Silhol F, Sarlon G, Deharo JC, Vaisse B. Downregulation of ACE2 induces overstimulation of the renin-angiotensin system in COVID-19: should we block the renin-angiotensin system? *Hypertens Res*. 2020;43(8):854-856.

36. Keidar S, Gamliel-Lazarovich A, Kaplan M, Pavlotzky E, Hamoud S, Hayek T, Karry R, Abassi Z. Mineralocorticoid receptor blocker increases angiotensin-converting enzyme 2 activity in congestive heart failure patients. *Circ Res*. 2005;97(9):946-953.

37. Yamamuro M, Yoshimura M, Nakayama M, Abe K, Sumida H, Sugiyma S, Saito Y, Nakao K, Yasue H, Ogawa H. Aldosterone, but not angiotensin II, reduces angiotensin converting enzyme 2 gene expression levels in cultured neonatal rat cardiomyocytes. *Circ J*. 2008;72(8):1346-1350.

38. Takeda Y, Zhu A, Yoneda T, Usukura M, Takata H, Yamagishi M. Effects of aldosterone and angiotensin II receptor blockade on cardiac angiotensinogen and angiotensin-
converting enzyme 2 expression in Dahl salt-sensitive hypertensive rats. *Am J Hypertens.* 2007;20(10):1119-1124.

39. Wu VC, Chang CH, Wang CY, Lin YH, Kao TW, Lin PC, Chu TS, Chang YS, Chen L, Wu KD, Chueh SJ. Risk of Fracture in Primary Aldosteronism: A Population-Based Cohort Study. *J Bone Miner Res.* 2017;32(4):743-752.

40. Wu VC, Chueh SJ, Chen L, Chang CH, Hu YH, Lin YH, Wu KD, Yang WS, Group TS. Risk of new-onset diabetes mellitus in primary aldosteronism: a population study over 5 years. *J Hypertens.* 2017;35(8):1698-1708.

41. Peng KY, Liao HW, Chan CK, Lin WC, Yang SY, Tsai YC, Huang KH, Lin YH, Chueh JS, Wu VC. Presence of Subclinical Hypercortisolism in Clinical Aldosterone-Producing Adenomas Predicts Lower Clinical Success. *Hypertension.* 2020;76(5):1537-1544.

42. Zhang Q, Cong M, Wang N, Li X, Zhang H, Zhang K, Jin M, Wu N, Qiu C, Li J. Association of angiotensin-converting enzyme 2 gene polymorphism and enzymatic activity with essential hypertension in different gender: A case-control study. *Medicine.* 2018;97(42):e12917.

43. Sama IE, Ravera A, Santema BT, van Goor H, Ter Maaten JM, Cleland JGF, Rienstra M, Friedrich AW, Samani NJ, Ng LL, Dickstein K, Lang CC, Filippatos G, Anker SD, Ponikowski P, Metra M, van Veldhuisen DJ, Voors AA. Circulating plasma concentrations of angiotensin-converting enzyme 2 in men and women with heart failure and effects of renin-angiotensin-aldosterone inhibitors. *European heart journal.* 2020;41(19):1810-1817.

44. Ramchand J, Patel SK, Kearney LG, Matalanis G, Farouque O, Srivastava PM, Burrell LM. Plasma ACE2 Activity Predicts Mortality in Aortic Stenosis and Is Associated With Severe Myocardial Fibrosis. *JACC Cardiovasc Imaging.* 2020;13(3):655-664.

45. Ferrario CM, Jessup J, Chappell MC, Averill DB, Brosnihan KB, Tallant EA, Diz DI, Gallagher PE. Effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockers on cardiac angiotensin-converting enzyme 2. *Circulation.* 2005;111(20):2605-2610.

46. Anguiano L, Riera M, Pascual J, Valdivielso JM, Barrios C, Betriu A, Mojal S, Fernandez E, Soler MJ, study N. Circulating angiotensin-converting enzyme 2 activity in patients with chronic kidney disease without previous history of cardiovascular disease. *Nephrol Dial Transplant.* 2015;30(7):1176-1185.

47. Zipeto D, Palmeira JDF, Arganaraz GA, Arganaraz ER. ACE2/ADAM17/TMPRSS2 Interplay May Be the Main Risk Factor for COVID-19. *Front Immunol.* 2020;11:576745.

48. Asselta R, Paraboschi EM, Mantovani A, Duga S. ACE2 and TMPRSS2 variants and expression as candidates to sex and country differences in COVID-19 severity in Italy. *Aging (Albany NY).* 2020;12(11):10087-10098.

49. Bunyavanich S, Grant C, Vicencio A. Racial/Ethnic Variation in Nasal Gene Expression of Transmembrane Serine Protease 2 (TMPRSS2). *JAMA.* 2020.

50. Lambert DW, Yarski M, Warner FJ, Thornhill P, Parkin ET, Smith AI, Hooper NM, Turner AJ. Tumor necrosis factor-alpha convertase (ADAM17) mediates regulated ectodomain shedding of the severe-acute respiratory syndrome-coronavirus (SARS-CoV) receptor, angiotensin-converting enzyme-2 (ACE2). *The Journal of biological chemistry.* 2005;280(34):30113-30119.
51. Wang K, Basu R, Poglitsch M, Bakal JA, Oudit GY. Elevated Angiotensin 1-7/Angiotensin II Ratio Predicts Favorable Outcomes in Patients With Heart Failure. *Circ Heart Fail*. 2020;13(7):e006939.

52. Tipnis SR, Hooper NM, Hyde R, Karran E, Christie G, Turner AJ. A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. *The Journal of biological chemistry*. 2000;275(43):33238-33243.

53. Santos RA, Simoes e Silva AC, Maric C, Silva DM, Machado RP, de Buhr I, Heringer-Walther S, Pinheiro SV, Lopes MT, Bader M, Mendes EP, Lemos VS, Campagnole-Santos MJ, Schultheiss HP, Speth R, Walther T. Angiotensin-(1-7) is an endogenous ligand for the G protein-coupled receptor Mas. *Proc Natl Acad Sci U S A*. 2003;100(14):8258-8263.

54. Biberoglu S, Ipekci A, Ikizceli I, Cakmak F, Akdeniz YS, Kanbakan A, Konukoglu D, Bolayirli IM, Borekci S, Urkmez S, Ozkan S. Role of plasma angiotensin II and angiotensin-converting enzyme 2 levels on prognosis and mortality in hypertensive patients with COVID-19. *Biomark Med*. 2021;15(17):1581-1588.


Figure legends

Figure 1. (A) The correlation matrix among the levels of various biomarkers. The correlations between the blood pressure, body mass index (BMI) and levels of aldosterone profiles at index enrollment when holding drugs that interfere RAAS were examined. On the contents of the diagonal are the value (logit) of the correlation. Blue color depicted positive correlation; while red color depicted negative correlation.

(B) Correlation network visualized the correlations of pACE2 versus other biochemical and baseline characteristics. pACE-2 was not correlated with other clinical parameters and did not cluster with previously recognized factors by Spearman correlation. Each path represented a correlation between the two variables that it joined. An orange path represented a positive correlation, and a blue path corresponded to a negative correlation. * The R function network_plot() was used to visualize and explore correlations (r). The width and transparency of the path represent the strength of the correlation (wider and less transparent indicated stronger correlation).

Abbreviations: ACE, angiotensin-converting enzyme; ARR, aldosterone to renin ratio; b, before confirmation test; BiPA, bilateral primary aldosteronism; BMI, body mass index; b, before confirmation test; Cre, creatinine; dBP, diastolic blood pressure; EH, essential hypertension, K, potassium; PAC, plasma aldosterone concentration; p, post-confirmation test; PRA, plasma renin activity; sBP, systolic blood pressure; uPA, unilateral primary aldosteronism.

(C). The differences of baseline biochemistry data in patients with PA, essential hypertension, when compared with normotensive controls. Violin plots showed the difference of plasma ACE2 levels, plasma aldosterone concentration, plasma renin activity, aldosterone/ratio, serum potassium concentrations and systemic blood pressure.

Abbreviations: ACE, angiotensin-converting enzyme; ARR, aldosterone to renin ratio; BiPA, bilateral primary aldosteronism; EH, essential hypertension, K, potassium; PAC, plasma aldosterone concentration; N, normotensive population; PRA, plasma renin activity; uPA, unilateral primary aldosteronism.

¶ the analysis consisted independent t-tests with normotensive population.

§ Log transformation was applied for skewed distributions, such as ARR and PRA.

*, p<0.05
Figure 2

Violin plots showed the differences of pACE2 levels of
(A) in patients with essential hypertension (EH), uPA patients at index date, who underwent
MRA therapy for 1 year, who had complete clinical remission after adrenalectomy, who were
uncured at 1 year after adrenalectomy, and normotensive controls‡.
(B) biPA patients with essential hypertension, biPA at index date, who underwent MRA therapy
after 1 year, and normotensive controls†.
¶ compared with uPA patients who received MRA for at least one year.
§ compared with uPA patients who were clinical remission after adrenalectomy for one year.
‡ compared with uncured uPA patients after adrenalectomy for one year.
++. compared with biPA patients received MRA for at least one year.
† using unpaired t test.

Abbreviations: pACE, plasma angiotensin-converting enzyme; APA, aldosterone producing
adenoma, BiPA, bilateral PA, EH, essential hypertension, K, potassium; MRA, minorcorticoid
receptor antagonist; PAC, plasma aldosterone concentration; PRA, plasma renin activity; uPA, unilateral PA.

(C). Forest plot depicts odds ratio (OR) and 95% confidence interval (CI) derived from
multivariate logistic regression analyzing the risk of increased pACE2 expression compared
with essential hypertension for multiple clinical variables¶.

Abbreviations: ACE, angiotensin-converting enzyme, ACEi, angiotensin-converting enzyme
inhibitor, APA, aldosterone-producing adenoma; ARB, Angiotensin Receptor Blocker; HTN,
hypertension; eGFR, estimated Glomerular filtration rate; OR, odds ratio.
¶ OR was adjusted with age, gender, body mass index, blood pressure, plasma aldosterone
concentration, plasma renin activity.

Figure 3. Plots depict (A) Quantity expression of the mRNA folds between unilateral PA,
essential hypertension and normotensive patients (B) the temporal fold change of the mRNA
before, post operation and MRA treatment in unilateral PA by using a quantitative real-time
polymerase chain reaction with gene-specific primers in the complementary DNA synthesis.

Abbreviations, ACE2, angiotensin-converting enzyme 2; ADAM17, Tumor Necrosis Factor-α
Convertase; APA, aldosterone producing adenoma; EH, essential hypertension, N,
normotensive controls; OP, operation; TMPRSS2, type II transmembrane serine protease.
*, p<0.05
Tables for Circulating Plasma Concentrations of ACE2/ TMPRSS2/ ADAM17 in Primary Aldosteronism and Cardiovascular Outcomes by Wu et al.

**Table 1.** Baseline characteristics of patients with essential hypertension and primary aldosteronism.

| Characteristics                        | EH     | PA     | Normotension | p(EH vs PA) | p(PA vs Nor) |
|----------------------------------------|--------|--------|--------------|-------------|--------------|
| Case numbers, n (%)                    | 40     | 168    | 24           |             |              |
| Age (yr)                               | 50.15±13.49 | 54.48±11.05 | 52.0±11.4   | 0.122       | 0.313        |
| Female, n (%)                          | 21 (52.5%) | 94 (56.0%) | 13 (54.2%)  | 0.665       | 0.845        |
| Body mass index (kg/m²)                | 24.72 [22.25-27.64] | 24.82 [22.06-27.56] | 23.5 [20.9-25.7] | 0.88       | <0.001       |
| Smoker, n (%)                          | 4 (10.0%) | 11 (6.5%) | 2 (9.1%)     | 0.48        | 0.635        |
| Duration of hypertension (yr) (log)    | 0.70 [0 - 1] | 0.78 [0.3 - 1.18] | NA          | 0.031       | NA           |
| Systolic blood pressure (mmHg)         | 143.12±20.60 | 152.51±20.59 | 117.1±12.4  | 0.01        | <0.001       |
| Diastolic blood pressure (mmHg)        | 86.53±15.01 | 91.36±13.34 | 71.0±10.4   | 0.046       | <0.001       |
| Diabetes mellitus, n (%)               | 5 (12.5%) | 33 (19.76%) | NA          | 0.287       | NA           |
| Creatinine (mg/dL)                     | 0.8 [0.75 - 0.9] | 0.8 [0.7 - 1] | 0.7 [0.6 - 0.9] | 0.595       | 0.011        |
| Serum potassium (mEq/L)                | 4.07±0.35 | 3.73±0.62 | 4.2±0.4     | <0.001      | <0.001       |
| Plasma ACE2 level (ng/ml)              | 54.7±20.8 | 53.9±16.6 | 30.7±13.9   | 0.937       | 0.007        |
| Before confirmation†                   |        |        |              |             |              |
| α-Blocker user                         | 8 (20.0%) | 34 (20.2%) | NA          | 0.96        | NA           |
| β-Blocker user                         | 8 (20.0%) | 63 (37.5%) | NA          | 0.04        | NA           |
| ARB user                               | 19 (47.5%) | 97 (57.7%) | NA          | 0.226       | NA           |
| ACEi user                              | 10 (25.0%) | 8 (4.8%)  | NA          | <0.001      | NA           |
| Plasma aldosterone concentration (ng/dL)| 34.75 [20.44 - 47.67] | 43.95 [30.32 - 60.44] | 13.5 [11.8 - 16.4] | 0.036      | <0.001       |
| Plasma renin activity (ng/mL/hr)       | 2.2 [0.2 - 6.3] | 0.2 [0.1 - 0.6] | 1.12 [0.58 - 1.34] | <0.001      | <0.001       |
| Log ARR§                                | 1.47±0.84 | 2.32±0.69 | 1.71±0.43   | <0.001      | <0.001       |
Data are presented as the mean [standard deviation] for normally distributed data and median [interquartile range] for non-normally distributed data.

**Abbreviations:** ACE, angiotensin-converting enzyme, ACEi, angiotensin-converting enzyme inhibitor, APA, aldosterone-producing adenoma; ARB, Angiotensin Receptor Blocker; ARR, aldosterone to renin ratio; EH, essential hypertension; PA, primary hyperaldosteronism; yr, year.

† All anti-hypertensive medications that will interfere the RAAS were discontinued before PA confirmation tests.

§ Log transformation was applied for skewed distributions, such as ARR.
Table 2. Baseline clinical and biochemical characteristics of PA patients after targeted treatment.

| Disease Type                  | uPA                  |                  |                       | uPA                  |                  |                       | biPA                 |                  |                       |
|-------------------------------|----------------------|------------------|-----------------------|----------------------|------------------|-----------------------|----------------------|------------------|-----------------------|
|                               | Pre-OP               | Post-OP          | p                     | Pre-OP               | Post-OP          | p                     | Pre-MRA             | Post-MRA         | p                     |
| Biochemistry and clinical     |                      |                  |                       |                      |                  |                       |                      |                  |                       |
| data†                         |                      |                  |                       |                      |                  |                       |                      |                  |                       |
| Case number, n                | 36                   | 36               |                       | 50                   | 46               |                       |
| Serum potassium (mmol/L)      | 3.8±0.7              | 4.4±0.3          | <0.001                | 3.8±0.6              | 4.2±0.4          | <0.001                | 3.62±0.51           | 4.22±0.45        | <0.001                |
| Plasma aldosterone level (ng/dL) | 55.7±43.4           | 31.1±19.7        | 0.007                 | 44.9±23.7           | 28.0±22.2        | 0.012                 | 56.6±32.6           | 63.4±37.7         | 0.293                |
| Plasma renin activity (ng/mL/hr) | 0.21±0.19           | 3.37±2.41        | <0.001                | 0.79±0.42           | 3.44±0.72        | 0.001                 | 0.55±1.01           | 1.72±1.97         | <0.001                |
| Systolic blood pressure (mmHg) | 139.8±16.8           | 121.7±11.7       | <0.001                | 164.1±19.5          | 142.2±19.2       | <0.001                | 153.8±223.6         | 146.0±23.0        | 0.025                |
| Diastolic blood pressure (mmHg) | 84.1±11.8           | 77.1±8.2         | <0.001                | 98.0±13.4           | 87.0±14.0        | <0.001                | 90.2±15.8           | 86.9±13.6         | 0.231                |
| Plasma ACE2 (ng/ml)           | 56.8±24.6            | 31.2±14.4        | <0.001                | 52.5±15.9           | 36.7±19.5        | 0.006                 | 45.7±22.8           | 39.9±27.7         | 0.085                |

Abbreviations: ACE, angiotensin-converting enzyme, OP, operation, MRA, mineralocorticoid receptor antagonist.
¶the analysis consisted of paired t-tests.
† Data after holding the medications that will interfere the renin-angiotensin-aldosterone system.
Figure 3

(A) mRNA fold change for ACE2, TMPRSS2, and ADAM17 under different conditions: BH, uPA, and N.

(B) mRNA fold change for ACE2 and TMPRSS2 under different conditions: preOP, postOP, preMRA, and postMRA.

* Indicates significant difference.