DIAGNOSIS AND TREATMENT OF PRIMARY ALDOSTERONISM: FROM CLINICAL ORIGIN TO TRANSLATIONAL RESEARCH

EDITED BY: Qiang Wei and Vin-Cent Wu
PUBLISHED IN: Frontiers in Endocrinology

frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88974-094-9
DOI 10.3389/978-2-88974-094-9

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: frontiersin.org/about/contact

DIAGNOSIS AND TREATMENT OF PRIMARY ALDOSTERONISM: FROM CLINICAL ORIGIN TO TRANSLATIONAL RESEARCH

Topic Editors:
Qiang Wei, Sichuan University, China
Vin-Cent Wu, National Taiwan University, Taiwan

Citation: Wei, Q., Wu, V.-C., eds. (2022). Diagnosis and Treatment of Primary Aldosteronism: From Clinical Origin to Translational Research. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88974-094-9

Table of Contents

frontiers in Endocrinology

EDITORIAL
published: 30 November 2021
doi: 10.3389/fendo.2021.781105

Editorial: Diagnosis and Treatment of Primary Aldosteronism: from Clinical Origin to Translational Research

Wan-Chen Wu[1], Qiang Wei[2] and Vin-Cent Wu[3] on behalf of TAIPAI, Taiwan Primary Aldosteronism Investigation (TAIPAI) Study Group*

[1] Division of Endocrinology and Metabolism, Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan, [2] Department of Urology, Institute of Urology, West China Hospital, Sichuan University, Chengdu, China, [3] Department of Internal Medicine, National Taiwan University Hospital, Taipei City, Taiwan

Keywords: primary aldosteronism, adrenal venous sampling, MRA, APCCs, CYP11B2

Editorial on the Research Topic

Diagnosis and Treatment of Primary Aldosteronism: from Clinical Origin to Translational Research

Primary aldosteronism (PA) is one of the common causes of secondary hypertension, increasing the risk of cardiovascular disease and renal events as compared to essential hypertension, independently of blood pressure control (1–3).

Primary aldosteronism (PA) is one of the common causes of secondary hypertension, and is associated with higher risks of cardiovascular, renal, and metabolic sequelae, including left ventricular hypertrophy, myocardial infarction, atrial fibrillation, stroke, microalbuminuria, osteoporosis, as well as metabolic syndrome (1, 4–7).

The present Research Topic highlights the interplay between clinical diagnosis, underlying genetic etiologies, and clinical outcome PA. Overall, it focuses on the histopathologic findings, gene mutation, the coexistence of cortisol, cosecretion, and targeted treatments of PA.

Nanba et al. provide a template for researchers to study aldosterone-producing adrenal using formalin-fixed paraffin-material embedded sections for DNA capture, sequencing, and mutation determination.

Although cortisol cosecretion in aldosterone-producing adenoma (APA) has been reported (8), the clinical relevance of such APA coexisting with cortisol-producing adenoma has not been illustrated (8). Inoue et al. summarize the current state of knowledge about cortisol cosecretion with PA. They conclude, there is increasing evidence about the relatively high prevalence of cortisol cosecretion in PA and its potential influence on adverse health outcomes.

Adrenal venous sampling (AVS) is the test of choice to identify patients with a surgically curable subtype of PA (9). Okamoto et al. assess 1586 PA patients without apparent adrenal tumors in the multicenter study and conclude adrenal venous sampling should be considered for male hypokalemic PA patients with high ARRs because of the rates of the lateralized subtype and cardiovascular events are high in these patients.

The major advance of understanding PA pathophysiology is the identification of several somatic driver mutations in ion channels and ATPases in lateralized aldosterone-producing adenoma (APA) (10). Utilizing the immunohistochemical (IHC) detection of aldosterone synthase (CYP11B2) has allowed the identification of aldosterone-producing cell clusters (APCCs) with unique focal localization positive for CYP11B2 expression in the subcapsular portion of the human adult adrenal cortex. Pauzi and Azizan reviewed APCCs associated aldosterone-stimulating somatic gene

OPEN ACCESS

Edited and reviewed by:
Andre Lacroix, Université de Montréal, Canada

****Correspondence:***
Vin-Cent Wu
q91421028@ntu.edu.tw

Specialty section:
This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Endocrinology

Received: *22 September 2021*
Accepted: *26 October 2021*
Published: *30 November 2021*

Citation:
Wu W-C, Wei Q and Wu V-C (2021) Editorial: Diagnosis and Treatment of Primary Aldosteronism: from Clinical Origin to Translational Research. Front. Endocrinol. 12:781105. doi: 10.3389/fendo.2021.781105

mutations (recently replaced by aldosterone-producing micronodules) and their accumulation during the aging process, raising the possibility that APCCs may play a role in the development of PA and age-related hypertension.

Recent studies indicate that somatic mutations of the potassium channel *KCNJ5* gene could be identified in 34 to 73% of APAs (11). Wang H. et al. find that gender, duration of hypertension, and the highest systolic blood pressure were independent predictors for the postoperative cure of APA identified based on HISTALDO histopathologic groups.

Lu et al. demonstrated that NP-59 adrenal scintigraphy could predict *KCNJ5* mutations in PA patients by two semiqualitative parameters [adrenal to liver ratio (ALR) and lesion to the contralateral ratio of bilateral adrenal glands (CON)] and provided more information in an individualized treatment plan.

In regarding possible variations in response to hormonal stimuli, APAs with ATPase-mutations are more responsive to ACTH than KCNJ5-mutated APAs was found in Lim et al. study.

PA patients also have a higher risk of cardiovascular diseases and greater cardiac remodeling compared to those with essential hypertension (7, 12). Pan et al. demonstrate extensive cardiac remodeling in APA patients through hemodynamic and non-hemodynamic causes. Adrenalectomy improved both hemodynamic and non-hemodynamic components of left ventricular remodeling, which also correlated with decreases in blood pressure and ARR.

Zhou et al. report the severity of diastolic dysfunction independently relates to the degree of diffuse myocardial fibrosis in PA patients with elevated aldosterone level.

The excess aldosterone causes atrial structural and electrical remodeling, which induce atrial fibrillation genesis, and PA was associated with a higher incidence of new-onset atrial fibrillation (NOAF) that could ameliorate after adrenalectomy (13). Tsai et al. performed a meta-analysis and showed different effects of PA treatment on NOAF risk. The PA patients receiving MRA treatment had a higher risk of NOAF compared to the PA patients receiving adrenalectomy and the patients with essential hypertension.

Chen et al. provide new insights into the relationship between adipose tissue and aldosterone excess in patients with APA and idiopathic hyperaldosteronism (IHA). Abdominal adiposity indexes were similar in patients with IHA and those with essential hypertension but were markedly lower in patients with APA. Aldosterone-to-renin ratio (ARR) was negatively correlated with abdominal adiposity indexes in patients with APA but not in patients with IHA.

The endocrine-gut interaction show PA patients had fewer short-chain fatty acids-producing genera and more inflammation-associated genera than healthy controls. Alteration of gut microbiota may contribute to the obesity and diabetics status in PA, as shown by Liu et al.

Some studies showed similar long-term cardiac effects of surgical or medical treatment in PA patients (14). However, other studies showed a lower incidence of adverse cardiovascular outcomes in PA patients treated by adrenalectomy (15). Huang et al. show superior performance of surgical treatment over medical treatment for patients with lateralized PA on both composite and individual clinical cardiovascular outcomes in this meta-analysis. An interesting study presented by Tezuka and Turcu on targeted treatment, they identified that lower baseline serum potassium, lower mineralocorticoid receptor antagonist (MRA) doses, and beta-blocker use was independently associated with lower odds of achieving target renin in PA. Their findings suggest that renin targets, when PRA was <1.0 ng/mL/h and DRC was <8.0 pg/mL, are followed in very few and are achieved in under half of such PA patients seen in an academic setting, with possibly even lower rates in community practices.

In the future, Wang C. et al. conclude international collaboration on the clinical and molecular mechanism of PA will be important to improve the investigation and therapy of patients with PA.

Highlighting the novel insights into the possible mechanism and outcome of PA, all the studies in this special issue fill some gaps of knowledge and give future challenges for research in this field.

AUTHOR CONTRIBUTIONS

W-CW wrote the draft of the article. V-CW and QW supervised the results. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by MOST 107-2314-B-002-026-MY3, 108-2314-B-002-058, 109-2314-B-002-174-MY3], National Taiwan University Hospital [109-S4634, PC-1264, PC-1309, VN109-09, UN109-041, UN110-030] Grant MOHW110-TDU-B-212-124005 and Mrs. Hsiu-Chin Lee Kidney Research Fund. The NGS experiments were performed by the NGS Core Laboratory, Molecular Medicine Research Center, Chang Gung University, Taiwan (grant CLRPD1J0012).

ACKNOWLEDGMENTS

The authors appreciate the Second Core Lab in National Taiwan University Hospital for technical assistance.

REFERENCES

1. Mulatero P, Monticone S, Bertello C, Viola A, Tizzani D, Iannaccone A, et al. Long-Term Cardio- and Cerebrovascular Events in Patients With Primary Aldosteronism. *J Clin Endocrinol Metab* (2013) 98(12):4826–33. doi: 10.1210/jc.2013-2805
2. Wu VC, Yang SY, Lin JW, Cheng BW, Kuo CC, Tsai CT, et al. Kidney Impairment in Primary Aldosteronism. *Clin Chim Acta* (2011) 412(15-16):1319–25. doi: 10.1016/j.cca.2011.02.018
3. Wu VC, Kuo CC, Wang SM, Liu KL, Huang KH, Lin YH, et al. Primary Aldosteronism: Changes in Cystatin C-Based Kidney Filtration, Proteinuria,

and Renal Duplex Indices With Treatment. *J Hypertens* (2011) 29(9):1778–86. doi: 10.1097/HJH.0b013e3283495cbb
4. Tsai CH, Pan CT, Chang YY, Chen ZW, Wu VC, Hung CS, et al. Left Ventricular Remodeling and Dysfunction in Primary Aldosteronism. *J Hum Hypertens* (2021) 35(2):131–47. doi: 10.1038/s41371-020-00426-y
5. Wang WJ, Chao CT, Huang YC, Wang CY, Chang CH, Huang TM, et al. The Impact of Acute Kidney Injury With Temporary Dialysis on the Risk of Fracture. *J Bone Miner Res* (2014) 29(3):676–84. doi: 10.1002/jbmr.2061
6. Chen YY, Lin YH, Huang WC, Chueh E, Chen L, Yang SY, et al. Adrenalectomy Improves the Long-Term Risk of End-Stage Renal Disease and Mortality of Primary Aldosteronism. *J Endocr Soc* (2019) 3(6):1110–26. doi: 10.1210/js.2019-00019
7. Huang WC, Chen YY, Lin YH, Chen L, Lin PC, Lin YF, et al. Incidental Congestive Heart Failure in Patients With Aldosterone-Producing Adenomas. *J Am Heart Assoc* (2019) 8(24):e012410. doi: 10.1161/JAHA.119.012410
8. Peng KY, Liao HW, Chan CK, Lin WC, Yang SY, Tsai YC, et al. Presence of Subclinical Hypercortisolism in Clinical Aldosterone-Producing Adenomas Predicts Lower Clinical Success. *Hypertension* (2020) 76(5):1537–44. doi: 10.1161/HYPERTENSIONAHA.120.15328
9. Chang CC, Lee BC, Liu KL, Chang YC, Wu VC, Huang KH, et al. The Influence of the Peripheral Cortisol Fluctuation on the Success Rate of Adrenal Venous Sampling. *Sci Rep* (2018) 8(1):2664. doi: 10.1038/s41598-018-20647-z
10. Wu VC, Wang SM, Chueh SJ, Yang SY, Huang KH, Lin YH, et al. The Prevalence of CTNNB1 Mutations in Primary Aldosteronism and Consequences for Clinical Outcomes. *Sci Rep* (2017) 7:39121. doi: 10.1038/srep39121
11. Peng KY, Liao HW, Chueh JS, Pan CY, Lin YH, Chen YM, et al. Pathophysiological and Pharmacological Characteristics of KCNJ5 157-159delite Somatic Mutation in Aldosterone-Producing Adenomas. *Biomedicines* (2021) 9(8):1026–39. doi: 10.3390/biomedicines9081026
12. Hung CS, Sung SH, Liao CW, Pan CT, Chang CC, Chen ZW, et al. Aldosterone Induces Vascular Damage. *Hypertension* (2019) 74(3):623–9. doi: 10.1161/HYPERTENSIONAHA.118.12342
13. Pan CT, Liao CW, Tsai CH, Chen ZW, Chen L, Hung CS, et al. Influence of Different Treatment Strategies on New-Onset Atrial Fibrillation Among Patients With Primary Aldosteronism: A Nationwide Longitudinal Cohort-Based Study. *J Am Heart Assoc* (2020) 9(5):e013699. doi: 10.1161/JAHA.119.013699
14. Catena C, Colussi G, Lapenna R, Nadalini E, Chiuch A, Gianfagna P, et al. Long-Term Cardiac Effects of Adrenalectomy or Mineralocorticoid Antagonists in Patients With Primary Aldosteronism. *Hypertension* (2007) 50(5):911–8. doi: 10.1161/HYPERTENSIONAHA.107.095448
15. Wu VC, Wang SM, Chang CH, Hu YH, Lin LY, Lin YH, et al. Long Term Outcome of Aldosteronism After Target Treatments. *Sci Rep* (2016) 6:32103. doi: 10.1038/srep32103

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Endocrinology

REVIEW
published: 03 March 2021
doi: 10.3389/fendo.2021.631848

Functional Characteristic and Significance of Aldosterone-Producing Cell Clusters in Primary Aldosteronism and Age-Related Hypertension

Fatin Athirah Pauzi and Elena Aisha Azizan *

Department of Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Cheras, Malaysia

Primary aldosteronism (PA) is one of the most frequent curable forms of secondary hypertension. It can be caused by the overproduction of aldosterone in one or both adrenal glands. The most common subtypes of PA are unilateral aldosterone overproduction due to aldosterone-producing adenomas (APA) or bilateral aldosterone overproduction due to bilateral hyperaldosteronism (BHA). Utilizing the immunohistochemical (IHC) detection of aldosterone synthase (CYP11B2) has allowed the identification of aldosterone-producing cell clusters (APCCs) with unique focal localization positive for CYP11B2 expression in the subcapsular portion of the human adult adrenal cortex. The presence of CYP11B2 supports that synthesis of aldosterone can occur in these cell clusters and therefore might contribute to hyperaldosteronism. However, the significance of the steroidogenic properties of APCCs especially in regards to PA remains unclear. Herein, we review the available evidence on the presence of APCCs in normal adrenals and adrenal tissues adjacent to APAs, their aldosterone-stimulating somatic gene mutations, and their accumulation during the ageing process; raising the possibility that APCCs may play a role in the development of PA and age-related hypertension.

Keywords: primary aldosteronism, aldosterone, adrenal, aldosterone-producing cell clusters, hypertension

OPEN ACCESS

Edited by:
Vin-Cent Wu,
National Taiwan University, Taiwan

Reviewed by:
Silvia Monticone,
University of Turin, Italy
Tetsuo Nishikawa,
Yokohama Rosai Hospital, Japan
Celso E. Gomez-Sanchez,
University of Mississippi Medical Center School of Dentistry, United States

***Correspondence:**
Elena Aisha Azizan
elena.azizan@ukm.edu.my

Specialty section:
This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Endocrinology

Received: *23 November 2020*
Accepted: *01 February 2021*
Published: *03 March 2021*

Citation:
Pauzi FA and Azizan EA (2021) Functional Characteristic and Significance of Aldosterone-Producing Cell Clusters in Primary Aldosteronism and Age-Related Hypertension. Front. Endocrinol. 12:631848. doi: 10.3389/fendo.2021.631848

INTRODUCTION

Arterial hypertension is a worldwide health problem that affects approximately 1.13 billion of the global population (1) and is estimated to cause 7.5 million deaths or approximately 12.8% of the total all deaths (2). In more than 90% of patients, there is not a single cause for arterial hypertension, known as essential or primary hypertension (3). However, in certain cases, hypertension may emerge from a specific disease and therefore known as secondary hypertension, which includes endocrine hypertension that develops following a dysregulation of one or more hormones that are implicated in blood pressure regulation (4). Primary aldosteronism (PA), also known as Conn's syndrome, is one of the most frequent curable forms of secondary/endocrine hypertension (5). This syndrome accounts for 7–10% of all referred hypertensive patients (6–8), 4% in primary care (9), and 15–20% of patients with resistant hypertension (10, 11). PA is caused by the autonomous aldosterone production in one

or both adrenal glands, in which patients with PA are clinically associated with high levels of aldosterone despite being under suppressed renin conditions (12).

Under physiological conditions, the two primary systems that regulate aldosterone production are the renin-angiotensin-aldosterone system (RAAS) and the hypothalamic-pituitary-adrenal axis, in addition to blood potassium levels (13, 14). However, in PA, excess aldosterone independent of the RAAS acts on the distal tubule and medullary collecting duct to increase sodium reabsorption as well as potassium excretion (13). Water reabsorption follows salt, causing volume expansion that develops into hypertension and in some cases hypokalemic metabolic alkalosis (14). Hence, clinical presentation for hypokalemia, though not specific for PA, may also present as cramping and muscle weakness, palpitations, polydipsia, and polyuria among others (13). Furthermore, chronic inappropriate elevations of aldosterone levels in PA patients can increase pro-inflammatory cytokines (15), causing oxidative stress (16), which in the long-term leads to tissue damage and fibrosis (17). Compared to the age-, sex-, and blood pressure-matched essential hypertension patients, the risk for cardiovascular complications is significantly higher in PA patients (18, 19). This suggests the impact of autonomous aldosterone production on cardiovascular complications is beyond the increase in blood pressure (20–22).

Due to the high prevalence of PA among hypertensive patients and the increased risk for cardiovascular complications, early diagnosis as well as targeted treatment is of importance (12). The biochemical picture of patients with PA comprises of high aldosterone to renin ratio which has become one of the primary PA's screening tools alongside different confirmatory tests, that includes fludrocortisone suppression test, intravenous saline infusion test, oral salt-loading test, or captopril test, which are all to be performed after a positive screening test (i.e. high aldosterone to renin ratio) (13, 18). The current guidelines recommend that when aldosterone is overproduced unilaterally, as detected by adrenal vein sampling (AVS), or in some exceptions when CT-scan detects a unilateral adenoma in a young hypokalemic PA patient (<35 years old), unilateral adrenalectomy is the treatment of choice. Conversely, mineralocorticoid receptor antagonists are utilized when AVS implies bilateral hyperaldosteronism or when the patient is not suitable for surgery (18). As identifying the most suitable therapy for PA is heavily influenced by the laterality of aldosterone overproduction, AVS, the gold standard to determine unilateralization (13), should be used where possible.

The majority of unilateral PA is due to an aldosterone-producing adenoma (APA), whereas bilateral PA, also known as bilateral hyperaldosteronism (BHA), are mostly due to adrenal zona glomerulosa hyperplasia or bilateral micronodular hyperplasia (23, 24). Bilateral PA can be further categorized into CT-negative idiopathic hyperaldosteronism (IHA) and rarely CT-positive bilateral APA (25–27). Approximately, APA and BHA account for 35%–40% and 60% of PA cases, respectively (28). In addition, there are less common subtypes of PA that include adrenal carcinoma, rare familial subtypes, and unilateral adrenal hyperplasia (29). It is postulated that bilateral PA could be caused by the accumulation of aldosterone-producing cell clusters (APCCs) (23), i.e. clusters of cells autonomously expressing aldosterone synthase (CYP11B2). Interestingly, these APCCs are not only found in the cells adjacent to an APA of which excised tissue is available (24, 30–33) but also in normal adrenal glands (30, 34, 35), with the prevalence seeming to increase with age (34–36). Hence, it is of importance to understand whether these clusters could contribute to the state of hyperaldosteronism or affect the increased prevalence of hypertension occurring with age.

CHARACTERISTICS OF ALDOSTERONE-PRODUCING CELL CLUSTERS (APCCs)

Physiologically, aldosterone synthesis occurs in the most outer zone of the adrenal, the zona glomerulosa (ZG) (23). This is because the cells in this zone can express CYP11B2, the enzyme aldosterone synthase, which is essential for the final steps of aldosterone synthesis. The synthesis of aldosterone is primarily stimulated by the activation of intracellular calcium signaling in the ZG that can be mediated either by angiotensin II from the renin-angiotensin system or by extracellular potassium levels. Similar to aldosterone, adrenal cortisol steroid production physiologically occurs in distinct zones of the adrenal cortex, the zona fasciculata (ZF), due to the expression of 11β-hydroxylase (CYP11B1), the enzyme responsible for the final reaction in cortisol production. The synthesis of cortisol is primarily regulated by the hypothalamus-pituitary-adrenal axis mainly through the adrenocorticotropic hormone (ACTH) (4). Hence, in conventional adrenal zonation, CYP11B2 is only expressed in the ZG but not in ZF, whereas CYP11B1 is expressed in the ZF but not in the ZG (30, 37–40). However, immunohistochemical characterization of the adrenal gland by Nishimoto et al. (30) has led to the novel discovery of microscopic clusters of subcapsular adrenal cells that express CYP11B2 that extends from the ZG into the ZF which have been termed as APCCs. However, as the term "cell cluster" in APCCs does not describe the histology and pathology of the feature a recent consensus on histopathology of PA adrenals recommends the usage of the term "aldosterone-producing micronodule" instead of APCCs (41).

Nevertheless, whether termed APCCs or aldosterone-producing micronodule, the definition of this feature varies between research groups and a clear description that can distinguish this unique structure from APA and adjacent normal ZG cells has yet to be established. The size of APCCs has been reported to be smaller, approximately 0.2–1.5 mm in length, than APAs that are typically more than 3 mm in length (30, 36, 42). Further, APCCs have been characterized as having non-adenomatous features unlike that seen frequently with APA, e.g. not having a fibrous capsule or having cellular/tissue atypia (12). APCCs have also been described to be composed of morphological ZG cells in contact with the capsule and inner

columnar ZF like cells forming cords along sinusoids (30). The current common definition for APCCs is to have positive CYP11B2 expression in a unique focal localization (30). As the morphology of APCC cells appears to be remarkably close to those of non-APCCs (33, 35), they cannot be easily distinguished from adjacent normal cortical cells by routine hematoxylin and eosin staining (30, 36, 42). Instead, immunohistochemistry (IHC) of CYP11B2 has been the method of choice to identify the presence of APCCs (30, 43).

IHC analysis has shown that compared to ZF cells that are generally negative for CYP11B2 and unrelated to aldosterone production, ZF-like looking cells of APCCs in the ZF has high levels of CYP11B2 and low levels of CYP11B1 or CYP17A1, both enzymes being involved in cortisol production (33, 35, 37, 38). However, according to Lim & Rainey (44), APCCs may manifest various patterns of enzyme expression with some exhibiting homogeneous expression of CYP11B2 while others exhibit polarity with a declining expression of CYP11B2 aligned with the development of CYP17A1 expression. This pattern of expression indicates that some APCCs cells may go through a transition to a more ZF-like phenotype, at least partially (44), thus making CYP11B1 and CYP17A1 IHC useful to characterize APCCs (33, 35). Another published method to detect APCCs is *in situ* hybridization of CYP11B2. Utilizing this method, Boulkroun et al. (45) distinguished three CYP11B2-expressing structures beneath the adrenal capsule that were termed as foci, megafoci and APCCs based on the size of cell clusters and their relative expression of a glomerulosa marker, Disable-2 (DAB2). In this study, APCCs were described as large clusters of CYP11B2 cells that did not express DAB2 indicating that APCCs contain cells presenting with an intermediate phenotype between ZG and ZF cells (45). However, on the transcriptomic level, Omata et al. (12) reported APCCs to be more similar to the adrenal ZG transcriptome than ZF or zona reticularis transcriptomes.

PHYSIOLOGICAL AND PATHOLOGICAL ASPECTS OF APCCs

APCCs are now accepted as a common feature in adrenal tissues adjacent to an APA (24, 30–33). Analysis of the adrenal glands of PA patients demonstrated that 40% to 50% of adrenals with APAs contained APCCs in the non-tumor portions of APA (30, 31). Interestingly, in Kometani et al. (32) study that found a 94% prevalence of APCCs in 16 adrenal tissue adjacent to the APA; the number and summed-area of APCCs in APAs were significantly higher in patients with discordant AVS results whose diagnosis changed to bilateral PA post-adrenocorticotropic hormone (ACTH) stimulation. The authors therefore proposed that APA patients with multiple APCCs might have similar adrenocortical pathological conditions in the contralateral adrenal gland. This assumption would explain their finding that in the pre-ACTH group having multiple APCCs, the post- to pre-ACTH plasma aldosterone concentrations (PACs) ratio was markedly higher on the non-dominant than the dominant side. Although the aldosterone responsiveness of APCCs towards ACTH has not been thoroughly described, it is conceivable that ACTH stimulation triggers the synthesis of aldosterone by APCCs in the contralateral adrenal gland, resulting in a decreased lateralization index (32). As RAAS is systemically suppressed in PA patients, these findings indicate that APCCs might play a role in autonomous aldosterone production (12). Concurringly, APCCs were also reported to be increased in adrenals of unilateral CT-negative PA compared to normotensive adrenals, implying that the elevated number of adrenal CYP11B2-expressing nodules might be the cause of hyperaldosteronism in these patients (33). Moreover, in one study that investigated the presence of APCCs in adrenals from IHA PA patients, all IHA adrenals were found to have at least one APCC or a microAPA (24). The number of APCCs in IHA adrenals were markedly larger compared to the cohorts of age-matched normotensive adrenals (24). Thus, these cell clusters could be the cause of hyperaldosteronism in these patients. This was proved to be the case in four patients with unilateral APCCs (aldosterone-producing micronodules not more than 3 mm in greatest dimension) whereby unilateral adrenalectomy resulted in a complete amelioration of blood pressure and hormonal abnormalities (46). Due to the small sizes of APCCs, patients with this feature may often be misdiagnosed as IHA as standard imaging test may not identify tiny anomalies of adrenals. Thus AVS, or if possible segmental selective AVS, should be used as part of the diagnostic procedure (47).

On the other hand, APCCs have not only been found in pathological adrenal tissues but also in normal adrenals where no tumors were present (30, 34, 35). In situ hybridization studies have found that APCCs in normal adrenals have a very similar profile to APCCs found in adrenal tissue adjacent to an APA (48, 49). In a study of nine Japanese patients with renal cell carcinoma (RCC) or upper urinary tract urothelial carcinoma (n=1), the presence of APCCs was found in eight of nine normal adrenals (30). Concurringly, another study from Japan on normotensive adrenal glands procured from an autopsy cohort found that out of 107 adrenals, 61 APCCs were detected in 31 autopsy cases (35). Similarly, a larger study by Nanba et al. (34) that analyzed the relationship between age and adrenal CYP11B2 expression, reported 69% of normal adrenal glands (88/127) had at least one APCC. Interestingly, this study also reported that although the total CYP11B2-expressing area negatively correlated with age (r=-0.431, P<0.0001), the total APCCs area positively correlated with age (r=0.390, P<0.0001) (34).

SIGNIFICANCE OF APCCs TO AGE-RELATED HYPERTENSION

Owing to the fact that APCCs are frequently seen in non-hypertensive as well as pathological human adrenal glands expressing CYP11B2, several studies have investigated if these so-called APCCs detected through CYP11B2 expression could produce aldosterone. By utilizing *in situ* hybridization method,

Shigematsu et al. (49) reported the presence of APCC-like subcapsular micronodules, showing intense transcript expression for HSD3B2, CYP11B1, and CYP11B2, but not CYP17A1, implying that the nodules have the steroidogenic enzymatic property needed to synthesize aldosterone and thus might be responsible for hyperaldosteronism. This was supported by Sugiura et al. (50) study of PA adrenal sections using Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) and tandem mass spectrometry imaging that demonstrated high levels of aldosterone and 18-oxocortisol, a potential serum marker of APA, in APCCs. Similarly, a recent study using tandem mass spectrometry imaging verified Suguira et al's findings in normal adrenals as elevated levels of aldosterone and 18-oxocortisol were found in APCC's relative to the adjacent adrenal tissue in an adrenal obtained from a patient that had undergone radical nephrectomy for renal cell carcinoma (51). These studies support the steroidogenic activity of APCCs in producing aldosterone in normal adrenals as well as under suppressed renin conditions, as shown in PA patients' adrenals (44). Accordingly, APCCs has been proposed to be the transitioning step to APAs (50).

Based on aldosterone studies from their groups and others, Sugiura et al. (50) suggested that with ageing, APCCs accumulate aldosterone-driver mutations causing autonomous aldosterone production that can lead to APCC-to-APA translational lesions (pAATL). This is as with age, there is a loss of the classical subcapsular CYP11B2-positive ZG due to the effects of trophic hormones that lead to the decline in total aldosterone production. Thus within this background of decreased ZG CYP11B2 expression, the number of CYP11B2-positive APCCs grows (34, 44). Finally, the expansion of the mutation bearing APCCs leads to the generation of an aldosterone-producing adrenal lesion. The age-related progressive pattern of APCCs has been validated by several studies that showed the number of adrenal APCCs increased with ageing in normal adrenals from kidney donors (34) and non-hypertensive Japanese cohorts (33), signifying the contribution of APCCs in the early stages of PA in ageing adults. Altogether, the findings of APCCs in normal adrenals and the adrenal glands of PA patients with APAs, as well as their accumulation during the ageing process, has brought up the possibility that APCCs might play a role in the development of APAs and therefore is a continuum of PA.

SOMATIC GENE MUTATIONS OF APCCs

Whole exome sequencing studies on APAs have defined recurrent somatic mutations in genes coding for ion channels (*KCNJ5*, *CACNA1H*, and *CACNA1D*) and ATPases (*ATP1A1* and *ATP2B3*) that either caused cytosolic acidification (for *ATP1A1*) or activated intracellular calcium signaling by depolarization of ZG cell membrane that opens the voltage-gated calcium channels or by affecting the intracellular calcium recycling (4, 52–56). This ultimately results in an increase of CYP11B2 expression that leads to aldosterone overproduction. The promising discovery of recurrent somatic mutations in APAs through the application of next-generation sequencing (52–55) has driven several studies to utilize this platform to determine the prevalence of aldosterone-driving somatic mutations in APCCs. From a cohort of 42 normal adrenals among kidney donors, 8 of 23 APCCs (35%) were found to harbor known APA-associated driver mutations, predominantly in *CACNA1D* (26%) followed by *ATP1A1* (9%) (43). However, this study did not find any APCCs to harbor the *KCNJ5* mutations, the most common mutations found in APAs (43). Similarly, in another study that performed next-generation sequencing analysis on CYP11B2-expressing nodules from the adrenals of PA patients that were CT-negative for adenomas, APCC-like micronodules were found to pre-dominantly harbor *CACNA1D* somatic mutations (33).

Compared to *KCNJ5* mutant APAs that have been described as more prevalent in young women with higher levels of plasma aldosterone and larger ZF-like APAs (12, 57–60), *CACNA1D* mutations are associated with smaller ZG-like APAs in older men that are more difficult to be diagnosis (43, 53, 60). *CACNA1D* encodes for the voltage-dependent L-type (long-lasting) calcium channel subunit alpha-1D (the Cav1.3 calcium channel) (52, 53). This calcium channel is composed of 4 homologous repeated domains with 6 transmembrane segments (S1-S6) each and a membrane-associated loop between S5 and S6 (52, 53, 61). Mutations occurring in *CACNA1D* are gain of function mutations that lead to a shift of the voltage-dependent channel activation to more negative voltages or delay the inactivation of the channel. The resulting net effect is increased intracellular calcium concentrations and thereby induction of excessive aldosterone synthesis (52, 53). Hence, the high prevalence of APCCs with *CACNA1D* mutations suggests that an intracellular increase of calcium is causal for the high CYP11B2 expression in APCCs (62).

A study on 107 adrenal glands of normotensive Japanese patients from an 837 consecutive autopsy cohort was conducted to test the hypothesis that hyperaldosteronism in the group of CT-negative PA patients was induced by the elevated number of adrenal CYP11B2-expressing nodules (33, 35). The study in adrenals from non-hypertensive Japanese patients demonstrated that APCCs were common with 34% harboring known aldosterone-driver somatic mutations, *CACNA1D* being the most frequent mutations while there being no detections of *KCNJ5* mutant APCCs (35). The mutations were found to be present in both the ZG and ZF-like components of APCCs and absent in neighboring cells negative for CYP11B2 (35). The similarity of APCCs somatic mutation spectrum observed in this normotensive cohort to that found in PA patients that had adrenals that were CT-negative for adenomas implied that normal adrenal glands may progress to PA through an increase in the APCCs frequency (12). Concurringly, a study on the IHA subtype of PA found that in 15 IHA adrenals, 99 APCCs were detected of which 58% had a *CACNA1D* mutation thus supporting the notion that PA in IHA may result from the accumulation or enlargement of CT-undetectable APCCs harboring APA-associated somatic mutations that increases aldosterone production (24).

The high presence of aldosterone-driver mutations in APCCs, especially in *CACNA1D* and *ATP1A1* (as shown in **Table 1**), supports the suggestion that APCCs are capable of autonomous aldosterone production and therefore could be the origin of APAs. Nishimoto et al. (42, 43) proposed that the series of events contributing to emergence of APAs from APCCs arises through the formation of possible APCC-to-APA transitional lesions (pAATLs) that has been characterized to consist of an outer APCC-like portion and an inner micro-APA (mAPA)-like portion (42). Interestingly, in one pAATL, the same *ATP1A1* mutation has been found in the APCC-like portion and the mAPA-like portion of the pAATL, indicating that these portions have a clonal origin (42). However, the genetic characteristic of pAATL is heterogeneous. In one adrenal gland, Nishimoto et al. (42) found the mAPA-like portions of pAATLs examined harbored *KCNJ5* mutations whereas their corresponding APCC-like portions did not, with the mutation being different from that identified in the APA within the same adrenal. This indicates that the APA and the pAATL had different origins and that the mAPA-like portion arises from existing APCCs due to the introduction of a *KCNJ5* mutation that differentiated the mAPA portion from the APCC. The lack of detection of *KCNJ5* mutation in the APCC-like portion could be due to a rapid progression to APA, therefore hard to be witnessed prior to its development (43, 63). However, some authors postulate that *KCNJ5* mutation could rather be a second hit mutation (than a causal mutation of pAATL), as only pAATL nodules larger than 3 mm have been reported to harbor *KCNJ5* mutations (63). Thus, these observational findings require further functional analysis to better elucidate the genomic events involved in the transition of APCCs to APA.

TABLE 1 | Somatic gene mutations detected in APCCs.

Gene	Mutation	Reference
ATP1A1	L104R*	(33)
	L104V*	(40)
	V332G*	(40)
	E687K	(33)
	M734L	(40)
ATP2B3	D77N	(33)
	E135K	(33)
	G270D	(33)
	G325D	(33)
	R345Q	(40)
	A790V	(33)
	S1137F	(33)
	P1150L	(33)
	A1157V	(33)
CACNA1D	E124K	(30)
	L248F	(30)
	V259G	(30)
	L272R	(30)
	G323R	(30)
	V401L*	(30)
	G403R**	(30, 33, 40)
	S410L	(30, 33)
	G457R	(33)
	R510X	(33)
	P548L	(33)
	L613Q	(40)
	R619W	(40)
	S652L*	(30)
	L653P	(30)
	S724L	(30)
	F747C	(33)
	F747L**	(30, 33, 40)
	F747V**	(30, 33, 40)
	L748S	(30)
	755_757del	(30)
	S969L	(30)
	R990H**	(30, 33)
	A998V**	(30, 33)
	A1011T	(30)
	I1015V	(30)
	F1147C	(33)
	F1147L	(30, 33)
	R1183H	(30)
	F1248L	(30, 33)
	D1273N	(30)
	P1336R*	(33)
	V1338M**	(30, 33, 40)
	I1352T	(33)
	P1499L	(33)
	T1835I	(30)
	W1836X	(33)

*Mutations that have been previously reported in sporadic APAs.
**Mutations that have been previously reported in APAs and functionally characterized.

CONCLUSIONS

In conclusion, several studies have found the presence of aldosterone-driving somatic gene mutations, similar to those found in APAs, among APCCs which supports the autonomous secretion of aldosterone by these clusters of cells and its role in the pathology of PA. The high frequency of *CACNA1D* and *ATP1A1* mutations, but not *KCNJ5* mutations in APCCs, suggest that these cells could be a potential precursor for ZG-like APAs rather than ZF-like APAs. Further, there is supporting evidence to suggest that accumulation of APCCs with age might contribute to the increased prevalence of hypertension occurring with age. More studies on APCCs are necessary to clarify the pathological mechanisms (and perhaps physiological as also found in "normal" adrenals) in regulating aldosterone production in these clusters of cells. As technology develops, whole-genome sequencing may be the important step forward to reveal novel gene mutations that lead to the development of aldosterone-driving somatic mutation. Identifications of germline variants may help to elucidate the potential mechanisms and pathways that lead to accumulation of aldosterone-driving somatic mutations and thus may consequently provide novel personalized treatment for age-related hypertension.

AUTHOR CONTRIBUTIONS

EA and FP conceived the presented idea. EA supervised and verified the concept. FP prepared the original draft. EA and FP

reviewed and edited the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

FP is supported by the program Malaysia Partnership and Alliances in Research (MyPAiR) Newton-MRC UK-MY (NEWTON-MRC/2020/002). EA is supported by the Royal Society-Newton Advanced Fellowship (FF-2018-033/NA170257).

ACKNOWLEDGMENTS

The authors would like to thank Dr. Suerialoasan Navanesan for proofreading this article.

REFERENCES

1. Zhou B, Bentham J, Di Cesare M, Bixby H, Danaei G, Cowan MJ, et al. Worldwide trends in blood pressure from 1975 to 2015: a pooled analysis of 1479 population-based measurement studies with 19·1 million participants. *Lancet* (2017) 389(10064):37–55. doi: 10.1016/S0140-6736(16)31919-5
2. Mendis S. World Health Organization. Global status report on non communicable diseases 2010. (2010). http://www.who.int/nmh/publications/ncd_report2010/en.
3. Zahedmehr A. "Chapter 17 - Hypertension". In: M Maleki, A Alizadehasl, M.B.T.-P.C. Haghjoo, editors. *Practical Cardiology*. St. Louis, Missouri: Eds.; Elsevier (2018). p. 291–302.
4. El Zein RM, Boulkroun S, Fernandes-Rosa FL, Zennaro MC. Molecular genetics of Conn adenomas in the era of exome analysis. *Presse Med* (2018) 47(7-8P2):e151–8. doi: 10.1016/j.lpm.2018.07.006
5. Mohler ER, Townsend RR. "Elevated Aldosterone Renin Ratio". In: ER Mohler, RR Townsend, editors. *Advanced Therapy in Hypertension and Vascular Disease*. Hamilton, Ontario: Eds.; B.C. Decker (2006). p. 321–8.
6. Käyser SC, Dekkers T, Groenewoud HJ, van der Wilt GJ, Carel Bakx J, van der Wel MC, et al. Study Heterogeneity and Estimation of Prevalence of Primary Aldosteronism: A Systematic Review and Meta-Regression Analysis. *J Clin Endocrinol Metab* (2016) 101(7):2826–35. doi: 10.1210/jc.2016-1472
7. Monticone S, D'Ascenzo F, Moretti C, Williams TA, Veglio F, Gaita F, et al. Cardiovascular events and target organ damage in primary aldosteronism compared with essential hypertension: a systematic review and meta-analysis. *Lancet Diabetes Endocrinol* (2018) 6(1):41–50. doi: 10.1016/S2213-8587(17)30319-4
8. Williams B, MacDonald TM, Morant SV, Webb DJ, Sever P, McInnes GT, et al. Endocrine and haemodynamic changes in resistant hypertension, and blood pressure responses to spironolactone or amiloride: the PATHWAY-2 mechanisms substudies. *Lancet Diabetes Endocrinol* (2018) 6(6):464–75. doi: 10.1016/S2213-8587(18)30071-8
9. Hannemann A, Wallaschofski H. Prevalence of primary aldosteronism in patient's cohorts and in population-based studies–a review of the current literature. *Hormone Metab Res* (2012) 44(3):157–62. doi: 10.1055/s-0031-1295438
10. Calhoun DA, Nishizaka MK, Zaman MA, Thakkar RB, Weissmann P. Hyperaldosteronism Among Black and White Subjects With Resistant Hypertension. *Hypertension* (2002) 40(6):892–6. doi: 10.1161/01.HYP.0000040261.30455.B6
11. Douma S, Petidis K, Doumas M, Papaefthimiou P, Triantafyllou A, Kartali N, et al. Prevalence of primary hyperaldosteronism in resistant hypertension: a retrospective observational study. *Lancet* (2008) 371(9628):1921–6. doi: 10.1016/S0140-6736(08)60834-X
12. Omata K, Tomlins SA, Rainey WE. Aldosterone-producing cell clusters in normal and pathological states. *Hormone Metab Res* (2017) 49(12):951–6. doi: 10.1055/s-0043-122394
13. Deipolyi AR, Oklu R. Adrenal vein sampling in the diagnosis of aldosteronism. *J Vasc Diagn Interventions* (2015) 3:17–23. doi: 10.2147/JVD.S79302
14. Bassett MH, White PC, Rainey WE. The regulation of aldosterone synthase expression. *Mol Cell Endocrinol* (2004) 217(1–2):67–74. doi: 10.1016/j.mce.2003.10.011
15. Lim JS, Park S, Park SII, Oh YT, Choi E, Kim JY, et al. Cardiac Dysfunction in Association with Increased Inflammatory Markers in Primary Aldosteronism. *Endocrinol Metab (Seoul Korea)* (2016) 31(4):567–76. doi: 10.3803/EnM.2016.31.4.567
16. Zia AA, Kamalov G, Newman KP, McGee JE, Bhattacharya SK, Ahokas RA, et al. From aldosteronism to oxidative stress: the role of excessive intracellular calcium accumulation. *Hypertens Res* (2010) 33(11):1091–101. doi: 10.1038/hr.2010.159
17. Herrada AA, Campino C, Amador CA, Michea LF, Fardella CE, Kalergis AM. Aldosterone as a modulator of immunity: implications in the organ damage. *J Hypertens* (2011) 29(9):1684–92. doi: 10.1097/HJH.0b013e32834a4c75
18. Funder JW, Carey RM, Mantero F, Murad MH, Reincke M, Shibata H, et al. The Management of Primary Aldosteronism: Case Detection, Diagnosis, and Treatment: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab* (2016) 101(5):1889–916. doi: 10.1210/jc.2015-4061
19. Milliez P, Girerd X, Plouin P-F, Blacher J, Safar M, Mourad J-J. Evidence for an Increased Rate of Cardiovascular Events in Patients With Primary Aldosteronism. *J Am Coll Cardiol* (2005) 45(8):1243–8. doi: 10.1016/j.jacc.2005.01.015
20. Yang Y, Reincke M, Williams TA. Prevalence, diagnosis and outcomes of treatment for primary aldosteronism. *Best Pract Res Clin Endocrinol Metab* (2019) 34(2):101365. doi: 10.1016/j.beem.2019.101365
21. Rossi GP. Primary Aldosteronism. *J Am Coll Cardiol* (2019) 74(22):2799–811. doi: 10.1016/j.jacc.2019.09.057
22. Hundemer GL, Curhan GC, Yozamp N, Wang M, Vaidya A. Cardiometabolic outcomes and mortality in medically treated primary aldosteronism: a retrospective cohort study. *Lancet Diabetes Endocrinol* (2018) 6(1):51–9. doi: 10.1016/S2213-8587(17)30367-4
23. Gomez-Sanchez C, Kuppusamy M, Reincke M, Williams T. Disordered CYP11B2 Expression in Primary Aldosteronism. *Hormone Metab Res* (2017) 49:957–62. doi: 10.1055/s-0043-122238
24. Omata K, Satoh F, Morimoto R, Ito S, Yamazaki Y, Nakamura Y, et al. Cellular and genetic causes of idiopathic hyperaldosteronism. *Hypertension* (2018) 72(4):874–80. doi: 10.1161/HYPERTENSIONAHA.118.11086
25. Satoh F, Morimoto R, Seiji K, Satani N, Ota H, Iwakura Y, et al. Is there a role for segmental adrenal venous sampling and adrenal sparing surgery in patients with primary aldosteronism? *Eur J Endocrinol* (2015) 173(4):465–77. doi: 10.1530/EJE-14-1161
26. Wu VC, Chueh SC, Chang HW, Lin WC, Liu KL, Li HY, et al. Bilateral aldosterone-producing adenomas: differentiation from bilateral adrenal hyperplasia. *QJM: Int J Med* (2008) 101(1):13–22. doi: 10.1093/qjmed/hcm101
27. Sonoyama T, Sone M. Regulatory Role of ACTH on Aldosterone in Aldosterone-Producing Adenoma. *J Rare Dis Res Treat* (2017) 2(3):30–3. doi: 10.29245/2572-9411/2017/3.1101
28. Young WF. Primary aldosteronism: renaissance of a syndrome. *Clin Endocrinol* (2007) 66(5):607–18. doi: 10.1111/j.1365-2265.2007.02775.x
29. Vonend O, Ockenfels N, Gao X, Allolio B, Lang K, Mai K, et al. Adrenal Venous Sampling Evaluation of the German Conn's Registry. *Hypertension* (2011) 57(5):990–5. doi: 10.1161/HYPERTENSIONAHA.110.168484
30. Nishimoto K, Nakagawa K, Li D, Kosaka T, Oya M, Mikami S, et al. Adrenocortical Zonation in Humans under Normal and Pathological Conditions. *J Clin Endocrinol Metab* (2010) 95(5):2296–305. doi: 10.1210/jc.2009-2010
31. Nanba K, Tsuiki M, Sawai K, Mukai K, Nishimoto K, Usui T, et al. Histopathological diagnosis of primary aldosteronism using CYP11B2 immunohistochemistry. *J Clin Endocrinol Metab* (2013) 98(4):1567–74. doi: 10.1210/jc.2012-3726
32. Kometani M, Yoneda T, Aono D, Karashima S, Demura M, Nishimoto K, et al. Impact of aldosterone-producing cell clusters on diagnostic discrepancies in primary aldosteronism. *Oncotarget* (2018) 9(40):26007–18. doi: 10.18632/oncotarget.25418

33. Yamazaki Y, Nakamura Y, Omata K, Ise K, Tezuka Y, Ono Y, et al. Histopathological classification of cross-sectional image-negative hyperaldosteronism. *J Clin Endocrinol Metab* (2017) 102(4):1182–92. doi: 10.1210/jc.2016-2986
34. Nanba K, Vaidya A, Williams GH, Zheng I, Else T, Rainey WE. Age-Related Autonomous Aldosteronism. *Circulation* (2017) 136(34):347–55. doi: 10.1161/CIRCULATIONAHA.117.028201
35. Omata K, Anand SK, Hovelson DH, Liu C-J, Yamazaki Y, Nakamura Y, et al. Aldosterone-Producing Cell Clusters Frequently Harbor Somatic Mutations and Accumulate With Age in Normal Adrenals. *J Endocrine Soc* (2017) 1 (7):787–99. doi: 10.1210/js.2017-00134
36. Nishimoto K, Seki T, Hayashi Y, Mikami S, Al-eyd G, Nakagawa K, et al. Human Adrenocortical Remodeling Leading to Aldosterone-Producing Cell Cluster Generation. *Int J Endocrinol* (2016) 2016(7834356):6. doi: 10.1155/2016/7834356
37. Nakamura Y, Maekawa T, Felizola SJA, Satoh F, Qi X, Velarde-Miranda C, et al. Adrenal CYP11B1/2 expression in primary aldosteronism: Immunohistochemical analysis using novel monoclonal antibodies. *Mol Cell Endocrinol* (2014) 392(1):73–9. doi: 10.1016/j.mce.2014.05.002
38. Gomez-Sanchez C, Qi X, Velarde-Miranda C, Plonczynski MW, Parker CR, Rainey W, et al. Development of monoclonal antibodies against human CYP11B1 and CYP11B2. *Mol Cell Endocrinol* (2014) 383(1–2):111–7. doi: 10.1016/j.mce.2013.11.022
39. Miller WL, Auchus RJ. The Molecular Biology, Biochemistry, and Physiology of Human Steroidogenesis and Its Disorders. *Endocrine Rev* (2011) 32(1):81–151. doi: 10.1210/er.2010-0013
40. Hattangady NG, Olala LO, Bollag WB, Rainey WE. Acute and chronic regulation of aldosterone production. *Mol Cell Endocrinol* (2012) 350 (2):151–62. doi: 10.1016/j.mce.2011.07.034
41. Williams TA, Gomez-Sanchez CE, Rainey WE, Giordano TJ, Lam AK, Marker A, et al. International histopathology consensus for unilateral primary aldosteronism. *J Gerontol Ser A: Biol Sci Med Sci* (2021) 106(1):42–54. doi: 10.1210/clinem/dgaa484
42. Nishimoto K, Seki T, Kurihara I, Yokota K, Omura M, Nishikawa T, et al. Case Report: Nodule Development From Subcapsular Aldosterone-Producing Cell Clusters Causes Hyperaldosteronism. *J Clin Endocrinol Metab* (2016) 101 (1):6–9. doi: 10.1210/jc.2015-3285
43. Nishimoto K, Tomlins SA, Kuick R, Cani AK, Giordano TJ, Hovelson DH, et al. Aldosterone-stimulating somatic gene mutations are common in normal adrenal glands. *Proc Natl Acad Sci U S A* (2015) 112(33):E4591–9. doi: 10.1073/pnas.1505529112
44. Lim JS, Rainey WE. The Potential Role of Aldosterone-Producing Cell Clusters in Adrenal Disease. *Hormone Metab Res* (2020) 52(6):427–34. doi: 10.1055/a-1128-0421
45. Boulkroun S, Samson-Couterie B, Golib Dzib JF, Lefebvre H, Louiset E, Amar L, et al. Adrenal cortex remodeling and functional zona glomerulosa hyperplasia in primary aldosteronism. *Hypertension* (2010) 56(5):885–92. doi: 10.1161/HYPERTENSIONAHA.110.158543
46. Omura M, Sasano H, Fujiwara T, Yamaguchi K, Nishikawa T. Unique cases of unilateral hyperaldosteronemia due to multiple adrenocortical micronodules, which can only be detected by selective adrenal venous sampling. *Metabol: Clin Exp* (2002) 51(3):350–5. doi: 10.1053/meta.2002.30498
47. Kitamoto T, Kitamoto KK, Omura M, Takiguchi T, Tsurutani Y, Kubo H, et al. Precise Mapping of Intra-Adrenal Aldosterone Activities Provides a Novel Surgical Strategy for Primary Aldosteronism. *Hypertension* (2020) 76 (3):976–84. doi: 10.1161/HYPERTENSIONAHA.119.14341
48. Enberg U, Volpe C, Hoog A, Wedell A, Farnebo LO, Thoren M, et al. Postoperative differentiation between unilateral adrenal adenoma and bilateral adrenal hyperplasia in primary aldosteronism by mRNA expression of the gene CYP11B2. *Eur J Endocrinol* (2004) 151(1):73–85. doi: 10.1530/eje.0.1510073
49. Shigematsu K, Kawai K, Irie J, Sakai H, Nakashima O, Iguchi A, et al. Analysis of Unilateral Adrenal Hyperplasia with Primary Aldosteronism from the Aspect of Messenger Ribonucleic Acid Expression for Steroidogenic Enzymes: A Comparative Study with Adrenal Cortices Adhering to Aldosterone-Producing Adenoma. *Endocrinology* (2006) 147(2):999–1006. doi: 10.1210/en.2005-0765
50. Sugiura Y, Takeo E, Shimma S, Yokota M, Higashi T, Seki T, et al. Aldosterone and 18-oxocortisol coaccumulation in aldosterone-producing lesions. *Hypertension* (2018) 72(6):1345–54. doi: 10.1161/HYPERTENSIONAHA.118.11243
51. Takeo E, Sugiura Y, Uemura T, Nishimoto K, Yasuda M, Sugiyama E, et al. Tandem Mass Spectrometry Imaging Reveals Distinct Accumulation Patterns of Steroid Structural Isomers in Human Adrenal Glands. *Analyt Chem* (2019) 91(14):8918–25. doi: 10.1021/acs.analchem.9b00619
52. Scholl UI, Goh G, Oliveira RC, De Choi M, Overton JD, Fonseca AL, et al. Somatic and germline CACNA1D calcium channel mutations in aldosterone-producing adenomas and primary aldosteronism. *Nat Genet* (2013) 45: (9):1050–4. doi: 10.1038/ng.2695
53. Azizan EAB, Poulsen H, Tuluc P, Zhou J, Clausen MV, Lieb A, et al. Somatic mutations in ATP1A1 and CACNA1D underlie a common subtype of adrenal hypertension. *Nat Genet* (2013) 45(9):1055–60. doi: 10.1038/ng.2716
54. Choi M, Scholl UI, Yue P, Björklund P, Zhao B, Nelson-Williams C, et al. K+ channel mutations in adrenal aldosterone-producing adenomas and hereditary hypertension. *Science (New York NY)* (2011) 331(6018):768–72. doi: 10.1126/science.1198785
55. Beuschlein F, Boulkroun S, Osswald A, Wieland T, Nielsen HN, Lichtenauer UD, et al. Somatic mutations in ATP1A1 and ATP2B3 lead to aldosterone-producing adenomas and secondary hypertension. *Nat Genet* (2013) 45 (4):440–4. doi: 10.1038/ng.2550
56. Stindl J, Tauber P, Sterner C, Tegtmeier I, Warth R, Bandulik S. Pathogenesis of Adrenal Aldosterone-Producing Adenomas Carrying Mutations of the Na+/K +-ATPase. *Endocrinology* (2015) 156(12):4582–91. doi: 10.1210/en.2015-1466
57. Boulkroun S, Beuschlein F, Rossi G-P, Golib-Dzib J-F, Fischer E, Amar L, et al. Prevalence, Clinical, and Molecular Correlates of KCNJ5 Mutations in Primary Aldosteronism. *Hypertension* (2012) 59(3):592–8. doi: 10.1161/HYPERTENSIONAHA.111.186478
58. Rossi GP, Cesari M, Letizia C, Seccia TM, Cicala MV, Zinnamosca L, et al. KCNJ5 gene somatic mutations affect cardiac remodelling but do not preclude cure of high blood pressure and regression of left ventricular hypertrophy in primary aldosteronism. *J Hypertens* (2014) 32(7):1514–21. doi: 10.1097/HJH.0000000000000186
59. Lenzini L, Rossitto G, Maiolino G, Letizia C, Funder JW, Rossi GPA. Meta-Analysis of Somatic KCNJ5 K+ Channel Mutations In 1636 Patients With an Aldosterone-Producing Adenoma. *J Clin Endocrinol Metab* (2015) 100(8): E1089–95. doi: 10.1210/jc.2015-2149
60. Fernandes-Rosa FL, Williams TA, Riester A, Steichen O, Beuschlein F, Boulkroun S, et al. Genetic Spectrum and Clinical Correlates of Somatic Mutations in Aldosterone-Producing Adenoma. *Hypertension* (2014) 64 (2):354–61. doi: 10.1161/HYPERTENSIONAHA.114.03419
61. Catterall WA. Signaling complexes of voltage-gated sodium and calcium channels. *Neurosci Lett* (2010) 486(2):107–16. doi: 10.1016/j.neulet.2010.08.085
62. Zennaro M-C, Boulkroun S, Fernandes-Rosa F. Genetic Causes of Functional Adrenocortical Adenomas. *Endocrine Rev* (2017) 38(6):516–37. doi: 10.1210/er.2017-00189
63. Nishimoto K, Koga M, Seki T, Oki K, Gomez-sanchez EP, Gomez-sanchez CE, et al. Immunohistochemistry of aldosterone synthase leads the way to the pathogenesis of primary aldosteronism. *Mol Cell Endocrinol* (2017) 441:124–33. doi: 10.1016/j.mce.2016.10.014

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

SYSTEMATIC REVIEW
published: 16 March 2021
doi: 10.3389/fendo.2021.645488

Cortisol Co-Secretion and Clinical Usefulness of ACTH Stimulation Test in Primary Aldosteronism: A Systematic Review and Biases in Epidemiological Studies

Kosuke Inoue[1,2], *Takumi Kitamoto*[2,3], *Yuya Tsurutani*[2], *Jun Saito*[2], *Masao Omura*[2] *and Tetsuo Nishikawa*[2*]

[1] Department of Epidemiology, University of California, Los Angeles (UCLA) Fielding School of Public Health, Los Angeles, CA, United States, [2] Endocrinology and Diabetes Center, Yokohama Rosai Hospital, Yokohama, Japan, [3] Division of Endocrinology, Department of Medicine, Columbia University, New York, NY, United States

The hypothalamus-pituitary-adrenal (HPA) axis plays an important role in primary aldosteronism. Aldosterone biosynthesis is regulated not only by angiotensin II in the renin-angiotensin-aldosterone system, but also by adrenocorticotropic hormone (ACTH), one of the key components of the HPA axis. Although previous studies have reported cortisol cosecretion in primary aldosteronism, particularly aldosterone-producing adenoma (APA), the clinical relevance of such aldosterone and cortisol cosecretion from APA and hypertension or other metabolic disorders has not been fully established. Several somatic mutations including *KCNJ5* and *CACNA1D* are known to induce autonomous production of aldosterone in APA, and the aldosterone responsiveness to ACTH may vary according to each mutation. The ACTH stimulation test has been reported to be a useful tool to distinguish the subtypes of primary aldosteronism (e.g., unilateral vs bilateral) in some studies, but it has not been commonly applied in clinical practice due to limited evidence. Given the recent advancement of imaging, omics research, and computational approach, it is important to summarize the most updated evidence to disentangle the potential impact of cortisol excess in primary aldosteronism and whether the ACTH stimulation test needs to be considered during the diagnostic process of primary aldosteronism. In this article, we conducted a systematic review of epidemiological studies about (i) cortisol cosecretion in primary aldosteronism and (ii) the ACTH stimulation test for the diagnosis of primary aldosteronism (including subtype diagnosis). Then, we discussed potential biases (e.g., confounding bias, overadjustment, information bias, selection bias, and sampling bias) in the previous studies and introduced some advanced epidemiological/statistical methods to minimize these limitations. A better understanding of biases and epidemiological perspective on this topic would allow us to produce further robust evidence and balanced discussion about the causal mechanisms involving the HPA axis and clinical usefulness of the ACTH stimulation test among patients with primary aldosteronism.

Keywords: cortisol, ACTH, primary aldosteronism, systematic review, bias, epidemiological methods

OPEN ACCESS

Edited by:
Vin-Cent Wu,
National Taiwan University, Taiwan

Reviewed by:
Shao-Yu Yang,
National Taiwan University Hospital, Taiwan
Leay Kiaw Er,
Taipei Tzu Chi Hospital, Taiwan

***Correspondence:**
Tetsuo Nishikawa
tetsuon@yokohamah.johas.go.jp

Specialty section:
This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Endocrinology

Received: *23 December 2020*
Accepted: *15 February 2021*
Published: *16 March 2021*

Citation:
Inoue K, Kitamoto T, Tsurutani Y, Saito J, Omura M and Nishikawa T (2021) Cortisol Co-Secretion and Clinical Usefulness of ACTH Stimulation Test in Primary Aldosteronism: A Systematic Review and Biases in Epidemiological Studies. Front. Endocrinol. 12:645488. doi: 10.3389/fendo.2021.645488

INTRODUCTION

Primary aldosteronism (PA) is one of the common causes of secondary hypertension, increasing the risk of cardiovascular disease (CVD) and renal events (1–4). Since the first report of a patient with adrenal adenoma concurrently producing aldosterone and cortisol (5), cortisol cosecretion in PA has received substantial attention, particularly because it may cause severe complications due to cortisol excess in addition to aldosterone excess. A recent study using a mass spectrometry-based analysis in Europe showed that cortisol cosecretion in PA was associated with adverse metabolic risk factors (6), indicating the role of glucocorticoid on an increased risk of metabolic disorders among patients with PA (7–11). Moreover, while the actual prevalence of this phenotype is not clear, some studies have suggested a relatively high prevalence of PA with hypercortisolism than expected (6, 10–15). Therefore, it is imperative to understand the current state of knowledge about cortisol cosecretion in PA, and consider whether the evaluation of cortisol excess needs be recommended in general PA screening or not.

Although the hypothalamus-pituitary-adrenal (HPA) axis plays an important role in regulating aldosterone biosynthesis, the clinical usefulness of ACTH stimulation test during the management of PA, particularly for PA subtype diagnosis (e.g., aldosterone-producing adenoma [APA] vs. bilateral aldosterone hyperplasia [BAH]), has been unclear. In general, ACTH is known to stimulate both cortisol and aldosterone secretion acutely and transiently through binding to melanocortin type 2 receptor (MC2R) (16). Previous studies have shown the high performance of classifying subtype of PA based on plasma aldosterone concentrations during ACTH stimulation test under 1-mg dexamethasone suppression test (DST) (17, 18). Other studies have also reported a larger decrease in aldosterone levels following dexamethasone suppression of HPA axis among APA than BAH (19) or among *KCNJ5*-mutated APAs than *KCNJ5* wild-type APAs (20), indicating the regulating role of endogenous ACTH on aldosterone secretion among patients with PA. Because lateralization of hyperaldosteronism is crucial to determine the target treatment (i.e., adrenalectomy or medication therapy) (21), there has been a great interest in the clinical benefit of conducting ACTH stimulation test during the PA confirmation process before AVS—a gold standard but more invasive and challenging procedure for PA subtype diagnosis.

Over the last several decades, observational studies have played a key role in PA research. Because exposure or treatment is not randomized in observational studies, we always need to be careful about confounding bias, a bias due to unmeasured common causes of exposure and outcomes (22). This is also the case in the "big data" analysis or machine learning-based approach because they do not guarantee the adjustment of a sufficient set of confounders and could rather introduce *precisely wrong* conclusion due to small variance of the estimates under the presence of bias. Moreover, both observational studies and randomized controlled trials suffer from other sources of bias including information bias (e.g., measurement error of hormone levels, self-report of history of hypertension and medications) (23) and selection bias (e.g., loss to follow-up, selection of study sample based on both exposure and outcome assessment) (24). Limited generalizability due to sampling bias is another issue of previous PA-related studies because most of the studies have been conducted at single-center and/or specific cohorts.

This review has two goals. First, we will provide updated summaries of epidemiological studies about cortisol cosecretion in primary aldosteronism and the clinical usefulness of the ACTH stimulation test to diagnose primary aldosteronism and/or its subtypes. Then, we will explain possible biases (e.g., confounding bias, overadjustment, information bias, selection bias, and sampling bias) in these epidemiological studies and some methods to minimize such limitations in future PA-related studies. Recognizing these biases and applying the advanced epidemiological methods would help us to build a further balanced and profound discussion on this topic—cortisol cosecretion and clinical usefulness of the ACTH stimulation test in PA.

CORTISOL COSECRETION IN PRIMARY ALDOSTERONISM

Recently, there is increasing literature regarding cortisol cosecretion in PA and its impact on CVD risk factors (6, 25, 26). We conducted literature searches between January 2000 and November 2020 using the electronic databases MEDLINE and EMBASE for cohort studies investigating the cortisol cosecretion in primary aldosteronism. The following search terms were applied: ("cortisol" OR "cortisolemia" OR "Cushing") AND ("primary aldosteronism" OR "aldosterone-producing adenoma"). We extracted the following information: first author name, publication year, region of study, populations, exposures/comparators, outcomes, and study design. We restricted studies to those with a sample size ≥10 (to avoid case reports) and written in English. Flow of studies through review and summary of the identified 16 studies are shown in **Figure 1A** and **Table 1** (6, 10–15, 27–35).

Previous studies have shown a relatively high prevalence of subclinical Cushing's syndrome or subclinical hypercortisolemia among patients with PA although the definition of hypercortisolemia differs across studies and countries (**Table 1**). In 2009, Piaditis et al. reported 12.1% of patients with adrenal incidentalomas had PA with hypercortisolemia (36). Since then, several studies from the Asian cohort have reported a 5%–27% prevalence of subclinical hypercortisolemia among patients with PA or APA (11–15). A recent multicenter cohort study in Germany showed even 77.6% of subclinical hypercortisolemia among 161 patients with PA (10). While the difference in clinical characteristics between PA patients with and without hypercortisolemia is still not clear (mainly due to the small sample size in each previous study) and may vary by geographical area and definitions, most of these studies showed larger tumor size among PA patients with subclinical hypercortisolemia than those without.

In 2014, monoclonal antibodies against human CYP11B1 and CYP11B2 were developed and their expression was reported in APAs, indicating the cortisol synthesis in such adrenal tumors (37, 38). This finding was supported by a small cohort study

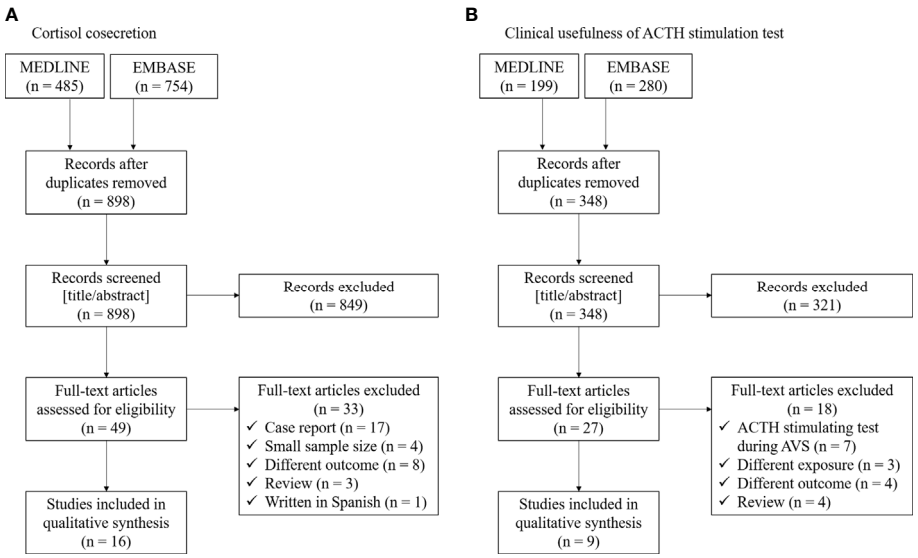

FIGURE 1 | Flow of studies through review for **(A)** cortisol cosecretion and **(B)** clinical usefulness of ACTH stimulation test in primary aldosteronism.

reporting a significant decrease in serum cortisol levels during 1 mg DST after the resection of APAs (29) and a large mass spectrometry-based analysis showing higher excretion of cortisol and total glucocorticoid metabolites in the PA group compared with the healthy control group (6). Moreover, a previous case series showed that the majority of APAs with subclinical hypercortisolemia was composed mainly of ZF-like cells (39). Given that ZF comprise larger cells that synthesize glucocorticoids compared with ZG that synthesize aldosterone (40), such histopathological feature may be related to the large tumor size in APAs with subclinical hypercortisolemia, that requires further investigation.

Recent studies also have reported health outcomes related to autonomous cortisol secretion in APAs. The above-mentioned mass spectrometry-based analysis also found the correlation between glucocorticoid output and several metabolic markers including body mass index, waist circumference, insulin resistance, high-density lipoprotein, and diastolic blood pressure (6). Tang et al. showed higher proportions of cardiovascular complications, glucose intolerance/diabetes, and osteopenia/osteoporosis among patients with APAs and cortisol producing adenoma (CPA) than patients with pure APAs (14). Adolf et al. scrutinized the possible impact of cortisol excess on cardiac remodeling among PA patients (30). Across patients in the German Conn's registry, they found the association of total glucocorticoid excretion with the decrease in LVMI a year after the initiation of PA treatment (30). The association between subclinical hypercortisolemia and impaired glucose metabolism in patients with PA was also reported by other cohort studies (10, 11). These results support the adverse effect of glucocorticoid excess on health through the activation of not only glucocorticoid receptor but also mineralocorticoid receptor (MR) through the impaired conversion of cortisol to its MR-inactive cortisone (41). Another study using the same German registry showed significant increases in anti-TPO levels after adrenalectomy in unilateral PA patients with cortisol cosecretion but the trend was not found in unilateral PA patients without cortisol cosecretion and bilateral PA patients receiving mineralocorticoid antagonist therapy (34). Their findings generate the hypothesis that cortisol cosecretion among unilateral PA may exhibit the immunosuppressive effect. Given these findings, it would be important to evaluate the cortisol cosecretion among PA patients in routine clinical practice. To produce robust evidence, more research is warranted to clarify whether the adverse health outcomes of APAs with subclinical hypercortisolemia differ between those with and without CPA.

The possible influence of mild cortisol excess in PA on the interpretation of AVS has been suggested in prior literature. In general, cannulation success is evaluated by selectivity index (the ratio of cortisol concentration for each adrenal vein and inferior vena cava [IVC]), and the lateralization is evaluated by lateralization index (the aldosterone to cortisol ratio on the dominant side with excess aldosterone secretion over aldosterone to cortisol ratio on the non-dominant side) and contralateral suppression index (the aldosterone to cortisol ratio on the non-dominant side over aldosterone to cortisol ratio in IVC) (21). Based on the findings from AVS in patients with CPA, but without PA, Goupil et al, suggested that autonomous unilateral cortisol hypersecretion could confound the accuracy of AVS by affecting the aldosterone/cortisol ratio in the adrenal vein (i.e., selectivity index, lateralization index, and contralateral suppression index), leading to misdiagnosis and suboptimal management (42). However, a recent single-center study focusing on PA with mildly elevated cortisol levels (mean serum cortisol levels during the 1-mg DST = 3.3 μg/dl) showed that the rates of cannulation success or lateralization did not differ between PA patients with mild cortisol excess and those without cortisol excess during AVS with ACTH stimulation (blood sample was obtained 1 h after the stimulation) (35). A multi-center cohort study on this topic would help us to validate their findings and

TABLE 1 | Summary of epidemiological studies about cortisol cosecretion in primary aldosteronism (PA).

Author	Year	Region	Populations	Exposures/Comparators	Characteristics/Outcomes	Study design
Hiraishi et al. (12)	2011	Japan	38 patients with PA	Coexistence of PA and subclinical Cushing's syndrome[a] (n=8)	Clinical and histopathological characteristics	Cross-sectional study
Nakajima et al. (27)	2011	Japan	76 patients with PA	Coexistence of PA and subclinical cortisol hypersecretion (n=22, serum cortisol levels during 1 mg DST ≥3.0 μg/dl)	Clinical characteristics including a history of cardiovascular events	Cross-sectional study
Fallo et al. (28)	2011	Italy	76 patients with PA	Coexistence of PA and subclinical cortisol hypersecretion (n=3, serum cortisol levels during 1 mg DST >1.8 μg/dl)	Clinical and histopathological characteristics	Cohort study
Fujimoto et al. (13)	2013	Japan	39 PA patients	Coexistence of PA and subclinical Cushing's syndrome[a] (n=5)	Clinical and histopathological characteristics	Cross-sectional study
Arlt et al. (6)	2017	Germany	174 patients with PA[b,c]	Cortisol cosecretion (24-h cortisol and total glucocorticoid outputs collected by quantitative gas chromatography-mass spectrometry)	Metabolic risk factors (BMI, blood pressure, fasting plasma glucose and insulin, 2-h glucose values during 75-g oral glucose tolerance test, HbA1c, total cholesterol, HDL, and triglycerides)	Cohort study
Inoue et al. (29)	2017	Japan	30 patients with APA and serum cortisol levels during 1 mg DST <3.0 μg/dl	Adrenalectomy	The change in serum cortisol levels during the 1-mg DST before and after adrenalectomy and histological characteristics	Cohort study
Tang et al. (14)	2018	China	414 patients with APA	Coexistence of APA and subclinical cortisol hypersecretion (n=22, serum cortisol levels during 1 mg DST >1.8 μg/dl)	Clinical and histopathological characteristics	Cross-sectional study
Adolf et al. (30)	2019	Germany	73 patients with PA [c]	Cortisol cosecretion (24-h total glucocorticoid outputs collected by quantitative gas chromatography-mass spectrometry)	Left ventricular hypertrophy	Cohort study
Ohno et al. (31)	2019	Japan	527 patients with bilateral PA. [d]	Bilateral PA cases with adrenal tumors (n=196) and without adrenal tumors (n=331)	Hormone levels including serum cortisol levels during 1 mg DST and clinical complications	Cross-sectional study
Kometani et al. (32)	2019	Japan	16 APAs	Coexistence of APA and subclinical cortisol hypersecretion (n=6, serum cortisol levels during 1 mg DST and at midnight >1.8 μg/dl)	Genetic and epigenetic characteristics	Cross-sectional study
Bhatt et al. (33)	2019	UK	25 patients with PA	Coexistence of PA and subclinical cortisol hypersecretion (n=4, serum cortisol levels during 1 mg DST >1.8 μg/dl)	Metabolic risk factors (ALT, total cholesterol, HDL, LDL and mean arterial blood pressure)	Cross-sectional study
Gerards et al. (10)	2019	Germany	161 patients with PA[c]	Coexistence of PA and subclinical cortisol hypersecretion (n=125, serum cortisol levels during 1 mg DST >1.8 μg/dl, or late-night salivary cortisol >1.45 ng/ml, or 24-h urinary free cortisol >150 μg/24h)	Glucose homeostasis evaluated by the standard oral glucose tolerance test	Cohort study
Akehi et al. (11)	2019	Japan	890 patients with PA who conducted 1 mg DST [d]	Coexistence of PA and subclinical cortisol hypersecretion (n=209, serum cortisol levels during 1 mg DST >1.8 μg/dl)	Prevalence of diabetes	Cross-sectional study
Handgriff et al. (34)	2020	Germany	97 patients with PA[c]	Coexistence of PA and subclinical cortisol hypersecretion (n=72, serum cortisol levels during 1 mg DST >1.8 μg/dl, or late-night salivary cortisol >1.5 ng/ml, or 24-h urinary free cortisol >150 μg/l [before 2015] or >83 μg/l [after 2015])	The kinetics of anti-thyroid peroxidase and thyroglobulin antibody before and after the therapy initiation	Cohort study
O'Toole et al. (35)	2020	UK	144 patients with PA	Coexistence of PA and subclinical cortisol hypersecretion (n=21, serum cortisol levels during 1 mg DST >1.8 μg/dl)	Parameters and interpretation in adrenal venous sampling	Cross-sectional study
Peng et al. (15)	2020	Taiwan	82 patients with APA	Coexistence of APA and subclinical cortisol hypersecretion (n=22, serum cortisol levels during 1 mg DST >1.8 μg/dl)	Clinical and biochemical outcomes after adrenalectomy	Cohort study

BMI, body mass index; HbA1c, glycohemoglobin; HDL, high-density lipoprotein cholesterol; APA, aldosterone-producing adenoma; A/CPA, aldosterone and cortisol-coproducing adenoma; DST, dexamethasone suppression test.

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

[b]This study also included 162 healthy controls, 56 patients with endocrine inactive adrenal adenoma, 104 patients with mild subclinical, and 47 with clinically overt adrenal cortisol excess as comparison groups.

[c]The German Conn's registry.

[d]The Japan Primary Aldosteronism Study.

further understand which AVS parameters are useful for the management of PA with cortisol cosecretion.

Lastly, some studies have investigated the genetic mutation of APA with cortisol cosecretion. A recent study based on a Taiwanese database showed that the presence of *KCNJ5* mutations was significantly lower among APA patients with subclinical hypercortisolemia than those without (15). Their immunohistochemistry analysis revealed the lower CYP11B1 expression among *KCNJ5* mutated APAs, which generally display a ZF-like phenotype (43). Although the underlying mechanism is unclear, enhanced 18-oxocortisol synthesis in *KCNJ5* mutated APAs may contribute to the findings of the lower cortisol levels in the mutated APAs than *KCNJ5* wild-type APAs. They also found that patients with subclinical hypercortisolemia and *KCNJ5* wild-type APAs exhibited the lowest complete clinical success rate (36.8%) after adrenalectomy (43), highlighting the importance of detecting these phenotypes for the management of APAs (15). Genetic and epigenetic analysis in a Japanese single-center study showed that APA with hypercortisolemia was not associated with the prevalence of *KCNJ5* mutations but associated with lower DNA methylation rate of the CYP11B1 promoter than APA without hypercortisolemia (32). However, this study was based on only 16 APAs and the causal relationship between DNA methylation and the regulation of gene expression is still unclear. Given that some somatic mutations, such as *CTNNB1* and *PRKACA* (44, 45), have also been reported in both APAs and CPAs, future research about these mutations and cortisol cosecretion among PA patients would be warranted.

ACTH STIMULATION TEST FOR THE DIAGNOSIS OF PRIMARY ALDOSTERONISM

Aldosterone production from the adrenal glomerulosa is regulated by ACTH as well as potassium, angiotensin II, and serotonin (46). Previous biological studies showed that acute ACTH administration increases plasma aldosterone through binding to MC2R (47), suggesting that ACTH may play a role in inappropriate hypersecretion of aldosterone in some PAs (16). A previous study showed aldosterone responsiveness to even very low dose ACTH (i.e., 0.003 IU), followed by a treadmill test (48). Since the first report of the ACTH stimulation test for patients with PA in 1978 (5), several epidemiological studies have investigated whether the ACTH stimulation test is useful to diagnose PA or its subtype. We conducted literature searches between January 2000 and November 2020 using the electronic databases MEDLINE and EMBASE for cohort studies investigating the cortisol cosecretion in primary aldosteronism. The following search terms were applied: ("ACTH" OR "adrenocorticotropic hormone" OR "corticotropin") AND ("primary aldosteronism" OR "aldosterone-producing adenoma"). We extracted the following information: first author name, publication year, region of study, populations, exposures/comparators, outcomes, and study design. We restricted studies to those with a sample size ≥10 (to avoid case reports) and written in English. Flow of studies through review and summary of the identified 9 studies are shown in **Figure 1B** and **Table 2** (17–19, 49–54).

In 1995, Stowasser et al. reported higher plasma aldosterone response after ACTH stimulation under dexamethasone suppression among angiotensin II responsive APA (n=16) or angiotensin II unresponsive APA (n=11) compared with BAH (n=19) (55). Although several studies mostly from Japan have shown moderate to high predictive performance (area under the curve [AUC] ranges from 0.70 to 0.95) to differentiate APA from BAH by using ACTH stimulation test (17, 18, 49–52, 54) over the last two decades, these findings are not comparable across studies due to the lack of uniformity in protocols in each study (e.g., ACTH dosage, with or without 1 mg DST, different cut-off time or values, etc), inconsistent definition of outcomes (e.g., diagnosis of APA and BAH based on AVS, histopathological findings, etc.), and study design (sample size, single-center or multi-center, prospective or retrospective, etc.). These limitations have made the clinical usefulness of the ACTH stimulation test inconclusive. For example, we previously demonstrated that the predictive performance of the ACTH stimulation test for subtype diagnosis of PA could vary by the definition of lateralization during AVS with ACTH stimulation (51); i.e., whether evaluating the ratio of aldosterone to cortisol at adrenal central veins (21) or evaluating the absolute value of aldosterone at adrenal tributary veins (56). Compared with the subtype diagnosis of PA, the evidence about the definite diagnosis of PA has been very limited and has not been consistent (49–51). As the current literature on this topic is mostly based on small sample size and a single-center or a specific country, further investigations with larger sample size from multi-center cohort are needed to understand whether the ACTH stimulation test should be routinely included during the process of PA diagnosis and which parameters are useful to minimize the use of AVS which is more invasive and challenging than peripheral blood examination.

Whether ACTH stimulation is recommended during AVS for subtype diagnosis or not is another important topic in PA management. ACTH stimulation may maximize cortisol secretion from the adrenal gland and minimize the pulsatile adrenocortical hormone secretion. A recent meta-analysis showed that AVS with ACTH stimulation did not significantly reduce the number of incorrect lateralization but significantly reduced the number of unsuccessful cannulations compared with AVS without ACTH stimulation (57). However, the evidence is still limited and under debate which is beyond the scope of this review and can be found in prior review literature (58).

Aldosterone responsiveness to ACTH may vary by a genetic mutation. Our group recently reported a larger decrease in aldosterone levels during 1 mg DST among *KCNJ5* mutated APAs than *KCNJ5* wild-type APAs, indicating that the ACTH pathway may be more sensitive and activated among *KCNJ5* mutated APAs (20). We also reported that aldosterone levels were more responsive to ACTH stimulation among APAs with somatic mutation of *CACNA1D* than those without the mutation (59). APAs with these mutations are likely to consist of ZF-like clear cells than others, and ZF cells are responsible for cortisol secretion and express a higher level of ACTH receptor than ZG cells (59, 60). Given that nearly 90% of APAs had somatic

TABLE 2 | Summary of epidemiological studies about ACTH stimulation test for the definite and the subtype diagnosis of primary aldosteronism (PA).

Author	Year	Region	Populations	Exposures/Comparators	Outcomes	Study design
Sonoyama et al. (17)	2011	Japan	39 patients with PA (unilateral, n=23; bilateral, n=16) and 20 patients without PA	ACTH stimulation (0.25mg [25IU] of cosyntropin) with 1 mg DST	The predictive accuracy for the subtype diagnosis of PA (i.e., unilateral or bilateral).	Cohort study
Jiang et al. (18)	2015	China	95 patients with PA (unilateral, n=56; bilateral, n=39)	ACTH stimulation (50IU) with 1 mg DST	The predictive accuracy for the subtype diagnosis of PA	Cohort study
Umakoshi et al. (49)	2016	Japan	121 patients with PA (unilateral, n=34; bilateral, n=87) and 66 patients without PA	ACTH stimulation (25IU)	The predictive accuracy for the definite and subtype diagnosis of PA	Cohort study
Terui et al. (50)	2016	Japan	138 patients with PA (unilateral, n=41; bilateral, n=57; no AVS, n=40) and 19 patients without PA	ACTH stimulation (25IU)	The predictive accuracy for the definite and subtype diagnosis of PA	Cohort study
Inoue et al. (51)	2017	Japan	30 patients with PA (unilateral, n=13; bilateral, n=17) and 18 patients without PA	ACTH stimulation (25IU) with and without 1 mg DST	The predictive accuracy for the definite and subtype diagnosis of PA	Cohort study
Moriya et al. (52)	2017	Japan	76 patients with PA (unilateral, n=17; bilateral, n=59)	ACTH stimulation (25IU)	The predictive accuracy for the subtype diagnosis of PA	Cohort study
Kita et al. (53)	2018	Japan	40 patients with PA (unilateral, n=22; bilateral, n=18)	ACTH stimulation (25IU)	The predictive accuracy for the subtype diagnosis of PA	Cohort study
Kidoguchi et al. (54)	2020	Japan	123 (unilateral, n=27; bilateral, n=96)	ACTH stimulation (25 IU)	The predictive accuracy for the subtype diagnosis of PA	Cohort study
St-Jean et al. (19)	2020	Canada	43 (unilateral, n=28; bilateral, n=11; undefined, n=4)	ACTH stimulation (25 IU)[a]	Aldosterone responsiveness after the stimulation	Cohort study

DST, dexamethasone suppression test.

[a]*The effect of endogenous ACTH on aldosterone secretion was indirectly evaluated by the relative percent of suppression of aldosterone following dexamethasone suppression during at least 48 h.*

mutations based on the CYP11B2 immunohistochemistry-guided, full gene-based next-generation sequencing (61–63), future multi-center studies are warranted to identify the heterogeneous aldosterone response to synthetic ACTH by these genetic mutations.

BIASES IN EPIDEMIOLOGICAL STUDIES RELATED TO PRIMARY ALDOSTERONISM

Although these previous studies were well-conducted and prismatic, they have suffered from biases that are sometimes substantial. Herein, we describe the common types of biases (confounding, overadjustment, information bias, selection bias, and sampling bias) in epidemiological studies related to PA by using directed acyclic graphs (DAGs), a graphical tool to represent causal relationships between variables by linking them through single-headed arrows (64). In this section, to make a simple explanation, we assume that the exposure of interest is PA with subclinical hypercortisolemia (PA/SH) and the outcome of interest is CVD.

Confounding

In observational studies, we generally cannot rule out the possibility of unmeasured confounding. This is also the case for PA studies when some common causes of PA/SH and CVD are missing (**Figure 2A**). To minimize this bias, researchers should include variables that are causes of the exposure and related to the outcome, or variables that are causes of the outcome and related to the exposure (65). If some important variables are missing, quantitative bias analysis may need to be considered to investigate the potential impact of uncontrolled confounding by the unmeasured confounders (22). This method can also be applied in the meta-analysis of observational studies (66, 67). Calculating E-value is an alternative approach to simply assess how much confounding is needed to explain away the observed association (68). Furthermore, if there is information on a genetic mutation that would affect CVD only through PA/SH phenotype and does not modify the effect of PA/SH on CVD, mendelian randomization may be considered to obtain a less biased estimate (69).

Overadjustment and Collider Bias

Another important point to select covariates in the model is overadjustment. Controlling for intermediate variable such as blood pressure (BP) that lies within the causal pathway between PA/SH and CVD would introduce a biased estimate (**Figure 2B**) because we (i) fail to include the effect of PA/SH on CVD that is mediated through BP, and (ii) introduce the nonexistent relationship between PA/SH and CVD through uncontrolled BP-CVD confounders by controlling for BP, so-called "collider bias" (70, 71). A data-driven approach (including machine learning algorithms) is sometimes useful to efficiently select covariates included in the model, but does not take account of this bias, and therefore, researchers need to carefully select the potential confounders based on prior knowledge and causal diagram (65).

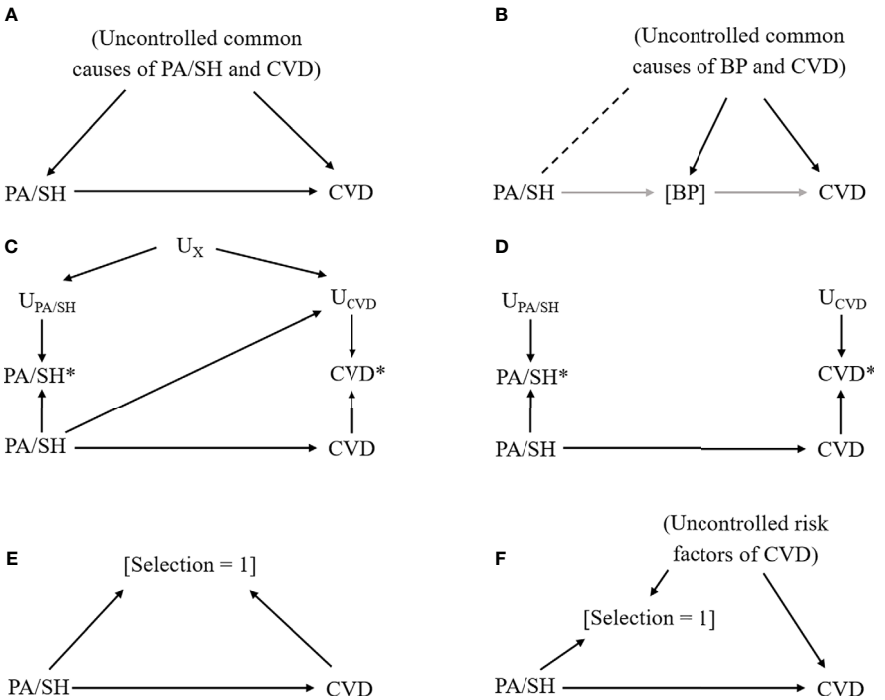

FIGURE 2 | Causal diagram representing each bias scenario in primary aldosteronism study. Notation: Exposure, primary aldosteronism/subclinical hypercortisolemia (PA/SH); Outcome, cardiovascular disease (CVD); solid arrow from X to Y, the causal effect of X on Y; [X], conditioning on X; dash line between X and Y, the nonexistent association between X and Y that could be introduced by conditioning on a variable affected by X and Y; U_X, factors that affect the measurement of X; X*, measured X (which could be different from the actual X). **(A)** Confounding bias due to uncontrolled common causes of exposure (PA/SH) and outcome (CVD). **(B)** Overadjustment and collider bias due to conditioning on an intermediate variable (blood pressure [BP]) that underestimate the total effect of PA/SH on CVD and introduce additional bias by linking PA/SH and uncontrolled common causes of BP and CVD (dashed line). **(C)** Measurement error, dependent (i.e., common factors affect measurements of exposure and outcome) and differential (i.e., exposure affects measurements of outcome, or vice versa). **(D)** Measurement error, independent and nondifferential. **(E)** Selection bias due to selecting participants by exposure and outcome status. **(F)** Selection bias due to loss to follow-up.

When there is an interest to estimate the mediation effect of such intermediate variables for the PA/SH-CVD association, causal mediation analysis may be a powerful tool to answer such research questions. For example, using causal mediation analysis, a recent study showed that elevated BP mediated 45% of the total association between serum aldosterone levels and coronary artery calcium score among general adults from a multi-ethnic group (72). This finding provided novel insight into both the direct effect (not through BP) and indirect effect (through BP) of elevated aldosterone on subclinical atherosclerosis. Meanwhile, it is also noteworthy that causal mediation analysis requires several strong assumptions and limitations. One of the hurdles of applying this method in PA studies is that relatively large sample size is generally needed to find the significant direct and indirect effect of the exposure on the outcome. Further details on this method can be found in the tutorial and methodological review paper (73).

Related to the overadjustment issue, temporal ordering between exposure, confounders, and outcome is crucial to obtain a less biased estimate. In PA studies, given the negative feedback system of RAAS and the HPA axis, it is often challenging to assume which variables (e.g., biomarker concentrations and medical conditions) occurred first at cross-sectional data. When the variables can be both confounders and mediators, researchers need to carefully interpret the results of stratified analysis by such variables because the seemingly heterogeneous findings could be biased (74). Temporal ordering can be established in longitudinal cohorts where the extraction of biosample and other information precedes the outcome assessment.

Measurement Error (Information Bias)

Because analyses are generally based on data, we need to be careful about the measurement error or misclassification of variables (23). When exposure status (i.e., whether subjects have PA/SH or not) affects the reporting of CVD and/or there are common features between exposure assessment and outcome assessment (e.g., physician diagnosis, subjects' awareness of diseases, subjects' cognitive performance, etc), our estimate would be biased (**Figure 2C**). For example, this type of bias could easily happen when the exposure assessment involves the results of the ACTH stimulation test in peripheral blood and the outcome assessment involves the results of ACTH stimulation during AVS. Recall bias is a similar type of bias that occurs when PA/SH assessment is affected by the presence of CVD. If the error for measured PA/SH status (or CVD status) is independent of the true CVD status (or PA/SH status) and there are no

common features that affect PA/SH and CVD measurement (**Figure 2D**), the bias is generally considered to be towards the null (i.e., underestimate the effect) particularly when the exposure is binary.

Selection Bias and Loss to Follow-Up

If the study restricts patients to those with confirmatory tests for PA/SH and the selection is also affected by health outcome status or its risk factors (which is often the case in a case-control study), such selection introduces collider bias limiting internal validity (**Figure 2E**). Another example is a loss to follow-up in a cohort study or a randomized controlled trial when enrollees drop the study due to health conditions related to CVD, as known as attrition bias (**Figure 2F**). Multiple imputations for missing data or inverse probability weighting approach would be helpful to minimize this type of bias when missing mechanisms can be explained by measured covariates (75).

Sampling Bias

Sampling bias is also caused by selecting participants that limit the generalizability of the study findings. Sampling bias needs to be considered in almost all studies (including clinical trials) because the study sample is often different from the population to which clinical interventions or guidelines are targeted in the real-world. This bias requires attention in PA studies given the different prevalence of PA/SH and somatic mutations in APA across countries (62, 63, 76); i.e., study results from a specific region may not simply be applied to other regions. Moreover, as shown in our literature review, some topic has tended to be heavily interested in a specific region or country, that also limits the external validity. Recently, the concept and method of generalizability and transportability have received renewed interest in causal inference literature to extend the study findings to the target population (77, 78).

CONCLUSION

In this review paper, we summarized the current state of knowledge about primary aldosteronism with cortisol cosecretion and the role of ACTH stimulation test in PA diagnosis. In summary, there is increasing evidence about the relatively high prevalence of cortisol cosecretion in PA and its potential influence on adverse health outcomes. Further studies are warranted to clarify whether there is a clinically useful cut-off points for cortisol cosecretion (i.e., serum cortisol levels during the 1-mg DST) for the PA management. The clinical usefulness of the ACTH stimulation test for PA (subtype) diagnosis has been suggested by some studies, but the evidence is limited to make a specific recommendation in clinical practice. Multicenter studies using the uniform protocol and consistent definition are needed to produce robust evidence on this topic. Moreover, heterogeneous response to ACTH signaling among patients with PA according to genetic mutations should be further investigated. We also explained the common type of biases in these epidemiological studies and also introduced some advanced methods to minimize the biases. As these epidemiological perspectives have not sufficiently been considered in PA-related literature, we hope this review would contribute to a better understanding of possible biases in epidemiological studies and would help clinicians and researchers to produce further robust epidemiological evidence and balanced discussion on these topics.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

Conceptualization, KI, TK, and TN. Methodology, KI. Formal analysis, KI. Investigation, KI, TK, and TN. Writing—original draft preparation, KI. Writing—review and editing, KI, TK, YT, JS, MO, and TN. Visualization, KI. Supervision, TN. All authors contributed to the article and approved the submitted version.

FUNDING

KI was supported by the National Institutes of Health (NIH)/ NIDDK grant F99DK126119, Toffler award at UCLA, and the Honjo International Foundation Scholarship. This article does not necessarily represent the views and policies of the NIH. The funders had no role in the design and conduct of the study, collection, management, analysis, and interpretation of the data, preparation, review, or approval of the manuscript, and decision to submit the manuscript for publication.

REFERENCES

1. Hundemer GL, Curhan GC, Yozamp N, Wang M, Vaidya A. Cardiometabolic outcomes and mortality in medically treated primary aldosteronism: a retrospective cohort study. *Lancet Diabetes Endocrinol* (2018) 6:51–9. doi: 10.1016/S2213-8587(17)30367-4
2. Mulatero P, Monticone S, Bertello C, Viola A, Tizzani D, Iannaccone A, et al. Long-term cardio- and cerebrovascular events in patients with primary aldosteronism. *J Clin Endocrinol Metab* (2013) 98:4826–33. doi: 10.1210/jc.2013-2805
3. Catena C, Colussi G, Nadalini E, Chiuch A, Baroselli S, Lapenna R, et al. Cardiovascular outcomes in patients with primary aldosteronism after treatment. *Arch Intern Med* (2008) 168:80–5. doi: 10.1001/archinternmed.2007.33
4. Rossi GP, Bernini G, Desideri G, Fabris B, Ferri C, Giacchetti G, et al. Renal damage in primary aldosteronism: results of the PAPY Study. *Hypertension* (2006) 48:232–8. doi: 10.1161/01.HYP.0000230444.01215.6a
5. Kem DC, Weinberger MH, Higgins JR, Kramer NJ, Gomez-Sanchez C, Holland OB. Plasma aldosterone response to ACTH in primary aldosteronism and in patients with low renin hypertension. *J Clin Endocrinol Metab* (1978) 46:552–60. doi: 10.1210/jcem-46-4-552

6. Arlt W, Lang K, Sitch AJ, Dietz AS, Rhayem Y, Bancos I, et al. Steroid metabolome analysis reveals prevalent glucocorticoid excess in primary aldosteronism. *JCI Insight* (2017) 2:e93136. doi: 10.1172/jci.insight.93136
7. Hanslik G, Wallaschofski H, Dietz A, Riester A, Reincke M, Allolio B, et al. Increased prevalence of diabetes mellitus and the metabolic syndrome in patients with primary aldosteronism of the German Conn's Registry. *Eur J Endocrinol* (2015) 173:665–75. doi: 10.1530/EJE-15-0450
8. Beuschlein F, Reincke M, Arlt W. The impact of Connshing's syndrome - mild cortisol excess in primary aldosteronism drives diabetes risk. *J Hypertens* (2017) 35:2548. doi: 10.1097/HJH.0000000000001550
9. Wu V-C, Chueh S-CJ, Chen L, Chang C-H, Hu Y-H, Lin Y-H, et al. Risk of new-onset diabetes mellitus in primary aldosteronism: a population study over 5 years. *J Hypertens* (2017) 35:1698–708. doi: 10.1097/HJH.0000000000001361
10. Gerards J, Heinrich DA, Adolf C, Meisinger C, Rathmann W, Sturm L, et al. Impaired Glucose Metabolism in Primary Aldosteronism Is Associated With Cortisol Cosecretion. *J Clin Endocrinol Metab* (2019) 104:3192–202. doi: 10.1210/jc.2019-00299
11. Akehi Y, Yanase T, Motonaga R, Umakoshi H, Tsuiki M, Takeda Y, et al. High Prevalence of Diabetes in Patients With Primary Aldosteronism (PA) Associated With Subclinical Hypercortisolism and Prediabetes More Prevalent in Bilateral Than Unilateral PA: A Large, Multicenter Cohort Study in Japan. *Diabetes Care* (2019) 42:938–45. doi: 10.2337/dc18-1293
12. Hiraishi K, Yoshimoto T, Tsuchiya K, Minami I, Doi M, Izumiyama H, et al. Clinicopathological features of primary aldosteronism associated with subclinical Cushing's syndrome. *Endocr J* (2011) 58:543–51. doi: 10.1507/endocrj.k10e-402
13. Fujimoto K, Honjo S, Tatsuoka H, Hamamoto Y, Kawasaki Y, Matsuoka A, et al. Primary aldosteronism associated with subclinical Cushing syndrome. *J Endocrinol Invest* (2013) 36:564–7. doi: 10.3275/8818
14. Tang L, Li X, Wang B, Ma X, Li H, Gao Y, et al. Clinical Characteristics of Aldosterone- and Cortisol-Coproducing Adrenal Adenoma in Primary Aldosteronism. *Int J Endocrinol* (2018) 2018:4920841. doi: 10.1155/2018/4920841
15. Peng K-Y, Liao H-W, Chan C-K, Lin W-C, Yang S-Y, Tsai Y-C, et al. Presence of Subclinical Hypercortisolism in Clinical Aldosterone-Producing Adenomas Predicts Lower Clinical Success. *Hypertension* (2020) 76:1537–44. doi: 10.1161/HYPERTENSIONAHA.120.15328
16. Funder JW. The Potential of ACTH in the Genesis of Primary Aldosteronism. *Front Endocrinol (Lausanne)* (2016) 7:40. doi: 10.3389/fendo.2016.00040
17. Sonoyama T, Sone M, Miyashita K, Tamura N, Yamahara K, Park K, et al. Significance of adrenocorticotropin stimulation test in the diagnosis of an aldosterone-producing adenoma. *J Clin Endocrinol Metab* (2011) 96:2771–8. doi: 10.1210/jc.2011-0573
18. Jiang Y, Zhang C, Wang W, Su T, Zhou W, Jiang L, et al. Diagnostic value of ACTH stimulation test in determining the subtypes of primary aldosteronism. *J Clin Endocrinol Metab* (2015) 100:1837–44. doi: 10.1210/jc.2014-3551
19. St-Jean M, Bourdeau I, Martin M, Lacroix A. Aldosterone is Aberrantly Regulated by Various Stimuli in a High Proportion of Patients with Primary Aldosteronism. *J Clin Endocrinol Metab* (2021) 106:e45–60. doi: 10.1210/clinem/dgaa703
20. Inoue K, Yamazaki Y, Kitamoto T, Hirose R, Saito J, Omura M, et al. Aldosterone Suppression by Dexamethasone in Patients with KCNJ5-Mutated Aldosterone-producing Adenoma. *J Clin Endocrinol Metab* (2018) 103:3477–85. doi: 10.1210/jc.2018-00738
21. Funder JW, Carey RM, Mantero F, Murad MH, Reincke M, Shibata H, et al. The Management of Primary Aldosteronism: Case Detection, Diagnosis, and Treatment: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab* (2016) 101:1889–916. doi: 10.1210/jc.2015-4061
22. Arah OA. Bias Analysis for Uncontrolled Confounding in the Health Sciences. *Annu Rev Public Health* (2017) 38:23–38. doi: 10.1146/annurev-publhealth-032315-021644
23. Hernán MA, Cole SR. Invited Commentary: Causal diagrams and measurement bias. *Am J Epidemiol* (2009) 170:959–962; discussion 963-964. doi: 10.1093/aje/kwp293
24. Hernán MA, Hernández-Díaz S, Robins JM. A structural approach to selection bias. *Epidemiology* (2004) 15:615–25. doi: 10.1097/01.ede.0000135174.63482.43
25. Fischer E, Adolf C, Pallauf A, Then C, Bidlingmaier M, Beuschlein F, et al. Aldosterone excess impairs first phase insulin secretion in primary aldosteronism. *J Clin Endocrinol Metab* (2013) 98:2513–20. doi: 10.1210/jc.2012-3934
26. Colussi G, Catena C, Lapenna R, Nadalini E, Chiuch A, Sechi LA. Insulin resistance and hyperinsulinemia are related to plasma aldosterone levels in hypertensive patients. *Diabetes Care* (2007) 30:2349–54. doi: 10.2337/dc07-0525
27. Nakajima Y, Yamada M, Taguchi R, Satoh T, Hashimoto K, Ozawa A, et al. Cardiovascular complications of patients with aldosteronism associated with autonomous cortisol secretion. *J Clin Endocrinol Metab* (2011) 96:2512–8. doi: 10.1210/jc.2010-2743
28. Fallo F, Bertello C, Tizzani D, Fassina A, Boulkroun S, Sonino N, et al. Concurrent primary aldosteronism and subclinical cortisol hypersecretion: a prospective study. *J Hypertens* (2011) 29:1773–7. doi: 10.1097/HJH.0b013e32834937f3
29. Inoue K, Yamazaki Y, Tsurutani Y, Suematsu S, Sugisawa C, Saito J, et al. Evaluation of Cortisol Production in Aldosterone-Producing Adenoma. *Horm Metab Res* (2017) 49:847–53. doi: 10.1055/s-0043-119878
30. Adolf C, Köhler A, Franke A, Lang K, Riester A, Löw A, et al. Cortisol Excess in Patients With Primary Aldosteronism Impacts Left Ventricular Hypertrophy. *J Clin Endocrinol Metab* (2018) 103:4543–52. doi: 10.1210/jc.2018-00617
31. Ohno Y, Sone M, Inagaki N, Takeda Y, Kurihara I, Tsuiki M, et al. Latent Autonomous Cortisol Secretion From Apparently Nonfunctioning Adrenal Tumor in Nonlateralized Hyperaldosteronism. *J Clin Endocrinol Metab* (2019) 104:4382–9. doi: 10.1210/jc.2018-02790
32. Kometani M, Yoneda T, Demura M, Aono D, Gondoh Y, Karashima S, et al. Genetic and epigenetic analyses of aldosterone-producing adenoma with hypercortisolemia. *Steroids* (2019) 151:108470. doi: 10.1016/j.steroids.2019.108470
33. Bhatt PS, Sam AH, Meeran KM, Salem V. The relevance of cortisol co-secretion from aldosterone-producing adenomas. *Hormones (Athens)* (2019) 18:307–13. doi: 10.1007/s42000-019-00114-8
34. Handgriff L, Adolf C, Heinrich DA, Braun L, Nirschl N, Sturm L, et al. The Impact of Glucocorticoid Co-Secretion in Primary Aldosteronism on Thyroid Autoantibody Titers During the Course of Disease. *Horm Metab Res* (2020) 52:404–11. doi: 10.1055/a-1164-1944
35. O'Toole SM, Sze W-CC, Chung T-T, Akker SA, Druce MR, Waterhouse M, et al. Low-grade Cortisol Cosecretion Has Limited Impact on ACTH-stimulated AVS Parameters in Primary Aldosteronism. *J Clin Endocrinol Metab* (2020) 105:e3776–84. doi: 10.1210/clinem/dgaa519
36. Piaditis GP, Kaltsas GA, Androulakis II, Gouli A, Makras P, Papadogias D, et al. High prevalence of autonomous cortisol and aldosterone secretion from adrenal adenomas. *Clin Endocrinol (Oxf)* (2009) 71:772–8. doi: 10.1111/j.1365-2265.2009.03551.x
37. Gomez-Sanchez CE, Qi X, Velarde-Miranda C, Plonczynski MW, Parker CR, Rainey W, et al. Development of monoclonal antibodies against human CYP11B1 and CYP11B2. *Mol Cell Endocrinol* (2014) 383:111–7. doi: 10.1016/j.mce.2013.11.022
38. Nakamura Y, Maekawa T, Felizola SJA, Satoh F, Qi X, Velarde-Miranda C, et al. Adrenal CYP11B1/2 expression in primary aldosteronism: immunohistochemical analysis using novel monoclonal antibodies. *Mol Cell Endocrinol* (2014) 392:73–9. doi: 10.1016/j.mce.2014.05.002
39. Fallo F, Castellano I, Gomez-Sanchez CE, Rhayem Y, Pilon C, Vicennati V, et al. Histopathological and genetic characterization of aldosterone-producing adenomas with concurrent subclinical cortisol hypersecretion: a case series. *Endocrine* (2017) 58:503–12. doi: 10.1007/s12020-017-1295-4
40. Gioco F, Seccia TM, Gomez-Sanchez EP, Rossi GP, Gomez-Sanchez CE. Adrenal histopathology in primary aldosteronism: is it time for a change? *Hypertension* (2015) 66:724–30. doi: 10.1161/HYPERTENSIONAHA.115.05873
41. Mihailidou AS, Loan Le TY, Mardini M, Funder JW. Glucocorticoids activate cardiac mineralocorticoid receptors during experimental myocardial infarction. *Hypertension* (2009) 54:1306–12. doi: 10.1161/HYPERTENSIONAHA.109.136242
42. Goupil R, Wolley M, Ahmed AH, Gordon RD, Stowasser M. Does concomitant autonomous adrenal cortisol overproduction have the potential to confound the interpretation of adrenal venous sampling in primary aldosteronism? *Clin Endocrinol (Oxf)* (2015) 83:456–61. doi: 10.1111/cen.12750
43. Mulatero P, Monticone S, Rainey WE, Veglio F, Williams TA. Role of KCNJ5 in familial and sporadic primary aldosteronism. *Nat Rev Endocrinol* (2013) 9:104–12. doi: 10.1038/nrendo.2012.230

44. Wu V-C, Wang S-M, Chueh S-CJ, Yang S-Y, Huang K-H, Lin Y-H, et al. The prevalence of CTNNB1 mutations in primary aldosteronism and consequences for clinical outcomes. *Sci Rep* (2017) 7:39121. doi: 10.1038/srep39121
45. Rhayem Y, Perez-Rivas LG, Dietz A, Bathon K, Gebhard C, Riester A, et al. PRKACA Somatic Mutations Are Rare Findings in Aldosterone-Producing Adenomas. *J Clin Endocrinol Metab* (2016) 101:3010–7. doi: 10.1210/jc.2016-1700
46. Young MJ, Funder JW. The renin-angiotensin-aldosterone system in experimental mineralocorticoid-salt-induced cardiac fibrosis. *Am J Physiology-Endocrinology Metab* (1996) 271:E883–8. doi: 10.1152/ajpendo.1996.271.5.E883
47. Young M, Funder JW. Eplerenone, But Not Steroid Withdrawal, Reverses Cardiac Fibrosis in Deoxycorticosterone/ Salt-Treated Rats. *Endocrinology* (2004) 145:3153–7. doi: 10.1210/en.2004-0005
48. Markou A, Sertedaki A, Kaltsas G, Androulakis II, Marakaki C, Pappa T, et al. Stress-induced Aldosterone Hyper-Secretion in a Substantial Subset of Patients With Essential Hypertension. *J Clin Endocrinol Metab* (2015) 100:2857–64. doi: 10.1210/jc.2015-1268
49. Umakoshi H, Xiaomei Y, Ichijo T, Kamemura K, Matsuda Y, Fujii Y, et al. Reassessment of the cosyntropin stimulation test in the confirmatory diagnosis and subtype classification of primary aldosteronism. *Clin Endocrinol (Oxf)* (2017) 86:170–6. doi: 10.1111/cen.13167
50. Terui K, Kageyama K, Nigawara T, Moriyama T, Sakihara S, Takayasu S, et al. Evaluation of the (1–24) adrenocorticotropin stimulation test for the diagnosis of primary aldosteronism. *J Renin Angiotensin Aldosterone Syst* (2016) 17:1470320315625703. doi: 10.1177/1470320315625703
51. Inoue K, Omura M, Sugisawa C, Tsurutani Y, Saito J, Nishikawa T. Clinical Utility of the Adrenocorticotropin Stimulation Test with/without Dexamethasone Suppression for Definitive and Subtype Diagnosis of Primary Aldosteronism. *Int J Mol Sci* (2017) 18:948. doi: 10.3390/ijms18050948
52. Moriya A, Yamamoto M, Kobayashi S, Nagamine T, Takeichi-Hattori N, Nagao M, et al. ACTH stimulation test and computed tomography are useful for differentiating the subtype of primary aldosteronism. *Endocrine J* (2017) 64:65–73. doi: 10.1507/endocrj.EJ16-0297
53. Kita T, Furukoji E, Sakae T, Kitamura K. Efficient screening of patients with aldosterone-producing adenoma using the ACTH stimulation test. *Hypertension Res* (2019) 42:801–6. doi: 10.1038/s41440-018-0191-5
54. Kidoguchi S, Sugano N, Kawauchi R, Nakashima D, Hayashi-Ishikawa N, Tokudome G, et al. Evaluation of various confirmatory tests for the diagnosis of aldosterone-producing adenoma. *J Renin Angiotensin Aldosterone Syst* (2020) 21:1470320320919610. doi: 10.1177/1470320320919610
55. Stowasser M, Klemm SA, Tunny TJ, Gordon RD. Plasma aldosterone response to ACTH in subtypes of primary aldosteronism. *Clin Exp Pharmacol Physiol* (1995) 22:460–2. doi: 10.1111/j.1440-1681.1995.tb02044.x
56. Kitamoto T, Kitamoto KK, Omura M, Takiguchi T, Tsurutani Y, Kubo H, et al. Precise Mapping of Intra-Adrenal Aldosterone Activities Provides a Novel Surgical Strategy for Primary Aldosteronism. *Hypertension* (2020) 76:976–84. doi: 10.1161/HYPERTENSIONAHA.119.14341.
57. Laurent I, Astère M, Zheng F, Chen X, Yang J, Cheng Q, et al. Adrenal venous sampling with or without adrenocorticotropic hormone stimulation: A meta-analysis. *J Clin Endocrinol Meta* (2018) 104:1060–8. doi: 10.1210/jc.2018-01324
58. Deinum J, Groenewoud H, van der Wilt GJ, Lenzini L, Rossi GP. Adrenal venous sampling: cosyntropin stimulation or not? *Eur J Endocrinol* (2019) 181:D15–26. doi: 10.1530/EJE-18-0844
59. Kitamoto T, Suematsu S, Yamazaki Y, Nakamura Y, Sasano H, Matsuzawa Y, et al. Clinical and Steroidogenic Characteristics of Aldosterone-Producing Adenomas With ATPase or CACNA1D Gene Mutations. *J Clin Endocrinol Metab* (2016) 101:494–503. doi: 10.1210/jc.2015-3284
60. Azizan EAB, Lam BYH, Newhouse SJ, Zhou J, Kuc RE, Clarke J, et al. Microarray, qPCR, and KCNJ5 sequencing of aldosterone-producing adenomas reveal differences in genotype and phenotype between zona glomerulosa- and zona fasciculata-like tumors. *J Clin Endocrinol Metab* (2012) 97:E819–829. doi: 10.1210/jc.2011-2965
61. Nanba K, Omata K, Else T, Beck PCC, Nanba AT, Turcu AF, et al. Targeted Molecular Characterization of Aldosterone-Producing Adenomas in White Americans. *J Clin Endocrinol Metab* (2018) 103:3869–76. doi: 10.1210/jc.2018-01004
62. Nanba K, Omata K, Gomez-Sanchez CE, Stratakis CA, Demidowich AP, Suzuki M, et al. Genetic Characteristics of Aldosterone-Producing Adenomas in Blacks. *Hypertension* (2019) 73:885–92. doi: 10.1161/HYPERTENSIONAHA.118.12070
63. Nanba K, Yamazaki Y, Bick N, Onodera K, Tezuka Y, Omata K, et al. Prevalence of Somatic Mutations in Aldosterone-Producing Adenomas in Japanese Patients. *J Clin Endocrinol Metab* (2020) 105:e4066–73. doi: 10.1210/clinem/dgaa595
64. Greenland S, Pearl J, Robins JM. Causal diagrams for epidemiologic research. *Epidemiology* (1999) 10(1):37–48.
65. VanderWeele TJ. Principles of confounder selection. *Eur J Epidemiol* (2019) 34:211–9. doi: 10.1007/s10654-019-00494-6
66. Goto A, Arah OA, Goto M, Terauchi Y, Noda M. Severe hypoglycaemia and cardiovascular disease: systematic review and meta-analysis with bias analysis. *BMJ* (2013) 347:f4533. doi: 10.1136/bmj.f4533
67. Inoue K, Beekley J, Goto A, Jeon CY, Ritz BR. Depression and cardiovascular disease events among patients with type 2 diabetes: A systematic review and meta-analysis with bias analysis. *J Diabetes Complicat* (2020) 34:107710. doi: 10.1016/j.jdiacomp.2020.107710
68. VanderWeele TJ, Ding P. Sensitivity Analysis in Observational Research: Introducing the E-Value. *Ann Intern Med* (2017) 167:268–74. doi: 10.7326/M16-2607
69. Davies NM, Holmes MV, Davey Smith G. Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. *BMJ* (2018) 362:k601. doi: 10.1136/bmj.k601
70. Greenland S. Quantifying biases in causal models: classical confounding vs collider-stratification bias. *Epidemiology* (2003) 14:300–6. doi: 10.1097/01.EDE.0000042804.12056.6C
71. Cole SR, Platt RW, Schisterman EF, Chu H, Westreich D, Richardson D, et al. Illustrating bias due to conditioning on a collider. *Int J Epidemiol* (2010) 39:417–20. doi: 10.1093/ije/dyp334
72. Inoue K, Goldwater D, Allison M, Seeman T, Kestenbaum BR, Watson KE. Serum Aldosterone Concentration, Blood Pressure, and Coronary Artery Calcium: The Multi-Ethnic Study of Atherosclerosis. *Hypertension* (2020) 76:113–20. doi: 10.1161/HYPERTENSIONAHA.120.15006
73. VanderWeele TJ. Mediation Analysis: A Practitioner's Guide. *Annu Rev Public Health* (2016) 37:17–32. doi: 10.1146/annurev-publhealth-032315-021402
74. Inoue K, Goto A, Sugiyama T, Ramlau-Hansen CH, Liew Z. The Confounder-Mediator Dilemma: Should We Control for Obesity to Estimate the Effect of Perfluoroalkyl Substances on Health Outcomes? *Toxics* (2020) 8:125. doi: 10.3390/toxics8040125
75. Howe LD, Tilling K, Galobardes B, Lawlor DA. Loss to Follow-up in Cohort Studies. *Epidemiology* (2013) 24:1–9. doi: 10.1097/EDE.0b013e31827623b1
76. Fernandes-Rosa FL, Williams TA, Riester A, Steichen O, Beuschlein F, Boulkroun S, et al. Genetic spectrum and clinical correlates of somatic mutations in aldosterone-producing adenoma. *Hypertension* (2014) 64:354–61. doi: 10.1161/HYPERTENSIONAHA.114.03419
77. Bareinboim E, Pearl J. Causal inference and the data-fusion problem. *PNAS* (2016) 113:7345–52. doi: 10.1073/pnas.1510507113
78. Dahabreh IJ, Robertson SE, Steingrimsson JA, Stuart EA, Hernán MA. Extending inferences from a randomized trial to a new target population. *Stat Med* (2020) 39:1999–2014. doi: 10.1002/sim.8426

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

ORIGINAL RESEARCH
published: 16 March 2021
doi: 10.3389/fendo.2021.644382

Aldosterone-Regulating Receptors and Aldosterone-Driver Somatic Mutations

Jung Soo Lim[1,2], *Samuel W. Plaska*[1], *Juilee Rege*[1], *William E. Rainey*[1,3] *and Adina F. Turcu*[3*]

[1] Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI, United States, [2] Division of Endocrinology and Metabolism, Department of Internal Medicine, Yonsei University Wonju College of Medicine, Wonju Severance Christian Hospital, Wonju, South Korea, [3] Division of Metabolism, Endocrine, and Diabetes, University of Michigan, Ann Arbor, MI, United States

Background: Somatic gene mutations that facilitate inappropriate intracellular calcium entrance have been identified in most aldosterone-producing adenomas (APAs). Studies suggest that angiotensin II and adrenocorticotropic hormone (ACTH) augment aldosterone production from APAs. Little is known, however, regarding possible variations in response to hormonal stimuli between APAs with different aldosterone-driver mutations.

Objective: To analyze the transcript expression of type 1 angiotensin II receptors (*AGTR1*), ACTH receptors (*MC2R*), and melanocortin 2 receptor accessory protein (*MRAP*) in APAs with known aldosterone-driver somatic mutations.

Methods: RNA was isolated from APAs with mutations in: *KCNJ5* (n = 14), *ATP1A1* (n = 14), *CACNA1D* (n = 14), and *ATP2B3* (n = 5), and from normal adjacent adrenal tissue (n = 45). Transcript expression of *MC2R*, *MRAP*, *AGTR1*, aldosterone synthase (*CYP11B2*), 17α-hydroxylase/17,20-lyase (*CYP17A1*), and 11β-hydroxylase (*CYP11B1*) were quantified using quantitative RT-PCR and normalized to β-actin.

Results: Compared to adjacent normal adrenal tissue, APAs had higher transcript levels of *CYP11B2* (2,216.4 [1,112.0, 2,813.5]-fold, $p < 0.001$), *MC2R* (2.88 [2.00, 4.52]-fold, $p < 0.001$), and *AGTR1* (1.80 [1.02, 2.80]-fold, $p < 0.001$]), and lower transcript levels of *MRAP*, *CYP17A1*, and *CYP11B1* (0.28–0.36, $p < 0.001$ for all). *MC2R* and *CYP11B2* transcripts were lower in APAs with *KCNJ5 vs.* other mutations ($p < 0.01$ for both). *MC2R* expression correlated positively with that of *AGTR1* in APAs harboring *KCNJ5* and *CACNA1D* mutations, and with *MRAP* expression in APAs harboring *ATP*ase mutations.

Conclusions: While *MC2R* and *AGTR1* are expressed in all APAs, differences were observed based on the underlying aldosterone-driver somatic mutations. In tandem, our findings suggest that APAs with *ATPase*-mutations are more responsive to ACTH than *KCNJ5*-mutated APAs.

Keywords: primary aldosteronism, aldosterone, angiotensin, adrenocorticotropic hormone (ACTH), adrenal, adrenal cortex

OPEN ACCESS

Edited by:
Vin-Cent Wu,
National Taiwan University, Taiwan

Reviewed by:
Masanori Murakami,
Tokyo Medical and Dental University, Japan
Silvia Monticone,
University of Turin, Italy

***Correspondence:**
Adina F. Turcu
aturcu@umich.edu

Specialty section:
This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Endocrinology

Received: *21 December 2020*
Accepted: *08 February 2021*
Published: *16 March 2021*

Citation:
Lim JS, Plaska SW, Rege J, Rainey WE and Turcu AF (2021) Aldosterone-Regulating Receptors and Aldosterone-Driver Somatic Mutations. Front. Endocrinol. 12:644382. doi: 10.3389/fendo.2021.644382

INTRODUCTION

Primary aldosteronism (PA) is characterized by inappropriate, renin-independent aldosterone production. PA is the most common curable form of secondary hypertension, accounting for up to 20% of resistant hypertension cases (1). Growing evidence suggests that PA increases the risk of cardiovascular and renal complications as compared to essential hypertension, independently of blood pressure control (2–4). Inappropriate mineralocorticoid receptor activation might promote the release of pro-inflammatory cytokines (5), oxidative stress (6), and, consequently, target organ damage (2, 4). Sporadic PA is broadly classified as bilateral adrenal hyperaldosteronism (BHA) or unilateral PA, which is often caused by an aldosterone-producing adenoma (APA). APAs account for 30–50% of PA cases and they can be cured by adrenalectomy, while BHA requires life-long targeted medical therapy (7). PA subtyping is typically established based on adrenal venous sampling (AVS) (7). In many centers, AVS is performed after administration of cosyntropin, a synthetic adrenocorticotropic hormone (ACTH), which enhances the confidence of successful adrenal vein catheterization and circumvents intrinsic ACTH fluctuations that might occur due to the stress of the procedure. Reports regarding the impact of ACTH on APAs, however, have been inconsistent (8–12).

Studies conducted over the past decade have identified a series of aldosterone–driver gene mutations in familial and sporadic forms of PA. Affected genes include: *KCNJ5* (13), *ATP1A1* (14, 15), *ATP2B3* (15), *CACNA1D* (16), *CACNA1H* (17), *CTNNB1* (18), and *CLCN2* (19, 20). Next-generation sequencing (NGS) of aldosterone-producing areas precisely mapped using immunohistochemistry (IHC) for aldosterone synthase (CYP11B2) has revealed aldosterone-driver somatic mutations in over 90% of APAs (21–23). A shared molecular feature of the somatic mutations found in APAs is that they facilitate intracellular calcium entrance, which then stimulates aldosterone production by augmenting CYP11B2 expression (23). Nonetheless, APAs harboring different aldosterone-driver somatic mutations have distinct histopathological features (24), steroidogenic potential (25), and responses to ACTH stimulation (26).

In addition to ion channel or pump mutations, some studies suggest that the aberrant expression of receptors in APAs, such as G-protein coupled receptors (GPCRs), might contribute to their dysregulated aldosterone production (27–29). Under physiological conditions, angiotensin II, serum potassium, and, to a lesser extent, ACTH control aldosterone synthesis from the adrenal zona glomerulosa (ZG) (30, 31). Variability in type 1 angiotensin II receptor (*AGTR1*) and melanocortin type 2 receptor (*MC2R*, also known as ACTH receptor) expression, which is abundant in both APAs and normal adrenals (29), might modulate aldosterone production (30, 31). Although cellular models of aldosterone-driver mutations showed that responses to angiotensin II are increased (32, 33), data on possible variations in response to hormonal stimuli between APAs with different somatic mutations are scarce. Herein, we investigated the transcript expression of *AGTR1*, *MC2R*, and melanocortin-2-receptor accessory protein (*MRAP*) in APAs with known aldosterone-driver somatic mutations and in adjacent normal adrenal tissue. In addition, we assessed the relationship between aldosterone-regulators and *CYP11B2* expression in APAs with different somatic mutations.

MATERIALS AND METHODS

Tissue Samples

The current study included adrenals from 47 patients with APA who underwent adrenalectomy at the University of Michigan between 2004 and 2018. Patients were selected based on availability of formalin-fixed paraffin-embedded (FFPE) adrenal tumor blocks. The clinical diagnosis of PA was made according to the institutional consensus available at the time or the Endocrine Society Clinical Practice guidelines (7). All adrenal specimens were pathologically diagnosed as adrenocortical adenomas. For comparison, we used adjacent normal adrenal tissue obtained from the same patients. Because the availability of adrenal tissue adjacent to the APA was limited, cortical and medullary tissue were not dissected separately. Sections from FFPE adrenal tumor blocks were used for IHC for CYP11B2 and 17α-hydroxylase/17,20-lyase (CYP17A1) and for genetic analysis, as previously described (21). This study was approved by Institutional Review Boards at the University of Michigan (HUM00106809, HUM00024461, HUM00083056). Written informed consent was obtained from all patients who underwent adrenalectomy after February, 2011. A waiver of consent was granted for the use of archival specimens (HUM00083056).

DNA/RNA Isolation

Genomic DNA (gDNA) and RNA were obtained from APAs with mutations in: *KCNJ5* (n = 14), *ATP1A1* (n = 14), *CACNA1D* (n = 14), and *ATP2B3* (n = 5), and from adjacent normal adrenal tissues (n = 45). Adrenocortical adenomas that displayed CYP11B2-expressing cells were considered APAs. After identification of CYP11B2-positive areas by IHC, four to nine unstained consecutive 5 μm FFPE slides were used to separately dissect corresponding CYP11B2-positive areas. Dissection of FFPE sections was performed using disposable scalpels under an Olympus SZ-40 microscope. The AllPrep DNA/RNA FFPE kit (QIAGEN, Hilden, Germany) was used to isolate gDNA and RNA, as previously described (34).

Next-Generation Sequencing

For mutation analysis, multiplexed PCR–based NGS was conducted using Ion Torrent Ampliseq sequencing (Thermo Fisher Scientific), as previously described (21, 34). The panel for library preparation included amplicons targeting the full coding regions of known aldosterone-driving genes, including the most commonly affected: *KCNJ5*, *ATP1A1*, *CACNA1D*, and *ATP2B3*. APAs with other aldosterone-driver mutations were not included in this analysis, due to their low prevalence.

Quantitative Real-Time RT-PCR (qPCR)

Total RNA was reverse transcribed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). qPCR was

performed using the ABI StepOnePlus Real-Time PCR systems (Applied Biosystems). *CYP11B2*, *CYP17A1*, and *CYP11B1* primer/probe mixtures were prepared as previously described (27, 35). For Human *MRAP* qPCR, the primer (qHsaCID0022591, Bio-Rad) was mixed with SYBR Green PCR master mix (Applied Biosystems). Primer/probe mixtures for the amplification of *AGTR1* (Hs00258938_m1), *MC2R* (Hs00300820_s1), and β-actin (*ACTB*; Hs01060665_g1) were purchased from Applied Biosystems. In this study, *ACTB* transcript was used as a reference gene for normalization between samples. Relative quantification was determined using the comparative threshold cycle method (36). The average ΔCT value of all adjacent normal tissues was used as reference when comparing gene expression between APAs with various underlying mutations.

Statistical Analysis

Statistical analyses were conducted using SAS 9.4 (SAS Institute, Cary, NC, USA), and GraphPad Prism 8 was used to generate figures. The Kruskal-Wallis test, followed by the Dwass-Steel-Critchlow-Fligner test were employed to compare continuous variables across multiple groups. Distribution of categorical variables across groups was assessed by the Chi-square or Fisher's exact test. Wilcoxon signed-rank test was used for paired comparison of transcript levels between APAs and the corresponding adjacent normal adrenal tissues. Correlations between gene expressions were examined with the Spearman correlation test. Two-sided p values below 0.05 were considered statistically significant.

RESULTS

Demographic and clinical characteristics of study participants are presented in **Table 1**. Most patients were Caucasian, with ages between 20 and 79 years (median age 52) and 62% were men. Patients with APAs harboring *KCNJ5* mutations were younger, leaner, and mostly women (**Table 1**).

AGTR1, MC2R, MRAP, CYP11B2, CYP17A1, and CYP11B1 Gene Expressions in Aldosterone-Producing Adenomas

Overall, APAs displayed higher transcript levels of *MC2R* (2.88 [2.00, 4.52]-fold, $p < 0.001$), *AGTR1* (1.80 [1.02, 2.80]-fold, $p < 0.001$), and *CYP11B2* (2216.4 [1112.0, 2813.5]-fold, $p < 0.001$) compared to the corresponding adjacent normal adrenal tissue, and these differences remained robust in APAs with *CACNA1D* and *ATP1A1* mutations (**Table 2**). *AGTR1* and *MC2R* transcript levels were only minimally, but not significantly higher in *KCNJ5*-mutated APAs as compared to the paired adjacent normal adrenal tissue. Conversely, APAs had lower transcript levels of *MRAP*, *CYP17A1*, and *CYP11B1* (0.28–0.36-fold, $p < 0.001$, **Table 2**) than the corresponding normal adjacent adrenal tissue and these differences were observed in all mutation subgroups.

APAs harboring *KCNJ5* mutations displayed lower *MC2R* and *CYP11B2* mRNA expressions compared to other APAs (**Figures 1B, C**), while *AGTR1* and *MRAP* transcript levels were relatively similar between mutation groups (**Figures 1A, D**).

Correlations Between Aldosterone Regulators and Steroidogenic Enzymes in Aldosterone-Producing Adenomas

Overall, APA *CYP11B2* expression correlated positively with *MC2R* (r = 0.77, $p < 0.0001$) and *AGTR1* (r = 0.52, $p = 0.0002$, **Figure 2**), and inversely with *CYP17A1* and *CYP11B1* (r = −0.3, $p < 0.05$ for both). The strongest correlations between *CYP11B2* and both *MC2R* and *AGTR1* were observed in *ATP1A1*-mutated APAs (r = 0.77, $p = 0.001$ and r = 0.61, $p = 0.021$, respectively).

APAs with *CACNA1D* and *KCNJ5* mutations displayed tight positive correlations between *MC2R* and *AGTR1* transcripts (r = 0.75, $p = 0.002$ and r = 0.65, $p = 0.012$, respectively), while no significant correlations were found in APAs with *ATPase* mutations. Conversely, *MC2R* and *MRAP* expressions correlated positively only in *ATP1A1*- and *ATP2B3*-mutated APAs (r = 0.62, $p = 0.018$ and r = 0.90, $p = 0.037$, respectively).

TABLE 1 | Baseline characteristics of patients with APA participating in this study.

	Total (n = 47)	*KCNJ5* (n = 14)	*ATP1A1* (n = 14)	*CACNA1D* (n = 14)	*ATP2B3* (n = 5)	*p* value
Age (years)	52.0 (20, 79)	42.0 (20, 56)	55.5 (41, 79)	53.0 (32, 78)	59.0 (53, 75)	0.002
Sex (n men, %)	29 (61.7%)	1 (7.1%)	12 (85.7%)	11 (78.6%)	5 (100%)	<0.001
Race (n)	C (38), AA (4), A (1), U (4)	C (10), AA (1), A (1), U (2)	C (13), U (1)	C (11), AA (2), U (1)	C (4), AA (1)	0.496
BMI (kg/m^2) [n = 33]	30.6 [26.2, 35.7]	25.2 [23.2, 33.4]	34.7 [31.9, 40.6]	30.6 [26.8, 33.9]	29.1 [26.1, 30.6]	0.024
SBP (mmHg) [n = 44]	145.5 [130.3, 167.5]	141.0 [128.0, 175.0]	158.5 [130.5, 182.0]	145.0 [134.3, 159.8]	149.0 [135.5, 165.5]	0.779
DBP (mmHg) [n = 44]	86.0 [74.0, 91.8]	76.0 [70.0, 92.5]	90.0 [83.0, 96.3]	85.5 [74.5, 98.0]	78.0 [73.0, 84.5]	0.270
Serum Cr (mg/dl) [n = 30]	0.90 [0.79, 1.10]	0.78 [0.69, 0.90]	0.94 [0.81, 1.09]	1.03 [0.83, 1.23]	1.50 [1.20, 3.43]	0.003
Serum potassium (mmol/L) [n = 43]	3.4 [2.9, 3.8]	3.3 [2.9, 3.9]	3.4 [2.9, 3.7]	3.6 [3.4, 3.8]	3.2 [3.0, 3.9]	0.462
PAC (ng/dl) [n = 44]	29.1 [21.7, 60.2]	26.2 [19.6, 36.1]	29.7 [23.3, 98.4]	27.4 [21.7, 48.1]	80.0 [27.1, 230.0]	0.296
PRA (ng/ml/hr) [n = 31]	0.20 [0.10, 0.60]	0.10 [0.07, 0.60]	0.10 [0.10, 0.40]	0.30 [0.15, 0.75]	0.30 [0.10, 0.73]	0.399

Continuous variables are expressed as median [interquartile range], except for age, which is expressed as median (range).

APA, aldosterone-producing adenoma; C, Caucasian; AA, African American; A, Asian; U, unknown; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; Cr, creatinine; PAC, plasma aldosterone concentration; PRA, plasma renin activity.

TABLE 2 | Paired comparisons of transcript levels of *AGTR1*, *MC2R*, *MRAP*, and steroidogenic enzymes between APAs and adjacent normal adrenal tissue.

	AGTR1	*MC2R*	*CYP11B2*	*MRAP*	*CYP17A1*	*CYP11B1*
All APAs						
APAs	1.80 [1.02, 2.80]	2.88 [2.00, 4.52]	2,216.40 [1,111.98, 2,813.45]	0.36 [0.18, 0.59]	0.30 [0.15, 0.43]	0.28 [0.19, 0.56]
Adjacent adrenal tissue	0.99 [0.64, 1.49]	0.99 [0.65, 1.43]	1.07 [0.35, 2.70]	0.97 [0.64, 1.65]	0.99 [0.78, 1.31]	1.04 [0.78, 1.27]
***p* value**	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
***KCNJ5*-mutated APAs**						
APAs	1.37 [1.06, 2.11]	1.90 [1.13, 2.52]	911.30 [502.92, 1,212.01]	0.41 [0.23, 0.66]	0.32 [0.28, 0.53]	0.53 [0.20, 0.81]
Adjacent adrenal tissue	0.99 [0.58, 1.35]	0.98 [0.53, 1.80]	0.38 [0.15, 2.31]	1.15 [0.83, 1.85]	0.92 [0.80, 1.17]	1.06 [0.75, 1.30]
***p* value**	0.101	0.064	0.001	0.004	0.002	0.002
***CACNA1D*-mutated APAs**						
APAs	2.25 [1.52, 2.89]	3.48 [2.57, 4.36]	2,559.10 [1,506.43, 3,273.80]	0.32 [0.12, 0.58]	0.28 [0.19, 0.45]	0.20 [0.15, 0.36]
Adjacent adrenal tissue	1.14 [0.79, 1.61]	1.08 [0.67, 1.51]	1.09 [0.49, 2.20]	1.29 [0.89, 2.36]	1.34 [0.90, 1.49]	1.04 [0.77, 1.20]
***p* value**	0.013	0.001	0.001	0.001	0.001	0.001
***ATP1A1*-mutated APAs**						
APAs	1.57 [0.98, 3.01]	5.13 [2.35, 7.55]	2,329.07 [1,519.96, 4,213.90]	0.43 [0.22, 0.58]	0.18 [0.10, 0.40]	0.31 [0.25, 0.48]
Adjacent adrenal tissue	1.16 [0.65, 1.56]	1.16 [0.73, 1.36]	1.59 [0.87, 7.77]	0.66 [0.60, 1.17]	0.85 [0.67, 1.24]	0.96 [0.76, 1.66]
***p* value**	0.013	0.001	0.001	0.002	0.001	0.001
***ATP2B3*-mutated APAs**						
APAs	2.91 [1.02, 6.97]	4.18 [2.58, 6.34]	2,736.94 [1,755.25, 4,163.27]	0.36 [0.14, 0.63]	0.20 [0.03, 0.49]	0.19 [0.16, 0.55]
Adjacent adrenal tissue	0.69 [0.57, 0.98]	0.73 [0.48, 1.14]	0.51 [0.25, 1.94]	0.65 [0.52, 0.78]	0.88 [0.72, 1.04]	1.07 [0.73, 1.09]
***p* value**	0.144	0.068	0.068	0.068	0.068	0.068

qPCR data are shown as fold changes normalized to β-actin (ACTB). Continuous variables are expressed as median [interquartile range].
APA, aldosterone-producing adenoma; AGTR1, type 1 angiotensin II receptor; MC2R, melanocortin type 2 receptors (ACTH receptors); CYP11B2, aldosterone synthase; MRAP, melanocortin 2 receptor accessory protein; CYP17A1, 17α-hydroxylase; CYP11B1, 11β-hydroxylase.

DISCUSSION

In this study, we delineate differential gene expression of the primary aldosterone regulatory receptors in APAs with different underlying mutations. We found that APAs displayed higher mRNA expression of both *MC2R* and *AGTR1* than adjacent normal adrenal tissue. In addition, we show that the expression patterns of *MC2R* and *AGTR1*, and their associations with *CYP11B2* transcripts differ between APAs with various underlying aldosterone-driver somatic mutations.

Under physiological conditions, angiotensin II induces Gi-mediated cell membrane depolarization and increases intracellular calcium signaling, thereby stimulating acute steroid production as a result of increased steroidogenic acute regulatory protein (StAR) protein expression (31). Furthermore, this elevation in intracellular calcium activates a cascade of signaling events that lead to increased *CYP11B2* transcription and aldosterone secretion from ZG cells (30, 37). Although PA is theoretically renin-independent, aldosterone excess may also result from aberrant receptor expression within APAs and/or hypersensitivity to physiological stimuli. A variety of autocrine and paracrine regulatory factors (38) can activate ectopic or aberrant receptors, which may govern aldosterone secretion independently from the suppressed renin-angiotensin system (29, 39). Indeed, mRNA expressions of *AGTRI* and *MC2R* were previously reported to be higher in APA tissues compared to healthy adult adrenals (27, 29, 40). The effects of posture, angiotensin II infusion, and angiotensin converting enzyme inhibitors have been shown to differ in APA when compared to BHA, although results have been variable (7, 41–43). In our study, *AGTR1* transcript levels tended to be higher in APAs as compared to adjacent normal adrenal tissue. Tunny and colleagues found that angiotensin II-unresponsive APAs were more common in women, while those responsive to angiotensin II were more prevalent in men (41). Indeed, we herein found that *KCNJ5*-mutated APAs, which are most prevalent in women of all races (44–46), expressed *AGTR1* transcript levels comparable to those found in the corresponding normal adrenal tissue.

In contrast with angiotensin II and potassium, ACTH stimulates aldosterone secretion acutely but transiently (31, 47). Aldosterone production follows a circadian rhythm that parallels that of ACTH both in normal individuals, as well as in patients with PA (48, 49). In patients with aldosterone-secreting tumors, plasma aldosterone concentration starts to fall around mid-morning, as ACTH levels decrease, in spite of upright posture (39). The relative impact of ACTH on aldosterone production from APA *vs.* BHA and normal ZG cells remains incompletely understood. Small studies suggest that APAs might be more sensitive to ACTH stimulation and suppression than BHA and normal adrenals (49). Asian studies (50–52) indicated that the response of aldosterone to cosyntropin stimulation, with or without *a priori* overnight suppression with 1mg dexamethasone, is higher in patients with APA than in those with BHA. Nevertheless, AVS data have shown that aldosterone lateralization might be apparent only prior to or exclusively after cosyntropin stimulation (8, 9, 53). Washout of a baseline aldosterone gradient between the two adrenal glands following cosyntropin stimulation indicates a relatively higher response

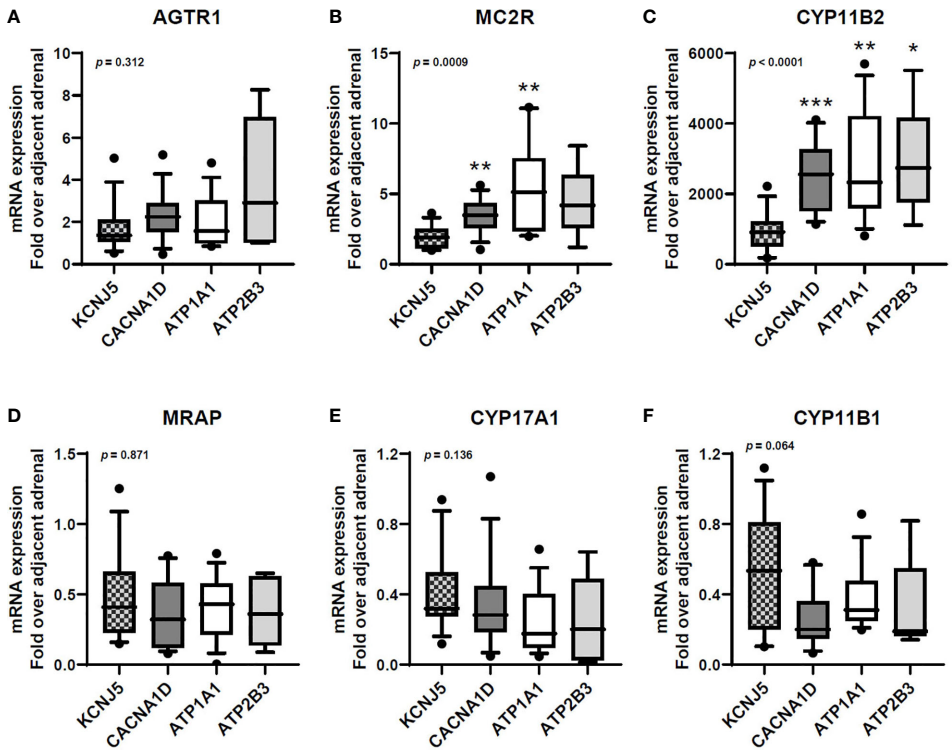

FIGURE 1 | Transcript expression of *AGTR1* **(A)**, *MC2R* **(B)**, *CYP11B2* **(C)**, *MRAP* **(D)**, *CYP17A1* **(E)**, and *CYP11B1* **(F)** in aldosterone-producing adenomas with different aldosterone-driver somatic mutations. qPCR data are shown as the fold changes normalized to β-actin (ACTB). *AGTR1*, type 1 angiotensin II receptor; *MC2R*, melanocortin type 2 receptors (ACTH receptors); *CYP11B2*, aldosterone synthase; *MRAP*, melanocortin 2 receptor accessory protein; *CYP17A1*, 17α-hydroxylase; *CYP11B1*, 11β-hydroxylase. Comparisons between groups were done using the Kruskal-Wallis test, followed by the Dwass-Steel-Critchlow-Fligner test. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, compared with *KCNJ5*-mutated APAs. The boxes contain the 25th and 75th percentiles, the whiskers mark the 10th and 90th percentiles, and the horizontal line within the box indicates the median, and the ● represent outliers.

from either normal ZG cells or from asymmetrical BHA. Conversely, amplification of a baseline aldosterone lateralization points towards a highly ACTH-sensitive APA.

The impact of ACTH on aldosterone secretion is dependent on the expression of *MC2R* in CYP11B2-positive cells (31). As ACTH is the primary regulator of cortisol synthesis, *MC2R* is abundantly expressed in the zona fasciculata (ZF) cells (54). Previous studies have shown that APAs have higher *MC2R* transcript levels than normal adrenal tissue, non-functional adrenal adenomas, or carcinomas (27, 29, 40, 55–57), although the levels reported have been somewhat variable. Our study is the first to quantify the expression of *MC2R* and *AGTR1* transcript levels in APAs confirmed by CYP11B2 IHC. Non-functional cortical adenomas can be present in patients with PA, and these tumors display lower *MC2R* expression than APAs or normal cortical tissue (40, 55); this might explain previously reported variability of *MC2R* expression in presumed APAs that were not functionally confirmed by examining CYP11B2 expression. Another cause of variability relates to the APA genotype. While all APAs had higher transcript levels of *MC2R* compared to adjacent normal adrenal tissue, *KCNJ5*-mutated APAs displayed lower *MC2R* transcripts than other APAs. Considering that BHA are often caused by multiple APCCs that harbor *CACNA1D* mutations (58), it is not surprising that East Asians studies that assessed the aldosterone response to ACTH stimulation or suppression in patients with APA *vs.* BHA found considerable overlap. As confirmed by several cohorts, *KCNJ5* mutations account for the vast majority of APAs in East Asian populations (45, 59). In line with these findings, we have previously reported that aldosterone lateralization during AVS often dampens following cosyntropin stimulation in patients with APAs harboring *KCNJ5* mutations, while the opposite happens in patients with *ATPase* mutations (26).

ACTH binds to its MC2R, and induces the activation of adenylate cyclase and the generation of intracellular cAMP (54, 60). Subsequently, the increased cAMP activates protein kinase A, which augments CREB phosphorylation and *CYP11B2* transcription (30, 31). MRAP, a small transmembrane protein, is an essential factor in regulating trafficking and functional expression of the MC2R in the adrenal gland (61, 62). Both *MC2R* and *MRAP* are known to be highly expressed in the undifferentiated zone as well as the ZF cells (63). Furthermore, the acute steroidogenic responses to ACTH stimulation depend on adequate amounts of *MC2R* and *MRAP* on the plasma membrane surface (61). In this study, *MC2R* transcripts correlated positively with *MRAP* expression only in *ATPase*-

	AGTR1	*MC2R*	*CYP11B2*	*MRAP*	*CYP17A1*	*CYP11B1*
All mutations						
AGTR1		0.55***	0.52***	0.11	-0.14	-0.06
MC2R	0.55***		0.77***	0.30*	-0.34*	-0.10
CYP11B2	0.52***	0.77***		0.12	-0.31*	-0.33*
MRAP	0.11	0.30*	0.12		0.19	0.12
CYP17A1	-0.14	-0.34*	-0.31*	0.19		0.54***
CYP11B1	-0.06	-0.10	-0.33*	0.12	0.54***	
KCNJ5-mutated APAs						
AGTR1		0.65*	0.53*	0.45	0.37	0.03
MC2R	0.65*		0.52	0.39	0.35	0.26
CYP11B2	0.53*	0.52		0.35	0.29	-0.50
MRAP	0.45	0.39	0.35		0.20	-0.09
CYP17A1	0.37	0.35	0.29	0.20		0.33
CYP11B1	0.03	0.26	-0.50	-0.09	0.33	
CACNA1D-mutated APAs						
AGTR1		0.75**	0.35	-0.20	-0.25	-0.19
MC2R	0.75**		0.59*	-0.09	-0.42	-0.33
CYP11B2	0.35	0.59*		0.13	-0.28	-0.13
MRAP	-0.20	-0.09	0.13		0.42	0.53
CYP17A1	-0.25	-0.42	-0.28	0.42		0.93***
CYP11B1	-0.19	-0.33	-0.13	0.53	0.93***	
ATP1A1-mutated APAs						
AGTR1		0.48	0.61*	0.16	-0.28	0.05
MC2R	0.48		0.77**	0.62*	-0.37	-0.23
CYP11B2	0.61*	0.77**		0.35	-0.16	0.09
MRAP	0.16	0.62*	0.35		-0.31	-0.41
CYP17A1	-0.28	-0.37	-0.16	-0.31		0.61*
CYP11B1	0.05	-0.23	0.09	-0.41	0.61*	
ATP2B3-mutated APAs						
AGTR1		0.60	0.40	0.30	0.10	0.10
MC2R	0.60		0.00	0.90*	0.70	0.30
CYP11B2	0.40	0.00		0.10	-0.70	-0.80
MRAP	0.30	0.90*	0.10		0.60	0.10
CYP17A1	0.10	0.70	-0.70	0.60		0.80
CYP11B1	0.10	0.30	-0.80	0.10	0.80	
All adjacent normal samples						
AGTR1		0.34*	0.06	-0.08	0.32*	0.32*
MC2R	0.34*		0.26	0.05	0.31*	0.67***
CYP11B2	0.06	0.26		-0.33*	-0.33*	0.10
MRAP	-0.08	0.05	-0.33*		0.27	0.10
CYP17A1	0.32*	0.31*	-0.33*	0.27		0.38*
CYP11B1	0.32*	0.67***	0.10	0.10	0.38*	

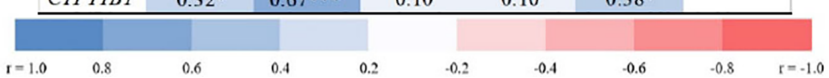

FIGURE 2 | Correlations between transcript levels of *AGTR1*, *MC2R*, *MRAP*, and steroidogenic enzymes in aldosterone-producing adenomas and adjacent normal adrenal tissue. *AGTR1*, type 1 angiotensin II receptor; *MC2R*, melanocortin type 2 receptors (ACTH receptors); *CYP11B2*, aldosterone synthase; *MRAP*, melanocortin 2 receptor accessory protein; *CYP17A1*, 17α-hydroxylase; *CYP11B1*, 11β-hydroxylase. Correlation analyses were done using the Spearman correlation test. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

mutated APAs. These findings further support the high responsivity of *ATPase*-mutated APAs to cosyntropin observed during AVS (26), in contrast with *KCNJ5* or *CACNA1D*-mutated APAs. Conversely, *MC2R* transcript levels correlated positively with those of *AGTR1* in APAs harboring *KCNJ5* or *CACNA1D* mutations, but not in those with *ATPase* mutations. Together these results highlight molecular differences between APAs, which go beyond those illustrated by recent histopathological studies (23, 24). Additional downstream molecular mechanisms might be impacted differently by various aldosterone-driver mutations and deserve further investigation. For example, *in vitro* studies suggest that angiotensin II upregulates *NR4A1*, *NR4A2*, and *NR4A3* gene expression (64, 65), and that *NR4A2* and *NR4A3* are upregulated in cell models overexpressing *KCNJ5* mutations (66, 67). Other transcriptome and methylome variations have been shown between APA with and without KCNJ5 mutations (68). In addition, differences in the expression of inhibitory regulators, such as dopamine receptors (69, 70) across APAs with various aldosterone-driver mutations deserve further investigation.

In summary, we found that ACTH and angiotensin II receptors are expressed in functionally confirmed APAs harboring the four most common aldosterone-driver somatic mutations. Additionally, we show that these key aldosterone regulatory receptors display several differences in expression across APAs with distinct underlying mutations. Specifically, *KCNJ5*-mutated APAs express lower mRNA transcript levels of both *MC2R* and *CYP11B2* as compared to other APAs, and they display no association between *MC2R* and *MRAP* expression, possibly explaining their relatively modest response to cosyntropin stimulation observed during AVS. Conversely, *ATP1A1*-mutated APAs showed robust positive correlation of *MC2R* with both *MRAP* and *CYP11B2* expression, supporting their ACTH-sensitivity. The relatively small number of tissue samples and individual variability from APAs with distinct somatic mutation are limitation of our study. Another important limitation is the lack of protein translation assessment, and thus conclusions regarding protein function remain limited. Such studies will be critical once highly selective human MC2R antibodies become available. Nevertheless, this initial study provides insight into the possible actions of ACTH and angiotensin II in APA with various aldosterone-driver mutations.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

This research was reviewed and approved by the Institutional Review Boards at the University of Michigan (HUM00106809, HUM00024461, HUM00083056). Written informed consent was obtained from all patients who underwent adrenalectomy prior to February, 2011. A waiver of consent was granted for the use of archival specimens (HUM00083056).

AUTHOR CONTRIBUTIONS

JSL, WER, and AFT conceived and designed the study. JSL and SP performed the experiment. JSL and AFT analyzed the data. JSL, JR, WR, and ADT interpreted the data. JL and AFT drafted and revised the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

AFT was supported by grants 1K08DK109116 from the NIDDK and DDCF_2019087 from the Doris Duke Charitable Foundation. WER was supported by grant R01DK106618 from the NIDDK.

ACKNOWLEDGMENTS

We thank Ms. Sarah Brand and former University of Michigan Adrenal Research Team members for assistance with regulatory processes and patient consent; Dr. Tom Giordano and Ms. Michelle Vinco for assistance with case identification and slide preparation; Ms. Amy R. Blinder for technical assistance; and all study participants.

REFERENCES

1. Douma S, Petidis K, Doumas M, Papaefthimiou P, Triantafyllou A, Kartali N, et al. Prevalence of primary hyperaldosteronism in resistant hypertension: a retrospective observational study. *Lancet* (2008) 371:1921–6. doi: 10.1016/S0140-6736(08)60834-X
2. Monticone S, D'Ascenzo F, Moretti C, Williams TA, Veglio F, Gaita F, et al. Cardiovascular events and target organ damage in primary aldosteronism compared with essential hypertension: a systematic review and meta-analysis. *Lancet Diabetes Endocrinol* (2018) 6:41–50. doi: 10.1016/S2213-8587(17)30319-4
3. Savard S, Amar L, Plouin PF, Steichen O. Cardiovascular complications associated with primary aldosteronism: a controlled cross-sectional study. *Hypertension* (2013) 62:331–6. doi: 10.1161/HYPERTENSIONAHA.113.01060
4. Mulatero P, Monticone S, Bertello C, Viola A, Tizzani D, Iannaccone A, et al. Long-term cardio- and cerebrovascular events in patients with primary aldosteronism. *J Clin Endocrinol Metab* (2013) 98:4826–33. doi: 10.1210/jc.2013-2805
5. Lim JS, Park S, Park SI, Oh YT, Choi E, Kim JY, et al. Cardiac Dysfunction in Association with Increased Inflammatory Markers in Primary Aldosteronism. *Endocrinol Metab (Seoul)* (2016) 31:567–76. doi: 10.3803/EnM.2016.31.4.567
6. Zia AA, Kamalov G, Newman KP, McGee JE, Bhattacharya SK, Ahokas RA, et al. From aldosteronism to oxidative stress: the role of excessive intracellular calcium accumulation. *Hypertens Res* (2010) 33:1091–101. doi: 10.1038/hr.2010.159
7. Funder JW, Carey RM, Mantero F, Murad MH, Reincke M, Shibata H, et al. The Management of Primary Aldosteronism: Case Detection, Diagnosis, and

Treatment: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab* (2016) 101:1889–916. doi: 10.1210/jc.2015-4061
8. El Ghorayeb N, Mazzuco TL, Bourdeau I, Mailhot JP, Zhu PS, Therasse E, et al. Basal and Post-ACTH Aldosterone and Its Ratios Are Useful During Adrenal Vein Sampling in Primary Aldosteronism. *J Clin Endocrinol Metab* (2016) 101:1826–35. doi: 10.1210/jc.2015-3915
9. Rossi GP, Pitter G, Bernante P, Motta R, Feltrin G, Miotto D. Adrenal vein sampling for primary aldosteronism: the assessment of selectivity and lateralization of aldosterone excess baseline and after adrenocorticotropic hormone (ACTH) stimulation. *J Hypertens* (2008) 26:989–97. doi: 10.1097/HJH.0b013e3282f9e66a
10. Seccia TM, Miotto D, De Toni R, Pitter G, Mantero F, Pessina AC, et al. Adrenocorticotropic hormone stimulation during adrenal vein sampling for identifying surgically curable subtypes of primary aldosteronism: comparison of 3 different protocols. *Hypertension* (2009) 53:761–6. doi: 10.1161/HYPERTENSIONAHA.108.128553
11. Young WF, Stanson AW, Thompson GB, Grant CS, Farley DR, van Heerden JA. Role for adrenal venous sampling in primary aldosteronism. *Surgery* (2004) 136:1227–35. doi: 10.1016/j.surg.2004.06.051
12. Wolley MJ, Ahmed AH, Gordon RD, Stowasser M. Does ACTH improve the diagnostic performance of adrenal vein sampling for subtyping primary aldosteronism? *Clin Endocrinol (Oxf)* (2016) 85:703–9. doi: 10.1111/cen.13110
13. Choi M, Scholl UI, Yue P, Bjorklund P, Zhao B, Nelson-Williams C, et al. K+ channel mutations in adrenal aldosterone-producing adenomas and hereditary hypertension. *Science* (2011) 331:768–72. doi: 10.1126/science.1198785
14. Azizan EA, Poulsen H, Tuluc P, Zhou J, Clausen MV, Lieb A, et al. Somatic mutations in ATP1A1 and CACNA1D underlie a common subtype of adrenal hypertension. *Nat Genet* (2013) 45:1055–60. doi: 10.1038/ng.2716
15. Beuschlein F, Boulkroun S, Osswald A, Wieland T, Nielsen HN, Lichtenauer UD, et al. Somatic mutations in ATP1A1 and ATP2B3 lead to aldosterone-producing adenomas and secondary hypertension. *Nat Genet* (2013) 45:440–4, 4e1-2. doi: 10.1038/ng.2550
16. Scholl UI, Goh G, Stolting G, de Oliveira RC, Choi M, Overton JD, et al. Somatic and germline CACNA1D calcium channel mutations in aldosterone-producing adenomas and primary aldosteronism. *Nat Genet* (2013) 45:1050–4. doi: 10.1038/ng.2695
17. Scholl UI, Stolting G, Nelson-Williams C, Vichot AA, Choi M, Loring E, et al. Recurrent gain of function mutation in calcium channel CACNA1H causes early-onset hypertension with primary aldosteronism. *Elife* (2015) 4:e06315. doi: 10.7554/eLife.06315
18. Wu VC, Wang SM, Chueh SJ, Yang SY, Huang KH, Lin YH, et al. The prevalence of CTNNB1 mutations in primary aldosteronism and consequences for clinical outcomes. *Sci Rep* (2017) 7:39121. doi: 10.1038/srep39121
19. Scholl UI, Stolting G, Schewe J, Thiel A, Tan H, Nelson-Williams C, et al. CLCN2 chloride channel mutations in familial hyperaldosteronism type II. *Nat Genet* (2018) 50:349–54. doi: 10.1038/s41588-018-0048-5
20. Fernandes-Rosa FL, Daniil G, Orozco IJ, Goppner C, El Zein R, Jain V, et al. A gain-of-function mutation in the CLCN2 chloride channel gene causes primary aldosteronism. *Nat Genet* (2018) 50:355–61. doi: 10.1038/s41588-018-0053-8
21. Nanba K, Omata K, Else T, Beck PCC, Nanba AT, Turcu AF, et al. Targeted Molecular Characterization of Aldosterone-Producing Adenomas in White Americans. *J Clin Endocrinol Metab* (2018) 103:3869–76. doi: 10.1210/jc.2018-01004
22. Nanba K, Omata K, Gomez-Sanchez CE, Stratakis CA, Demidowich AP, Suzuki M, et al. Genetic Characteristics of Aldosterone-Producing Adenomas in Blacks. *Hypertension* (2019) 73:885–92. doi: 10.1161/HYPERTENSIONAHA.118.12070
23. De Sousa K, Boulkroun S, Baron S, Nanba K, Wack M, Rainey WE, et al. Genetic, Cellular, and Molecular Heterogeneity in Adrenals With Aldosterone-Producing Adenoma. *Hypertension* (2020) 75:1034–44. doi: 10.1161/HYPERTENSIONAHA.119.14177
24. Ono Y, Yamazaki Y, Omata K, Else T, Tomlins SA, Rhayem Y, et al. Histological Characterization of Aldosterone-producing Adrenocortical Adenomas with Different Somatic Mutations. *J Clin Endocrinol Metab* (2020) 105(3):e282-9. doi: 10.1210/clinem/dgz235
25. Williams TA, Peitzsch M, Dietz AS, Dekkers T, Bidlingmaier M, Riester A, et al. Genotype-Specific Steroid Profiles Associated With Aldosterone-Producing Adenomas. *Hypertension* (2016) 67:139–45. doi: 10.1161/HYPERTENSIONAHA.115.06186
26. Wannachalee T, Zhao L, Nanba K, Nanba AT, Shields JJ, Rainey WE, et al. Three Discrete Patterns of Primary Aldosteronism Lateralization in Response to Cosyntropin During Adrenal Vein Sampling. *J Clin Endocrinol Metab* (2019) 104:5867–76. doi: 10.1210/jc.2019-01182
27. Ye P, Mariniello B, Mantero F, Shibata H, Rainey WE. G-protein-coupled receptors in aldosterone-producing adenomas: a potential cause of hyperaldosteronism. *J Endocrinol* (2007) 195:39–48. doi: 10.1677/JOE-07-0037
28. Perraudin V, Delarue C, Lefebvre H, Do Rego JL, Vaudry H, Kuhn JM. Evidence for a role of vasopressin in the control of aldosterone secretion in primary aldosteronism: *in vitro* and *in vivo* studies. *J Clin Endocrinol Metab* (2006) 91:1566–72. doi: 10.1210/jc.2005-1453
29. Zwermann O, Suttmann Y, Bidlingmaier M, Beuschlein F, Reincke M. Screening for membrane hormone receptor expression in primary aldosteronism. *Eur J Endocrinol* (2009) 160:443–51. doi: 10.1530/EJE-08-0711
30. Hattangady NG, Olala LO, Bollag WB, Rainey WE. Acute and chronic regulation of aldosterone production. *Mol Cell Endocrinol* (2012) 350:151–62. doi: 10.1016/j.mce.2011.07.034
31. El Ghorayeb N, Bourdeau I, Lacroix A. Role of ACTH and Other Hormones in the Regulation of Aldosterone Production in Primary Aldosteronism. *Front Endocrinol (Lausanne)* (2016) 7:72. doi: 10.3389/fendo.2016.00072
32. Oki K, Plonczynski MW, Lam ML, Gomez-Sanchez EP, Gomez-Sanchez CE. The potassium channel, Kir3.4 participates in angiotensin II-stimulated aldosterone production by a human adrenocortical cell line. *Endocrinology* (2012) 153:4328–35. doi: 10.1210/en.2012-1241
33. Reimer EN, Walenda G, Seidel E, Scholl UI. CACNA1H(M1549V) Mutant Calcium Channel Causes Autonomous Aldosterone Production in HAC15 Cells and Is Inhibited by Mibefradil. *Endocrinology* (2016) 157:3016–22. doi: 10.1210/en.2016-1170
34. Nishimoto K, Tomlins SA, Kuick R, Cani AK, Giordano TJ, Hovelson DH, et al. Aldosterone-stimulating somatic gene mutations are common in normal adrenal glands. *Proc Natl Acad Sci U.S.A.* (2015) 112:E4591–9. doi: 10.1073/pnas.1505529112
35. Bassett MH, Mayhew B, Rehman K, White PC, Mantero F, Arnaldi G, et al. Expression profiles for steroidogenic enzymes in adrenocortical disease. *J Clin Endocrinol Metab* (2005) 90:5446–55. doi: 10.1210/jc.2005-0836
36. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* (2001) 25:402–8. doi: 10.1006/meth.2001.1262
37. Gambaryan S, Butt E, Tas P, Smolenski A, Allolio B, Walter U. Regulation of aldosterone production from zona glomerulosa cells by ANG II and cAMP: evidence for PKA-independent activation of CaMK by cAMP. *Am J Physiol Endocrinol Metab* (2006) 290:E423–33. doi: 10.1152/ajpendo.00128.2005
38. Lefebvre H, Prevost G, Louiset E. Autocrine/paracrine regulatory mechanisms in adrenocortical neoplasms responsible for primary adrenal hypercorticism. *Eur J Endocrinol* (2013) 169:R115–38. doi: 10.1530/EJE-13-0308
39. St-Jean M, Ghorayeb NE, Bourdeau I, Lacroix A. Aberrant G-protein coupled hormone receptor in adrenal diseases. *Best Pract Res Clin Endocrinol Metab* (2018) 32:165–87. doi: 10.1016/j.beem.2018.01.003
40. Schubert B, Fassnacht M, Beuschlein F, Zenkert S, Allolio B, Reincke M. Angiotensin II type 1 receptor and ACTH receptor expression in human adrenocortical neoplasms. *Clin Endocrinol (Oxf)* (2001) 54:627–32. doi: 10.1046/j.1365-2265.2001.01253.x
41. Tunny TJ, Klemm SA, Stowasser M, Gordon RD. Angiotensin-responsive aldosterone-producing adenomas: postoperative disappearance of aldosterone response to angiotensin. *Clin Exp Pharmacol Physiol* (1993) 20:306–9. doi: 10.1111/j.1440-1681.1993.tb01690.x
42. Mantero F, Fallo F, Opocher G, Armanini D, Boscaro M, Scaroni C. Effect of angiotensin II and converting enzyme inhibitor (captopril) on blood pressure, plasma renin activity and aldosterone in primary aldosteronism. *Clin Sci (Lond)* (1981) 61 Suppl 7:289s–93s. doi: 10.1042/cs061289s
43. Stowasser M, Klemm SA, Tunny TJ, Gordon RD. Plasma aldosterone response to ACTH in subtypes of primary aldosteronism. *Clin Exp Pharmacol Physiol* (1995) 22:460–2. doi: 10.1111/j.1440-1681.1995.tb02044.x
44. Williams TA, Monticone S, Mulatero P. KCNJ5 mutations are the most frequent genetic alteration in primary aldosteronism. *Hypertension* (2015) 65:507–9. doi: 10.1161/HYPERTENSIONAHA.114.04636

45. Hong AR, Kim JH, Song YS, Lee KE, Seo SH, Seong MW, et al. Genetics of Aldosterone-Producing Adenoma in Korean Patients. *PloS One* (2016) 11: e0147590. doi: 10.1371/journal.pone.0147590
46. Fernandes-Rosa FL, Williams TA, Riester A, Steichen O, Beuschlein F, Boulkroun S, et al. Genetic spectrum and clinical correlates of somatic mutations in aldosterone-producing adenoma. *Hypertension* (2014) 64:354–61. doi: 10.1161/HYPERTENSIONAHA.114.03419
47. Funder JW. The Potential of ACTH in the Genesis of Primary Aldosteronism. *Front Endocrinol (Lausanne)* (2016) 7:40. doi: 10.3389/fendo.2016.00040
48. Schambelan M, Brust NL, Chang BC, Slater KL, Biglieri EG. Circadian rhythm and effect of posture on plasma aldosterone concentration in primary aldosteronism. *J Clin Endocrinol Metab* (1976) 43:115–31. doi: 10.1210/jcem-43-1-115
49. Sonoyama T, Sone M, Tamura N, Honda K, Taura D, Kojima K, et al. Role of endogenous ACTH on circadian aldosterone rhythm in patients with primary aldosteronism. *Endocr Connect* (2014) 3:173–9. doi: 10.1530/EC-14-0086
50. Sonoyama T, Sone M, Miyashita K, Tamura N, Yamahara K, Park K, et al. Significance of adrenocorticotropin stimulation test in the diagnosis of an aldosterone-producing adenoma. *J Clin Endocrinol Metab* (2011) 96:2771–8. doi: 10.1210/jc.2011-0573
51. Inoue K, Omura M, Sugisawa C, Tsurutani Y, Saito J, Nishikawa T. Clinical Utility of the Adrenocorticotropin Stimulation Test with/without Dexamethasone Suppression for Definitive and Subtype Diagnosis of Primary Aldosteronism. *Int J Mol Sci* (2017) 18(5):948. doi: 10.3390/ijms18050948
52. Jiang Y, Zhang C, Wang W, Su T, Zhou W, Jiang L, et al. Diagnostic value of ACTH stimulation test in determining the subtypes of primary aldosteronism. *J Clin Endocrinol Metab* (2015) 100:1837–44. doi: 10.1210/jc.2014-3551
53. Nanba AT, Nanba K, Byrd JB, Shields JJ, Giordano TJ, Miller BS, et al. Discordance between imaging and immunohistochemistry in unilateral primary aldosteronism. *Clin Endocrinol* (2017) 87(6):665–72. doi: 10.1111/cen.13442
54. Chan LF, Metherell LA, Clark AJ. Effects of melanocortins on adrenal gland physiology. *Eur J Pharmacol* (2011) 660:171–80. doi: 10.1016/j.ejphar.2010.11.041
55. Reincke M, Beuschlein F, Latronico AC, Arlt W, Chrousos GP, Allolio B. Expression of adrenocorticotrophic hormone receptor mRNA in human adrenocortical neoplasms: correlation with P450scc expression. *Clin Endocrinol* (1997) 46:619–26. doi: 10.1046/j.1365-2265.1997.1991009.x
56. Arnaldi G, Mancini V, Costantini C, Giovagnetti M, Petrelli M, Masini A, et al. ACTH receptor mRNA in human adrenocortical tumors: overexpression in aldosteronomas. *Endocr Res* (1998) 24:845–9. doi: 10.3109/07435809809032695
57. Murakami M, Yoshimoto T, Nakabayashi K, Tsuchiya K, Minami I, Bouchi R, et al. Integration of transcriptome and methylome analysis of aldosterone-producing adenomas. *Eur J Endocrinol/European Fed Endocr Societies* (2015) 173:185–95. doi: 10.1530/EJE-15-0148
58. Omata K, Satoh F, Morimoto R, Ito S, Yamazaki Y, Nakamura Y, et al. Cellular and Genetic Causes of Idiopathic Hyperaldosteronism. *Hypertension* (2018) 72:874–80. doi: 10.1161/HYPERTENSIONAHA.118.11086
59. Taguchi R, Yamada M, Nakajima Y, Satoh T, Hashimoto K, Shibusawa N, et al. Expression and mutations of KCNJ5 mRNA in Japanese patients with aldosterone-producing adenomas. *J Clin Endocrinol Metab* (2012) 97:1311–9. doi: 10.1210/jc.2011-2885
60. de Joussineau C, Sahut-Barnola I, Levy I, Saloustros E, Val P, Stratakis CA, et al. The cAMP pathway and the control of adrenocortical development and growth. *Mol Cell Endocrinol* (2012) 351:28–36. doi: 10.1016/j.mce.2011.10.006
61. Novoselova TV, Jackson D, Campbell DC, Clark AJ, Chan LF. Melanocortin receptor accessory proteins in adrenal gland physiology and beyond. *J Endocrinol* (2013) 217:R1–11. doi: 10.1530/JOE-12-0501
62. Cooray SN, Almiro Do Vale I, Leung KY, Webb TR, Chapple JP, Egertova M, et al. The melanocortin 2 receptor accessory protein exists as a homodimer and is essential for the function of the melanocortin 2 receptor in the mouse y1 cell line. *Endocrinology* (2008) 149:1935–41. doi: 10.1210/en.2007-1463
63. Gorrigan RJ, Guasti L, King P, Clark AJ, Chan LF. Localisation of the melanocortin-2-receptor and its accessory proteins in the developing and adult adrenal gland. *J Mol Endocrinol* (2011) 46:227–32. doi: 10.1530/JME-11-0011
64. Romero DG, Plonczynski M, Vergara GR, Gomez-Sanchez EP, Gomez-Sanchez CE. Angiotensin II early regulated genes in H295R human adrenocortical cells. *Physiol Genomics* (2004) 19:106–16. doi: 10.1152/physiolgenomics.00097.2004
65. Bassett MH, Suzuki T, Sasano H, White PC, Rainey WE. The orphan nuclear receptors NURR1 and NGFIB regulate adrenal aldosterone production. *Mol Endocrinol* (2004) 18:279–90. doi: 10.1210/me.2003-0005
66. Monticone S, Hattangady NG, Nishimoto K, Mantero F, Rubin B, Cicala MV, et al. Effect of KCNJ5 mutations on gene expression in aldosterone-producing adenomas and adrenocortical cells. *J Clin Endocrinol Metab* (2012) 97:E1567–72. doi: 10.1210/jc.2011-3132
67. Hattangady NG, Karashima S, Yuan L, Ponce-Balbuena D, Jalife J, Gomez-Sanchez CE, et al. Mutated KCNJ5 activates the acute and chronic regulatory steps in aldosterone production. *J Mol Endocrinol* (2016) 57:1–11. doi: 10.1530/JME-15-0324
68. Murakami M, Yoshimoto T, Nakabayashi K, Nakano Y, Fukaishi T, Tsuchiya K, et al. Molecular characteristics of the KCNJ5 mutated aldosterone-producing adenomas. *Endocr Relat Cancer* (2017) 24:531–41. doi: 10.1530/ERC-17-0117
69. Chang HW, Huang CY, Yang SY, Wu VC, Chu TS, Chen YM, et al. Role of D2 dopamine receptor in adrenal cortical cell proliferation and aldosterone-producing adenoma tumorigenesis. *J Mol Endocrinol* (2014) 52:87–96. doi: 10.1530/JME-13-0044
70. Rossitto G, Maiolino G, Lenzini L, Bisogni V, Seccia TM, Cesari M, et al. Subtyping of primary aldosteronism with adrenal vein sampling: Hormone- and side-specific effects of cosyntropin and metoclopramide. *Surgery* (2018) 163:789–95. doi: 10.1016/j.surg.2017.09.032

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer SM declared a past co-authorship with one of the authors WR to the handling editor.

frontiers in Endocrinology

ORIGINAL RESEARCH
published: 26 March 2021
doi: 10.3389/fendo.2021.625457

Real-World Effectiveness of Mineralocorticoid Receptor Antagonists in Primary Aldosteronism

Yuta Tezuka[1,2] *and Adina F. Turcu*[1]*

[1] Division of Metabolism, Endocrinology and Diabetes, University of Michigan, Ann Arbor, MI, United States, [2] Division of Nephrology, Endocrinology and Vascular Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan

Objective: To investigate how often target renin is pursued and achieved in patients with primary aldosteronism (PA) and other low renin hypertension (LRH) treated with mineralocorticoid receptor antagonists (MRAs), as reversal of renin suppression was shown to circumvent the enhanced cardiovascular and renal morbidity and mortality in these patients.

Patients and Methods: We conducted a retrospective cohort study of patients with PA and LRH treated with MRAs in an academic outpatient practice from January 1, 2000, through May 31, 2020.

Results: Of 30,777 patients with hypertension treated with MRAs, only 7.3% were evaluated for PA. 163 patients (123 with PA) had renin followed after MRA initiation. After a median follow-up of 124 [interquartile range, 65-335] days, 70 patients (43%) no longer had renin suppression at the last visit. The proportion of those who achieved target renin was higher in LRH than in PA (53% vs. 40%). Lower baseline serum potassium, lower MRA doses, and beta-blocker use were independently associated with lower odds of achieving target renin in PA, while male sex was associated with target renin in LRH. Overall, 50 patients (30.7%) had 55 adverse events, all from spironolactone, and 26 patients (52%) were switched to eplerenone or had a spironolactone dose reduction.

Conclusion: Despite evidence that reversal of renin suppression confers cardio-renal protection in patients with PA and LRH, renin targets are followed in very few and are achieved in under half of such patients seen in an academic setting, with possibly even lower rates in community practices.

Keywords: primary aldosteronism, renin, mineralocorticoid receptor (MR) antagonist, adrenal disorders, hypertension, aldosterone, adrenal, adrenal cortex

OPEN ACCESS

Edited by:
Vin-Cent Wu,
National Taiwan University, Taiwan

Reviewed by:
Gregory Hundemer,
Ottawa Hospital Research Institute (OHRI), Canada
Cristiana Catena,
University of Udine, Italy

***Correspondence:**
Adina F. Turcu
aturcu@umich.edu

Specialty section:
This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Endocrinology

Received: *03 November 2020*
Accepted: *23 February 2021*
Published: *26 March 2021*

Citation:
Tezuka Y and Turcu AF (2021) Real-World Effectiveness of Mineralocorticoid Receptor Antagonists in Primary Aldosteronism. Front. Endocrinol. 12:625457. doi: 10.3389/fendo.2021.625457

Abbreviations: CKD, chronic kidney disease; DRC, direct renin concentration; eGFR, estimated glomerular filtration ratio; LRH, low renin essential hypertension; MR, mineralocorticoid receptor; MRA, mineralocorticoid receptor antagonist; PA, primary aldosteronism; PAC, plasma aldosterone concentration; PRA, plasma renin activity; RAAS, renin-angiotensin-aldosterone system.

INTRODUCTION

Primary aldosteronism (PA) is a common form of secondary hypertension, accounting for up to 20% of resistant hypertension cases (1–3). Compared to sex- and age- matched individuals with essential hypertension and equivalent blood pressure, patients with PA have a higher risk of cardiovascular and renal complications, including atrial fibrillation, coronary artery disease, strokes, renal insufficiency, and death (4, 5). Such complications are partly mediated by excessive mineralocorticoid receptor (MR) activation in target tissues, which promotes myocardial fibrosis, left ventricular hypertrophy, increased carotid intima-media thickness, endothelial dysfunction, and microalbuminuria (6–12). Mineralocorticoid receptor antagonists (MRAs) are the mainstay of medical treatment for PA (13). While small observational studies showed that MRAs can be efficacious for blood pressure control and cardiovascular protection even in low doses (14–16), large retrospective cohort studies of patients with PA and essential hypertension suggest that the cardio-renal benefits of MRA therapy in PA patients are maximized when renin is no longer suppressed (4, 17, 18).

The clinical benefits of MRA therapy on blood pressure control and cardiovascular morbidity and mortality has also been demonstrated more broadly in resistant hypertension (19–21). Moreover, low renin has been associated with cardiovascular risk in patients with "essential hypertension" (22–24) and it has been shown to be a predictor of blood pressure response to MRAs in this population (25). Therefore, abrogation of renin suppression is suggestive of therapeutic MR blockade that overcomes the excessive amounts of aldosterone or other MR activators.

In this study, conducted in a large academic clinical practice that includes both primary care and specialty services, we aimed to: 1) assess how often MRA therapy is titrated to target renin levels; and 2) identify factors that preclude adequate MRA dose titration to overcome renin suppression, such as side effects or concomitant medications that contribute to alterations of renin-angiotensin-aldosterone-system (RAAS).

PATIENTS AND METHODS

Study Participants

We employed an internal database search engine, DataDirect (26), to identify patients with low-renin hypertension treated with MRA between January 1st, 2000 and June 1st, 2020 (**Figure 1**). We included patients with hypertension who had: 1) suppressed renin prior to initiation of MRA therapy, and 2) follow-up renin measurements after MRA initiation available in our medical records. We excluded patients with: end-stage renal disease; Cushing syndrome; glucocorticoid use; adrenal cortical carcinoma; congenital adrenal hyperplasia; and critically ill patients. We also excluded patients who did not have follow-up renin within 18 months after MRA initiation. This study was conducted with the University of Michigan Internal Review Boards approval (HUM00055821). A waiver of consent was granted for the retrospective review of medical records.

Clinical Information

Data extracted from medical records included: demographics, body mass index (BMI), blood pressure, medical diagnoses, medications, serum electrolytes, the estimated glomerular filtration ratio (eGFR), plasma aldosterone concentration (PAC), plasma renin, both before and after MRA initiation, as well as side effects related to MRA use. To compile results, eplerenone doses were converted to equivalent spironolactone doses by dividing by 2 (18, 27, 28).

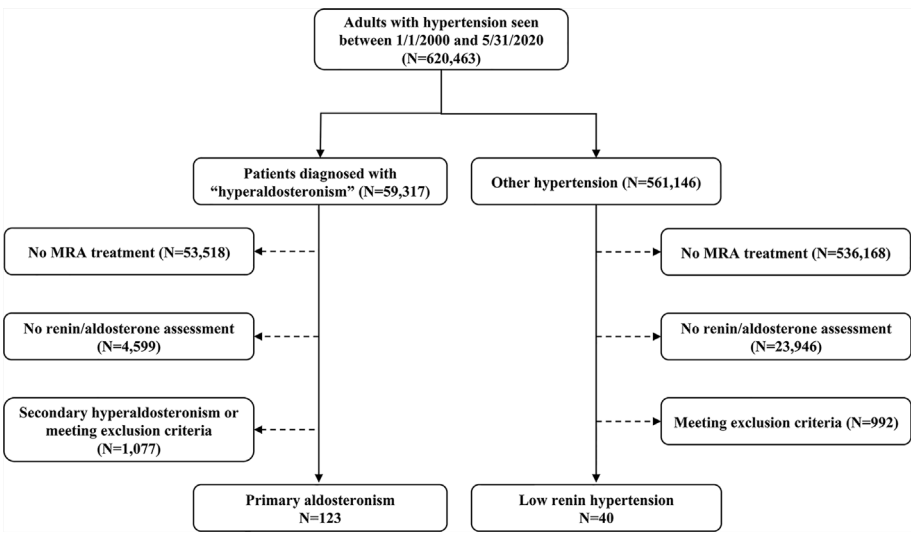

FIGURE 1 | Selection of study participants. MRA, mineralocorticoid receptor antagonist; PA, primary aldosteronism.

Measurement of Renin and Definition of PA Diagnosis

Plasma renin activity (PRA) was available in the majority of patients (74.8%), and it was measured as previously reported (29). Direct renin concentration (DRC) was measured after February 2018 and it was the only test available in the remaining patients (25.2%). DRC was measured with a DiaSorin Liaison competitive chemiluminescent immunoassay with a coefficient of variability <10%. A conversion factor of 1:8 was used to transform DRC values into PRA equivalents following rigorous in-house analysis of both assays over a 9-month transition period. Renin was considered suppressed when PRA was <1.0 ng/mL/h (to convert to pmol/L, multiply by 0.0030) and DRC was <8.0 pg/mL (to convert to pmol/L, multiply by 0.0237), and renin values above these thresholds were set as target following MRA therapy. The diagnosis of PA was established based on one of the following criteria: an oral salt loading test followed by a 24-hour urinary aldosterone >12 μg (to convert to μmol, multiply by 0.0028); an intravenous saline infusion test followed by a PAC >6.8 ng/dL (to convert to nmol/L, multiply by 0.0277) at 4 hours; or PAC ≥20 ng/dL along with suppressed renin (13). The laterality of hyperaldosteronism was confirmed by adrenal venous sampling, as previously reported (30).

Statistical Analysis

The Mann-Whitney *U* test was used for comparison of continuous variables between two independent groups. The paired *t*-test (for normal data distribution) or Wilcoxon signed-rank test (skewed data distribution) were used to compare continuous variables prior to and after MRA therapy within the same patients. The Chi-squared test and Fisher's exact test were used for comparison of proportions between two groups. Linear regression analysis was used to assess relationships between continuous variables. Multiple logistic regression with backward stepwise selection was employed to evaluate predictors of target renin achievement following MRA treatment. All statistical analyses were performed with StatFlex software (version 7.0; Artech Co, Ltd, Osaka, Japan) or Prism (version 8.0; GraphPad Software, La Jolla, CA). Statistical significance was accepted at p values smaller than 0.05.

RESULTS

Demographics and Clinical Characteristics of Study Participants

Of 620,463 adult patients with hypertension seen across our institution during the study period, 59,317 (10%) patients had a documented diagnosis of "hyperaldosteronism" (**Figure 1**). Only 5% of all patients and 9.8% of those with hyperaldosteronism were prescribed an MRA. In total, 123 patients with PA and 40 patients with other low-renin hypertension (LRH) met all inclusion criteria (**Figure 1**). The number of the patients meeting entry criteria increased progressively since 2013 (13 before 2010, 51 from 2011 through 2015, and 97 since 2016; **Supplemental Figure 1**). The median age of all participants at study entry was 56 (range, 19 to 84) years, and it was similar in both groups (**Table 1**). Patients with PA were more frequently men (60%), while those with LRH were more often women (67.5%, p=0.02). The median number of antihypertensive agents was 3 (interquartile range: 2 to 4) in both groups. Patients with LRH had a higher systolic blood pressure (159 [137, 182] vs.148 [132, 162] mmHg, p=0.03). Beta-blockers, RAAS inhibitors (including angiotensin converting enzyme inhibitors and angiotensin receptor blockers), and calcium channel blockers were prescribed in 64%, 53%, and 69% of the patients, respectively. Patients with PA were treated more often with calcium channel blockers (75% vs. 53%, p=0.008), and potassium supplements (45% vs. 20%, p=0.01) than those without PA (**Table 1**).

Rates of Target Renin Achievement After MRA Initiation

Patients were followed for a median of 124 (interquartile range: 65, 335) days while on MRA treatment. In 59% of cases (74/123 with PA and 22/40 with LRH), the MRA titration decision was based on renin values or side effects. In a minority of patients, MRA titration was based on other factors, such as blood pressure (14%, 11 with PA and 12 with LRH)) and serum potassium (4%, 3 with PA and 1 with LRH), or no documentation regarding the rationale for the titration was found (9%, 10 with PA and 4 with LRH). In the remaining patients, the initial MRA doses were not changed. The median MRA dose used at the last visit was 50 (range, 12.5 to 300) mg/day (**Table 1**). Most patients (82%) were treated with spironolactone. The overall cumulative proportion of patients in whom target renin was achieved was 43% at the last visit (**Figure 2A**). The proportion of patients with target renin increased gradually over time in both groups, but was higher across all stages in patients with LRH as compared to the PA group. In the PA group, target renin was achieved in 2% of patients at 2 weeks, and it reached 40% by the last visit (**Figure 2B**). In patients with LRH, target renin rates were reached in 8% at 2 weeks and 53% one year after MRA initiation (**Figure 2C**).

Factors Associated With Target Renin in PA and LRH

The clinical characteristics of PA patients with and without achievement of target renin levels are summarized in **Table 2A**. Compared to those who achieved target renin levels, patients with persistent renin suppression were younger (54 [44, 62] vs. 59 [51, 67] years, p=0.01), were more often treated with beta-blockers (74% vs. 51%, p=0.008), and had lower serum potassium concentrations (3.7 vs 3.9 mM, p=0.001). Patients with PA who reached a target renin value at the last visit were treated with higher doses of MRAs (50.0 [25.0, 81.3] vs. 31.3 [25.0, 50.0] mg/day, p=0.002), and had higher serum potassium levels (4.4 [4.2, 4.7] vs. 4.2 [3.9, 4.5] mM, p=0.01). Of the concomitant antihypertensive agents used, beta-blockers were associated with higher odds of persistent renin suppression

TABLE 1 | Baseline demographics of study participants.

Variables	All	PA	LRH	*p*
N	163	123	40	
Age (years)	56 [46, 64]	56 [47, 64]	56 [46, 67]	0.99
Women (N, %)	76 (46.6%)	49 (39.8%)	27 (67.5%)	0.02
BMI (kg/m^2)	32.9 [28.7, 36.5]	32.9 [29.0, 36.0]	33.3 [28.4, 37.1]	0.92
SBP (mmHg)	149 [134, 168]	148 [132, 162]	159 [137, 182]	0.03
DBP (mmHg)	84 [75, 92]	84 [75, 91]	85 [76, 99]	0.13
Serum potassium (mM)	3.8 [3.5, 4.2]	3.7 [3.5, 4.2]	4.0 [3.5, 4.2]	0.24
eGFR (mL/min/1.73m^2)	81 [64, 94]	81 [65, 92]	82 [61, 96]	0.86
PAC (ng/dL)	18.8 [13.2, 26.5]	21.0 [16.3, 29.7]	11.2 [8.4, 16.6]	<0.001
PRA (ng/mL/hr) [N = 122]	0.3 [0.1, 0.6]	0.2 [0.1, 0.6]	0.4 [0.2, 0.7]	0.06
DRC (pg/mL) [N = 41]	2.1 [2.1, 3.5]	2.1 [2.1, 2.1]	2.3 [2.1, 4.3]	0.052
ARR (ng/dL per ng/mL/h)	61.0 [32.2, 124.4]	79.5 [38.6, 170.0]	28.9 [18.5, 47.1]	<0.001
Follow-up duration (days)	124 [65, 335]	175 [73, 342]	80 [35, 173]	0.008
MRA dosage at the last visit (mg/day)	50.0 [25.0, 50.0]	50.0 [25.0, 50.0]	25.0 [25.0, 50.0]	0.18
MRA-related side effects (N, %)	50 (30.7%)	41 (33.3%)	9 (22.5%)	0.20
Smoking history (N, %)	59 (37.1%)	43 (35.8%)	16 (41.0%)	0.56
Diabetes mellitus (N, %)	52 (31.9%)	35 (28.5%)	17 (42.5%)	0.10
Cardiovascular disease (N, %)	33 (20.2%)	23 (18.7%)	10 (25.0%)	0.39
Stroke (N, %)	16 (9.8%)	10 (8.1%)	6 (15.0%)	0.20
Antihypertensive agents (N)	3.0 [2.0, 4.0]	3.0 [2.0, 4.0]	3.0 [2.0, 4.0]	0.58
Alpha-blocker (N, %)	22 (13.5%)	18 (14.6%)	4 (10.0%)	0.46
Beta-blocker (N, %)	105 (64.4%)	80 (65.0%)	25 (62.5%)	0.77
Central agonists (N, %)	39 (23.9%)	26 (21.1%)	13 (32.5%)	0.14
Potassium-wasting diuretics (N, %)	69 (42.3%)	54 (43.9%)	15 (37.5%)	0.48
Potassium-sparing diuretics (N, %)	10 (6.1%)	9 (7.3%)	1 (2.5%)	0.27
RAAS inhibitors (N, %)	87 (53.4%)	61 (49.6%)	26 (65.0%)	0.09
Calcium channel blocker (N, %)	113 (69.3%)	92 (74.8%)	21 (52.5%)	0.008
Other antihypertensives (N, %)	9 (5.5%)	7 (5.7%)	2 (5.0%)	0.87
Potassium replacement (N, %)	63 (38.7%)	55 (44.7%)	8 (20.0%)	0.005

PA, primary aldosteronism; LRH, low renin essential hypertension; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration ratio; PAC, plasma aldosterone concentration; PRA, plasma renin activity; DRC, direct renin concentration; ARR, aldosterone-to-renin ratio; RAAS, renin-angiotensin-aldosterone system; MRA, mineralocorticoid receptor antagonist. RAAS inhibitors include angiotensin-converting enzyme inhibitors and angiotensin receptor blockers. Continuous variables are shown as median [interquartile range].

(p=0.02), whereas neither RAAS inhibitors, nor potassium-wasting diuretics had an impact on the likelihood of target renin achievement. Multiple logistic regression determined that a lower baseline serum potassium, lower MRA dose, and beta-blocker use were independently associated with lower odds of achieving target renin levels (**Table 3A**).

Of patients with LRH, those who reached target renin levels had higher PRA (0.6 [0.3, 0.7] vs. 0.2 [0.1, 0.4] ng/mL/h, p=0.04) at baseline than the patients with persistent renin suppression (**Table 2B**). Intriguingly, patients with persistent renin suppression were more frequently women than those with target renin achievement (90% vs. 48%, p=0.005). There was no difference in age, beta-blocker use, or MRA doses between the two groups. In multiple logistic regression, only sex was independently associated with target renin levels (**Table 3B**).

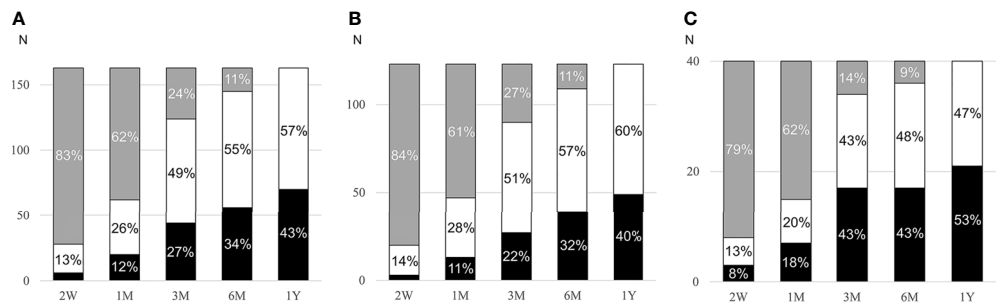

FIGURE 2 | Cumulative rates of target renin achievement during MRA therapy. MRA, mineralocorticoid receptor antagonist; PA, primary aldosteronism; LRH, low-renin essential hypertension; W, weeks; M, months; Y, year. Changes of target renin rates in all participants (**A**, n=163), PA patients (**B**, n=123), and LRH patients (**C**, n=40) during follow-up after initiation of MRA. Patients who reached target renin are shown in black bars, those who continued to have suppressed renin in white bars, and those in whom renin was not assessed in grey bars.

TABLE 2 | Comparison of patients with and without target renin following MRA therapy.

Variables	Target renin	Suppressed renin	*p*
A. PA patients			
N	49	74	
Age (years)	59 [51, 67]	54 [44, 62]	0.01
Women (N, %)	22 (44.9%)	27 (36.5%)	0.35
BMI (kg/m^2)	33.6 [29.9, 36.8]	32.7 [28.3, 35.9]	0.35
SBP (mmHg)	155 [137, 172]	143 [126, 156]	0.01
DBP (mmHg)	84 [78, 90]	82 [72, 92]	0.41
Serum potassium (mM)	3.9 [3.6, 4.3]	3.7 [3.4, 4.0]	0.001
eGFR (mL/min/1.73m^2)	84 [68, 96]	80 [63, 90]	0.38
PAC (ng/dL)	19.4 [15.9, 27.3]	22.0 [16.4, 30.8]	0.30
PRA (ng/mL/hr) [N=99]	0.3 [0.1, 0.6]	0.2 [0.1, 0.6]	0.33
DRC (pg/mL) [N=24]	2.1 [2.1, 5.1]	2.1 [2.1, 2.1]	0.50
ARR (ng/dL per ng/mL/h)	66.3 [38.3, 113.5]	100.2 [41.0, 210.0]	0.10
Confirmed unilateral PA cases (N, %)	5 (10.2%)	20 (27.0%)	0.02
Antihypertensive agents (N)	3.0 [1.0, 4.0]	3.0 [2.0, 4.0]	0.75
Beta-blocker (N, %)	25 (51.0%)	55 (74.3%)	0.008
Potassium replacement (N, %)	21 (42.9%)	34 (45.9%)	0.74
Cardiovascular disease (N, %)	8 (16.3%)	15 (20.3%)	0.58
Follow-up duration (days)	182 [78, 342]	164 [66, 342]	0.79
MRA dosage at the last visit (mg/day)	50.0 [25.0, 81.3]	31.3 [25.0, 50.0]	0.002
MRA-related side effect (N, %)	16 (32.7%)	25 (33.8%)	0.64
Serum potassium at the last visit (mM)	4.4 [4.2, 4.7]	4.2 [3.9, 4.5]	0.01
eGFR at the last visit (mL/min/1.73m^2)	66 [56, 80]	72 [60, 91]	0.08
PAC at the last visit (ng/dL) [N=82]	32.1 [23.5, 48.0]	28.7 [20.4, 38.6]	0.14
PRA at the last visit (ng/mL/h) [N=92]	2.3 [1.3, 5.9]	0.3 [0.2, 0.6]	<0.001
DRC at the last visit (pg/mL) [N=14]	15.0 [12.9, 26.5]	2.5 [2.1, 4.4]	<0.001
ARR at the last visit (ng/dL per ng/mL/h) [N=82]	15.4 [8.7, 20.4]	76.0 [44.3, 128.1]	<0.001
B. LRH patients			
N	21	19	
Age (years)	47 [36, 68]	59 [50, 64]	0.24
Women (N, %)	10 (47.6%)	17 (89.5%)	0.005
BMI (kg/m^2)	35.4 [29.7, 40.9]	30.2 [26.6, 35.6]	0.08
SBP (mmHg)	157 [143, 174]	160 [130, 184]	0.50
DBP (mmHg)	87 [80, 98]	83 [72, 106]	0.84
Serum potassium (mM)	4.0 [3.5, 4.2]	3.9 [3.5, 4.3]	0.71
eGFR (mL/min/1.73m^2)	77 [58, 95]	87 [75, 103]	0.26
PAC (ng/dL)	11.0 [8.6, 16.4]	11.3 [8.3, 17.3]	0.94
PRA (ng/mL/hr) [N=23]	0.6 [0.3, 0.7]	0.2 [0.1, 0.4]	0.04
DRC (pg/mL) [N=17]	2.7 [2.1, 6.6]	2.3 [2.1, 3.9]	0.79
ARR (ng/dL per ng/mL/h)	25.8 [13.6, 38.4]	36.1 [25.5, 58.8]	0.11
Antihypertensive agents (N)	3.0 [1.8, 4.0]	3.0 [2.0, 3.8]	0.72
Beta-blocker (N, %)	8 (38.1%)	7 (36.8%)	0.93
Potassium replacement (N, %)	4 (19.0%)	4 (21.1%)	0.87
Cardiovascular disease (N, %)	6 (28.6%)	4 (21.1%)	0.58
Follow-up duration (days)	80 [31, 160]	76 [37, 184]	0.99
MRA dosage at the last visit (mg/day)	25.0 [25.0, 50.0]	25.0 [25.0, 50.0]	0.84
MRA-related side effect (N, %)	6 (28.6%)	3 (15.8%)	0.33
Serum potassium at the last visit (mM)	4.5 [4.2, 4.9]	4.2 [4.0, 4.4]	0.02
eGFR at the last visit (mL/min/1.73m^2)	65 [43, 94]	75 [60, 82]	0.25
PAC at the last visit (ng/dL) [N=82]	19.0 [14.3, 26.5]	13.4 [8.5, 18.5]	0.02
PRA at the last visit (ng/mL/h) [N=92]	2.6 [1.5, 5.4]	0.3 [0.2, 0.5]	<0.001
DRC at the last visit (pg/mL) [N=14]	19.7 [18.4, 77.0]	3.1 [2.2, 3.4]	<0.001
ARR at the last visit (ng/dL per ng/mL/h) [N=82]	5.9 [1.9, 17.4]	47.0 [25.7, 54.0]	<0.001

PA, primary aldosteronism; LRH, low renin essential hypertension; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration ratio; PAC, plasma aldosterone concentration; PRA, plasma renin activity; DRC, direct renin concentration; ARR, aldosterone-to-renin ratio; MRA, mineralocorticoid receptor antagonist. Continuous variables are shown as median [interquartile range].

Safety and Side Effects of MRA Therapy

Overall, serum potassium concentrations increased (from 3.8 [3.5, 4.2] to 4.3 [4.1, 4.6] mM, $p<0.001$ for all) during MRA treatment. The percentage of patients taking potassium replacement therapy decreased from 39% to 23% ($p<0.001$), and 21 (13%) patients developed hyperkalemia (**Supplemental Table**). Of the latter, 16 (76%) patients had chronic kidney disease (CKD), and 12 (57%) were concurrently treated with RAAS inhibitors. Overall, eGFR decreased, from 81 [64, 94] prior to MRA initiation to 69 [57, 83] mL/min/1.73m^2 ($p<0.001$) at the last follow-up visit. The changes in eGFR and renin were inversely associated in PA (r=-0.3022, p=0.002,

TABLE 3 | Factors associated with target renin achievement during MRA therapy.

	β	SE	*p*	Odds ratio	95% CI
A. PA patients					
Serum potassium at baseline (mM)	1.67	0.48	<0.001	5.32	2.07-16.68
Beta-blocker use (Reference: no use)	-1.29	0.44	0.004	0.28	0.12-0.66
MRA dose (mg/day)	0.02	0.0056	0.002	1.018	1.01-1.03
B. LRH patients					
Sex (Reference: women)	2.24	0.87	0.01	9.35	1.71-51.03

PA, primary aldosteronism; LRH, low renin essential hypertension; MRA, mineralocorticoid receptor antagonist; SE, standard error; CI, confidence interval. Serum potassium, beta-blocker use and MRA daily dosage in PA and sex in LRH were chosen for multiple logistic regression analysis, using a backward stepwise selection.

Supplemental Figure 2), but not in LRH patients (data not shown).

Gynecomastia and/or breast tenderness occurred in 21 (13%) patients taking spironolactone, at doses between 25-200 mg daily (**Supplemental Table**). Other side effects associated with spironolactone included: acute elevation of creatinine, irregular vaginal bleeding, and decreased libido (**Supplemental Table**). The incidence of side effects was similar between patients with and without PA (33% vs. 23%, p=0.20). Overall, side effects occurred after a median follow up of 91 days and while taking MRA doses between 12.5-400 mg/day. In 17 patients (34%), spironolactone was changed to eplerenone, and 9 other patients (18%) stopped or reduced the doses of spironolactone.

DISCUSSION

Although MRAs have been used primarily for blood pressure control in patients with PA and resistant hypertension (31), recent data indicate that the risk of cardio-renal complications is reduced only in patients with adequate MR blockade, as suggested by reversal of renin suppression (17, 18). Several noteworthy findings emerge from this study of clinical practice patterns in an academic center, with a large volume of patients with hypertension: 1) a very small fraction of patients with hypertension are treated with MRAs; 2) in very few patients treated with MRAs the dose is titrated based on renin targets; 3) only 43% of patients with PA and other LRH treated with MRAs reached target renin after one year of treatment. These findings suggest that a large proportion of patients with hypertension are exposed to preventable risk of cardiovascular and renal morbidity and even death.

While patients with unilateral PA can be cured with surgery, patients with bilateral PA or those who do not undergo unilateral adrenalectomy require life-long medical management. In the absence of selective aldosterone synthase inhibitors, MRAs are the first line of treatment for all non-surgical PA cases (13). Evidence from cohort studies of patients with PA and essential hypertension suggests that, overall, PA patients treated with MRAs have an excess risk of developing atrial fibrillation and renal insufficiency as compared to those treated surgically or patients with essential hypertension with similar baseline blood pressure and risk factors (4, 18). The excessive risk of cardiovascular events and even mortality appeared to be annulled in patients treated with MRA doses that allowed renin elevations above 1 μg/L/h (17). MRAs are also highly effective add-on therapies for controlling resistant hypertension cases (19, 25), likely due to the high prevalence of unrecognized PA and other LRH (3). Moreover, mounting evidence suggests that PA spans a hormonal and clinical continuum, and that some cases of LRH might represent early stages of PA (32, 33). Yet, MRAs are prescribed infrequently. In line with previous data (34, 35), only 5% of all hypertensive patients and less than 10% of those diagnosed with hyperaldosteronism were prescribed MRAs in our cohort. Furthermore, of those who received MRAs, renin goals were followed in a minority of patients and were achieved in under half of the latter subgroup.

In addition to the lack of awareness regarding the benefits of MRA treatment in patients with low-renin hypertension, concerns about hyperkalemia might limit their broader use. The risk of hyperkalemia increases in patients with renal insufficiency. PA is associated with intravascular volume expansion and glomerular hyperfiltration. In such patients, correction of the hyperaldosteronism, either by surgery or medical treatment with MRA, can unmask underlying CKD, by reinstalling normal intravascular volume states (36, 37). Adrenalectomy for aldosterone-producing adenomas leads to a decline in eGFR by 11 to 15 mL/min/1.73m^2, and increase the proportion of apparent CKD (37–39). Similarly, MRA therapy in bilateral PA might cause a relatively acute fall of eGFR, but contributes to long-term preservation of renal function (40, 41). The inverse association between changes of renin and eGFR observed in our study is in line with previous data. Taken together these results indicate that eGFR should be carefully monitored after MRA initiation, and that a mild decline in eGFR should not prompt MRA discontinuation, as cardio-protective benefits occur with long-term MRA therapy.

Simultaneously with the escalation of MRA doses, adjustments of other medications that influence eGFR and/or serum potassium concentrations are essential to prevent hyperkalemia while attempting to reverse renin suppression. In order to reduce the risk of hyperkalemia, discontinuation of potassium supplements and reduction of RAAS inhibitors must be anticipated when suppressed renin renders intensification of MRA therapy. In our study, 21 patient developed hyperkalemia, and of these, 57% were concomitantly taking angiotensin converting enzyme inhibitors or angiotensin receptor blockers, and 76% had CKD.

In addition to the impact of potassium and renal function, concomitant drugs that interfere with RAAS might alter the time to achieving goal renin levels (13, 42). In particular, beta-blockers decrease both renin activity and concentration by blocking

sympathetic stimulation (42, 43). Supporting these effects, our study demonstrates that beta-blockers are associated with lower odds of overcoming renin suppression during MRA treatment. Beta-blockers are often used in patients with cardiac pathology, including heart failure, coronary artery disease, and atrial fibrillation or other tachyarrhythmias, and have survival benefits in such patients (44, 45). Thus, clinical trials designed to answer how to best manage patients with low renin hypertension and cardiac history are greatly needed.

In addition to concerns related to polypharmacy, medication-specific side effects can impact patients' adherence to recommended treatments. In this study, 14% of patients experienced breast tenderness, breast enlargement and/or sexual dysfunction while taking spironolactone. Spironolactone was the first MRA developed and it has been commercialized since the 1950s. While spironolactone is a potent MRA, it can also block the androgen and progesterone receptors (46, 47), which explain its side effect profile. Eplerenone has superior MR selectivity and low propensity for gynecomastia (47, 48), but its efficacy is lower than that of spironolactone (27, 28). Nevertheless, prospective and retrospective studies have shown that both MRAs are efficacious for blood pressure control and reduction of morbidity and mortality associated with heart failure (49–51). Consequently, eplerenone might offer an advantage over spironolactone, particularly in men.

Our study has several limitations, including its retrospective design, heterogeneity of concomitant antihypertensive regimens, and variability in follow-up. In addition, due to the overwhelming number of hypertensive patients and limitation of our medical records search engine, it was not feasible to determine the exact number of patients with resistant hypertension. Nevertheless, extrapolating from existing data, we speculate that a large number of patients who could benefit from MRA are never prescribed these agents.

In summary, in this study of patients with hypertension seen in a large academic center, including primary care and hypertension-focused services, we found that MRAs are rarely prescribed. Despite compelling evidence that inappropriate MR activation enhances the risk of cardiorenal morbidity and mortality, MRA titration to doses that overcome MR activation and reverse renin suppression is pursued in a minority of patients with PA and LRH, and is achieved in less than half of those followed. Until large prospective longitudinal studies of patients with PA and other low-renin hypertension will elucidate the optimal parameters for guiding medical therapy titration, incorporating renin targets within PA practice guidelines is likely to benefit patient care.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available due to patient privacy. Requests to access the datasets should be directed to aturcu@umich.edu.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by University of Michigan IRB. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

AFT conceptualized the study. AFT and YT designed the study. YT collected the data and performed the statistical analysis. AFT was responsible for reviewing the data and providing scientific input. YT and AFT wrote the article. All authors contributed to the article and approved the submitted version.

FUNDING

AFT was supported by grants: 1K08DK109116 from the NIDDK, 2019087 from the Doris Duke Charitable Foundation, and U070002 from the Michigan Institute for Clinical & Health Research (MICHR).

ACKNOWLEDGMENTS

We thank the University of Michigan (UM) Medical School Research Data Warehouse and DataDirect for providing data aggregation and management services in support of the research reported in this publication.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.625457/full#supplementary-material

REFERENCES

1. Florczak E, Prejbisz A, Szwench-Pietrasz E, Sliwiński P, Bieleń P, Klisiewicz A, et al. Clinical characteristics of patients with resistant hypertension: the RESIST-POL study. *J Hum Hypertens* (2013) 27:678–85. doi: 10.1038/jhh.2013.32
2. Strauch B, Zelinka T, Hampf M, Bernhardt R, Widimsky J Jr. Prevalence of primary hyperaldosteronism in moderate to severe hypertension in the Central Europe region. *J Hum Hypertens* (2003) 17:349–52. doi: 10.1038/sj.jhh.1001554
3. Brown JM, Siddiqui M, Calhoun DA, Carey RM, Hopkins PN, Williams GH, et al. The Unrecognized Prevalence of Primary Aldosteronism: A Cross-sectional Study. *Ann Intern Med* (2020) 173:10–20. doi: 10.7326/M20-0065
4. Hundemer GL, Curhan GC, Yozamp N, Wang M, Vaidya A. Renal Outcomes in Medically and Surgically Treated Primary Aldosteronism. *Hypertension* (2018) 72:658–66. doi: 10.1161/HYPERTENSIONAHA.118.11568

5. Monticone S, D'Ascenzo F, Moretti C, Williams TA, Veglio F, Gaita F, et al. Cardiovascular events and target organ damage in primary aldosteronism compared with essential hypertension: a systematic review and meta-analysis. *Lancet Diabetes Endocrinol* (2018) 6:41–50. doi: 10.1016/S2213-8587(17)30319-4
6. Brown NJ. Contribution of aldosterone to cardiovascular and renal inflammation and fibrosis. *Nat Rev Nephrol* (2013) 9:459–69. doi: 10.1038/nrneph.2013.110
7. Nishizaka MK, Zaman MA, Green SA, Renfroe KY, Calhoun DA. Impaired endothelium-dependent flow-mediated vasodilation in hypertensive subjects with hyperaldosteronism. *Circulation* (2004) 109:2857–61. doi: 10.1161/01.CIR.0000129307.26791.8E
8. Rossi GP, Bernini G, Desideri G, Fabris B, Ferri C, Giacchetti G, et al. Renal damage in primary aldosteronism: results of the PAPY Study. *Hypertension* (2006) 48:232–8. doi: 10.1161/01.HYP.0000230444.01215.6a
9. Kozakova M, Buralli S, Palombo C, Bernini G, Moretti A, Favilla S, et al. Myocardial ultrasonic backscatter in hypertension: relation to aldosterone and endothelin. *Hypertension* (2003) 41:230–6. doi: 10.1161/01.HYP.0000052542.68896.2B
10. Freel EM, Mark PB, Weir RA, McQuarrie EP, Allan K, Dargie HJ, et al. Demonstration of blood pressure-independent noninfarct myocardial fibrosis in primary aldosteronism: a cardiac magnetic resonance imaging study. *Circ Cardiovasc Imaging* (2012) 5:740–7. doi: 10.1161/CIRCIMAGING.112.974576
11. Stehr CB, Mellado R, Ocaranza MP, Carvajal CA, Mosso L, Becerra E, et al. Increased levels of oxidative stress, subclinical inflammation, and myocardial fibrosis markers in primary aldosteronism patients. *J Hypertension* (2010) 28:2120–6. doi: 10.1097/HJH.0b013e32833d0177
12. Mulatero P, Monticone S, Bertello C, Viola A, Tizzani D, Iannaccone A, et al. Long-term cardio- and cerebrovascular events in patients with primary aldosteronism. *J Clin Endocrinol Metab* (2013) 98:4826–33. doi: 10.1210/jc.2013-2805
13. Funder JW, Carey RM, Mantero F, Murad MH, Reincke M, Shibata H, et al. The Management of Primary Aldosteronism: Case Detection, Diagnosis, and Treatment: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab* (2016) 101:1889–916. doi: 10.1210/jc.2015-4061
14. Rossi GP, Ceolotto G, Rossitto G, Maiolino G, Cesari M, Seccia TM. Effects of Mineralocorticoid and AT1 Receptor Antagonism on The Aldosterone-Renin Ratio In Primary Aldosteronism-the EMIRA Study. *J Clin Endocrinol Metab* (2020) 105:2060–7. doi: 10.1210/clinem/dgaa080
15. Parthasarathy HK, Ménard J, White WB, Young WF Jr, Williams GH, Williams B, et al. A double-blind, randomized study comparing the antihypertensive effect of eplerenone and spironolactone in patients with hypertension and evidence of primary aldosteronism. *J Hypertens* (2011) 29:980–90. doi: 10.1097/HJH.0b013e3283455ca5
16. Pilz S, Trummer C, Verheyen N, Schwetz V, Pandis M, Aberer F, et al. Mineralocorticoid Receptor Blockers and Aldosterone to Renin Ratio: A Randomized Controlled Trial and Observational Data. *Horm Metab Res* (2018) 50:375–82. doi: 10.1055/a-0604-3249
17. Hundemer GL, Curhan GC, Yozamp N, Wang M, Vaidya A. Cardiometabolic outcomes and mortality in medically treated primary aldosteronism: a retrospective cohort study. *Lancet Diabetes Endocrinol* (2018) 6:51–9. doi: 10.1016/S2213-8587(17)30367-4
18. Hundemer GL, Curhan GC, Yozamp N, Wang M, Vaidya A. Incidence of Atrial Fibrillation and Mineralocorticoid Receptor Activity in Patients With Medically and Surgically Treated Primary Aldosteronism. *JAMA Cardiol* (2018) 3:768–74. doi: 10.1001/jamacardio.2018.2003
19. Williams B, MacDonald TM, Morant S, Webb DJ, Sever P, McInnes G, et al. Spironolactone versus placebo, bisoprolol, and doxazosin to determine the optimal treatment for drug-resistant hypertension (PATHWAY-2): a randomised, double-blind, crossover trial. *Lancet* (2015) 386:2059–68. doi: 10.1016/S0140-6736(15)00257-3
20. Chapman N, Dobson J, Wilson S, Dahlöf B, Sever PS, Wedel H, et al. Effect of spironolactone on blood pressure in subjects with resistant hypertension. *Hypertension* (2007) 49:839–45. doi: 10.1161/01.HYP.0000259805.18468.8c
21. de Souza F, Muxfeldt E, Fiszman R, Salles G. Efficacy of spironolactone therapy in patients with true resistant hypertension. *Hypertension* (2010) 55:147–52. doi: 10.1161/HYPERTENSIONAHA.109.140988
22. Kisaka T, Ozono R, Ishida T, Higashi Y, Oshima T, Kihara Y. Association of elevated plasma aldosterone-to-renin ratio with future cardiovascular events in patients with essential hypertension. *J Hypertens* (2012) 30:2322–30. doi: 10.1097/HJH.0b013e328359862d
23. Maule S, Mulatero P, Milan A, Leotta G, Caserta M, Bertello C, et al. QT interval in patients with primary aldosteronism and low-renin essential hypertension. *J Hypertension* (2006) 24:2459–64. doi: 10.1097/01.hjh.0000251908.93298.a0
24. Weinberger MH, Fineberg NS, Fineberg SE, Weinberger M. Salt sensitivity, pulse pressure, and death in normal and hypertensive humans. *Hypertension* (2001) 37:429–32. doi: 10.1161/01.HYP.37.2.429
25. Williams B, MacDonald TM, Morant SV, Webb DJ, Sever P, McInnes GT, et al. Endocrine and haemodynamic changes in resistant hypertension, and blood pressure responses to spironolactone or amiloride: the PATHWAY-2 mechanisms substudies. *Lancet Diabetes Endocrinol* (2018) 6:464–75. doi: 10.1016/S2213-8587(18)30071-8
26. Kheterpal S. *RDW/DataDirect: a self-serve tool for data retrieval*. Ann Arbor, MI: University of Michigan (2015).
27. Funder JW. Mineralocorticoid receptor antagonists: emerging roles in cardiovascular medicine. *Integr Blood Press Control* (2013) 6:129–38. doi: 10.2147/IBPC.S13783
28. Weinberger MH, Roniker B, Krause SL, Weiss RJ. Eplerenone, a selective aldosterone blocker, in mild-to-moderate hypertension. *Am J Hypertens* (2002) 15:709–16. doi: 10.1016/S0895-7061(02)02957-6
29. Nanba AT, Nanba K, Byrd JB, Shields JJ, Giordano TJ, Miller BS, et al. Discordance between imaging and immunohistochemistry in unilateral primary aldosteronism. *Clin Endocrinol (Oxf)* (2017) 87:665–72. doi: 10.1111/cen.13442
30. Wannachalee T, Zhao L, Nanba K, Nanba AT, Shields JJ, Rainey WE, et al. Three Discrete Patterns of Primary Aldosteronism Lateralization in Response to Cosyntropin During Adrenal Vein Sampling. *J Clin Endocrinol Metab* (2019) 104:5867–76. doi: 10.1210/jc.2019-01182
31. Garthwaite SM, McMahon EG. The evolution of aldosterone antagonists. *Mol Cell Endocrinol* (2004) 217:27–31. doi: 10.1016/j.mce.2003.10.005
32. Vaidya A, Mulatero P, Baudrand R, Adler GK. The Expanding Spectrum of Primary Aldosteronism: Implications for Diagnosis, Pathogenesis, and Treatment. *Endocr Rev* (2018) 39:1057–88. doi: 10.1210/er.2018-00139
33. Vaidya A, Carey RM. Evolution of the Primary Aldosteronism Syndrome: Updating the Approach. *J Clin Endocrinol Metab* (2020) 105:3771–83. doi: 10.1210/clinem/dgaa606
34. Hwang AY, Dave C, Smith SM. Trends in Antihypertensive Medication Use Among US Patients With Resistant Hypertension, 2008 to 2014. *Hypertension* (2016) 68:1349–54. doi: 10.1161/HYPERTENSIONAHA.116.08128
35. Carey RM, Sakhuja S, Calhoun DA, Whelton PK, Muntner P. Prevalence of Apparent Treatment-Resistant Hypertension in the United States. *Hypertension* (2019) 73:424–31. doi: 10.1161/HYPERTENSIONAHA.118.12191
36. Ribstein J, Du Cailar G, Fesler P, Mimran A. Relative glomerular hyperfiltration in primary aldosteronism. *J Am Soc Nephrol* (2005) 16:1320–5. doi: 10.1681/ASN.2004100878
37. Iwakura Y, Morimoto R, Kudo M, Ono Y, Takase K, Seiji K, et al. Predictors of decreasing glomerular filtration rate and prevalence of chronic kidney disease after treatment of primary aldosteronism: renal outcome of 213 cases. *J Clin Endocrinol Metab* (2014) 99:1593–8. doi: 10.1210/jc.2013-2180
38. Kobayashi H, Abe M, Nakamura Y, Takahashi K, Fujita M, Takeda Y, et al. Association Between Acute Fall in Estimated Glomerular Filtration Rate After Treatment for Primary Aldosteronism and Long-Term Decline in Renal Function. *Hypertension* (2019) 74:630–8. doi: 10.1161/HYPERTENSIONAHA.119.13131
39. Kramers BJ, Kramers C, Lenders JW, Deinum J. Effects of Treating Primary Aldosteronism on Renal Function. *J Clin Hypertens (Greenwich)* (2017) 19:290–5. doi: 10.1111/jch.12914
40. Sechi LA, Novello M, Lapenna R, Baroselli S, Nadalini E, Colussi GL, et al. Long-term renal outcomes in patients with primary aldosteronism. *JAMA* (2006) 295:2638–45. doi: 10.1001/jama.295.22.2638
41. Sechi LA, Di Fabio A, Bazzocchi M, Uzzau A, Catena C. Intrarenal hemodynamics in primary aldosteronism before and after treatment. *J Clin Endocrinol Metab* (2009) 94:1191–7. doi: 10.1210/jc.2008-2245

42. Mulatero P, Rabbia F, Milan A, Paglieri C, Morello F, Chiandussi L, et al. Drug effects on aldosterone/plasma renin activity ratio in primary aldosteronism. *Hypertension* (2002) 40:897–902. doi: 10.1161/01.HYP.0000038478.59760.41
43. Griffin TP, Browne GA, Wall D, Dennedy MC, O'Shea PM. A cross-sectional study of the effects of beta-blocker therapy on the interpretation of the aldosterone/renin ratio: can dosing regimen predict effect? *J Hypertens* (2016) 34:307–15. doi: 10.1097/HJH.0000000000000775
44. Packer M, Colucci WS, Sackner-Bernstein JD, Liang CS, Goldscher DA, Freeman I, et al. Double-blind, placebo-controlled study of the effects of carvedilol in patients with moderate to severe heart failure. The PRECISE Trial. Prospective Randomized Evaluation of Carvedilol on Symptoms and Exercise. *Circulation* (1996) 94:2793–9. doi: 10.1161/01.CIR.94.11.2793
45. McMurray J, Køber L, Robertson M, Dargie H, Colucci W, Lopez-Sendon J, et al. Antiarrhythmic effect of carvedilol after acute myocardial infarction: results of the Carvedilol Post-Infarct Survival Control in Left Ventricular Dysfunction (CAPRICORN) trial. *J Am Coll Cardiol* (2005) 45:525–30. doi: 10.1016/j.accreview.2005.05.018
46. Janmohamed S, Bouloux PM. The pharmacological treatment of primary aldosteronism. *Expert Opin Pharmacother* (2006) 7:563–73. doi: 10.1517/14656566.7.5.563
47. Menard J. The 45-year story of the development of an anti-aldosterone more specific than spironolactone. *Mol Cell Endocrinol* (2004) 217:45–52. doi: 10.1016/j.mce.2003.10.008
48. Pitt B, Remme W, Zannad F, Neaton J, Martinez F, Roniker B, et al. Eplerenone, a selective aldosterone blocker, in patients with left ventricular dysfunction after myocardial infarction. *N Engl J Med* (2003) 348:1309–21. doi: 10.1056/NEJMoa030207
49. Pitt B, Williams G, Remme W, Martinez F, Lopez-Sendon J, Zannad F, et al. The EPHESUS trial: eplerenone in patients with heart failure due to systolic dysfunction complicating acute myocardial infarction. Eplerenone Post-AMI Heart Failure Efficacy and Survival Study. *Cardiovasc Drugs Ther* (2001) 15:79–87. doi: 10.1023/a:1011119003788
50. Karagiannis A, Tziomalos K, Papageorgiou A, Kakafika AI, Pagourelias ED, Anagnostis P, et al. Spironolactone versus eplerenone for the treatment of idiopathic hyperaldosteronism. *Expert Opin Pharmacother* (2008) 9:509–15. doi: 10.1517/14656566.9.4.509
51. Pitt B, Zannad F, Remme WJ, Cody R, Castaigne A, Perez A, et al. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized Aldactone Evaluation Study Investigators. *N Engl J Med* (1999) 341:709–17. doi: 10.1056/NEJM199909023411001

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

ORIGINAL RESEARCH
published: 12 April 2021
doi: 10.3389/fendo.2021.645395

Should Adrenal Venous Sampling Be Performed in PA Patients Without Apparent Adrenal Tumors?

Kentaro Okamoto[1], Youichi Ohno[1], Masakatsu Sone[1,2], Nobuya Inagaki[1], Takamasa Ichijo[3], Takashi Yoneda[4], Mika Tsuiki[5], Norio Wada[6], Kenji Oki[7], Kouichi Tamura[8], Hiroki Kobayashi[9], Shoichiro Izawa[10], Akiyo Tanabe[11] and Mitsuhide Naruse[5,12]*

[1] Department of Diabetes, Endocrinology and Nutrition, Kyoto University Graduate School of Medicine, Kyoto, Japan, [2] Division of Metabolism and Endocrinology, Department of Internal Medicine, St. Marianna University School of Medicine, Kawasaki, Japan, [3] Department of Diabetes and Endocrinology, Saiseikai Yokohamashi Tobu Hospital, Yokohama, Japan, [4] Department of Health Promotion and Medicine of the Future, Graduate School of Medical Sciences, Kanazawa University, Kanazawa, Japan, [5] Department of Endocrinology and Metabolism, National Hospital Organization Kyoto Medical Center, Kyoto, Japan, [6] Department of Diabetes and Endocrinology, Sapporo City General Hospital, Sapporo, Japan, [7] Department of Molecular and Internal Medicine, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan, [8] Department of Medical Science and Cardiorenal Medicine, Yokohama City University Graduate School of Medicine, Yokohama, Japan, [9] Division of Nephrology, Hypertension and Endocrinology, Nihon University School of Medicine, Tokyo, Japan, [10] Division of Endocrinology and Metabolism, Tottori University Faculty of Medicine, Yonago, Japan, [11] Division of Endocrinology, National Center for Global Health and Medicine, Tokyo, Japan, [12] Endocrine Center, Ijinkai Takeda General Hospital, Kyoto, Japan

OPEN ACCESS

Edited by:
Vin-Cent Wu, National Taiwan University, Taiwan

Reviewed by:
Olivier Steichen, Assistance Publique Hopitaux De Paris, France
Chin-Chen Chang, National Taiwan University Hospital, Taiwan

***Correspondence:**
Youichi Ohno
bpm4567@kuhp.kyoto-u.ac.jp

Specialty section:
This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Endocrinology

Received: *23 December 2020*
Accepted: *19 March 2021*
Published: *12 April 2021*

Citation:
Okamoto K, Ohno Y, Sone M, Inagaki N, Ichijo T, Yoneda T, Tsuiki M, Wada N, Oki K, Tamura K, Kobayashi H, Izawa S, Tanabe A and Naruse M (2021) Should Adrenal Venous Sampling Be Performed in PA Patients Without Apparent Adrenal Tumors? Front. Endocrinol. 12:645395. doi: 10.3389/fendo.2021.645395

Introduction: Some aldosterone-producing micro-adenomas cannot be detected through image inspection. Therefore, adrenal venous sampling (AVS) is often performed, even in primary aldosteronism (PA) patients who have no apparent adrenal tumors (ATs) on imaging. In most of these cases, however, the PA is bilateral.

Objective: To clarify the clinical need for AVS in PA patients without apparent ATs, taking into consideration the rates of adrenalectomy.

Methods: This is a retrospective cross-sectional study assessing 1586 PA patients without apparent ATs in the multicenter Japan PA study (JPAS). We analyzed which parameters could be used to distinguish unilateral PA patients without apparent ATs from bilateral patients. We also analyzed the prevalences of adrenalectomy in unilateral PA patients.

Results: The unilateral subtype without an apparent AT was diagnosed in 200 (12.6%) of 1586 PA patients. Being young and female with a short hypertension duration, normokalemia, low creatinine level, low plasma aldosterone concentration, and low aldosterone-to-renin ratio (ARR) was significantly more common in bilateral than unilateral PA patients. If PA patients without apparent ATs were female and normokalemic with a low ARR (<560 pg/ml per ng/ml/h), the rate of unilateral PA was only 5 (1.1%) out of 444. Moreover, 77 (38.5%) of the 200 did not receive adrenalectomy, despite being diagnosed with the unilateral subtype based on AVS.

Conclusion: The low prevalence of the unilateral subtype in PA patients without apparent ATs suggests AVS is not indicated for all of these patients. AVS could be skipped in female

normokalemic PA patients without apparent ATs if their ARRs are not high. However, AVS should be considered for male hypokalemic PA patients with high ARRs because the rates of the unilateral subtype are high in these patients.

Keywords: adrenalectomy, adrenal venous sampling, cardiovascular disease, hyperaldosteronism, primary aldosteronism

INTRODUCTION

Primary aldosteronism (PA) is characterized by inappropriate aldosterone production leading to renin suppression, which, if prolonged and severe, may in turn lead to hypertension and hypokalemia. The reported prevalence of PA varies, but recent studies suggest it accounts for about 6% of patients with hypertension (1). The etiologies of PA include two main subtypes diagnosed through adrenal venous sampling (AVS): unilateral, which is often treated surgically, and bilateral, which is most often treated medically (2). Aldosterone is reported to do substantial damage to cardiovascular organs under conditions of high salt intake (3), and previous clinical studies have reported that the prevalence of cardiovascular disease (CVD) was higher in PA patients than patients with essential hypertension (4). In particular, among patients with unilateral PA, which is often caused by an aldosterone producing adenoma, the prevalence of CVD is reportedly higher than among those with bilateral PA (4). And because unilateral PA patients can be treated with adrenalectomy, a diagnosis of PA subtype is relevant.

However, aldosterone-producing micro-adenomas are not always detected through image inspection, necessitating the use of AVS (5, 6). Although AVS is the most reliable method for accurate determination of PA subtype, it has several limitations, including its high cost, invasiveness, associated radiation exposure, and technical difficulty (7–10). On the other hand, probably because PA screening has become more popular, AVS is often performed proactively, even in PA patients without resistant hypertension, a high plasma aldosterone concentration (PAC) or hypokalemia. Consequently, the rate at which PA patients are being diagnosed with unilateral PA on AVS is on the decline and is only about 30% in the multicenter Japan PA study (JPAS) database (11). There is thus a need to be cautious about the indication for AVS in PA patients, especially those without ATs.

In the present study, we analyzed which parameters could be used to distinguish unilateral PA patients without ATs from bilateral patients. We also assessed the rate of adrenalectomy in unilateral PA patients.

MATERIALS AND METHODS

Study Design and Patients

This study was conducted as a part of the JPAS and was a retrospective cross-sectional analysis. A nation-wide PA registry has been established at 40 centers in Japan, including 21 university hospitals and 19 city hospitals. We used a dataset valid as of March 2019. PA patients who were diagnosed and underwent AVS between January 2006 and January 2019 were enrolled. Patients eligible for enrollment in the JPAS were men and women aged 20-90 years. Patients whom the investigators deemed unsuitable were excluded. The clinical characteristics, biochemical findings, results of confirmatory testing, imaging findings, AVS results, treatments, surgical findings, and related follow-up data were electronically collected using the WEB registry system. System construction, data security, and maintenance of the registered data were outsourced to EPS Corporation (Tokyo, Japan). The data that support the findings of this study are available from the corresponding author upon reasonable request.

From among 4050 PA patients, 1586 were ultimately included in the present study. The reasons for exclusion of the other 2464 patients were as follows: the patient lacked imaging data or the imaging revealed an apparent AT (n=2099); AVS was without ACTH stimulation or was unsuccessful, which means there was a low selectivity index or no data on the selectivity index (n=285); or there was not enough data for analysis (n=80).

Diagnosis of PA

The diagnosis of PA was made in accordance with guidelines from the Japan Endocrine Society and the Japan Society of Hypertension (12, 13). PA was diagnosed based on positive case detection when the ratio of the PAC (measured in pg/mL) to the plasma renin activity (PRA) (measured in ng/mL per h) was greater than 200, or when the ratio of the PAC to the active renin concentration (ARC) (measured in pg/mL) was greater than 40 and there was a positive result from at least one confirmatory test, such as the captopril-challenge test, saline-infusion test, furosemide-upright test, or the oral salt-loading test. Antihypertensive medications were usually changed to Ca^{2+} channel blockers and/or α–adrenergic blockers, as appropriate, until a final diagnosis was made.

Subtype Diagnosis

A diagnosis of the PA subtype was made based on an AVS with ACTH (Cosyntropin) stimulation, the procedures for which have been described elsewhere (14). Adrenal vein cannulation was defined as successful if the selectivity index was greater than five (6). The selectivity index was defined as the ratio of the cortisol concentration in the adrenal vein to that in the inferior vena cava. The unilateral PA subtype was diagnosed when the lateralization index was greater than four. The bilateral PA subtype was diagnosed when the lateralization index was equal to or lower than four. The lateralization index was calculated by dividing the aldosterone-to-cortisol ratio on the dominant side by that on the non-dominant side.

Adrenal Lesions on Image Inspection

The findings on CT scans were evaluated by radiologists at each institution. An apparent nodule on a CT scan was deemed to be a nodule if it was greater than or equal to 10 mm in diameter following a previous report (15). The appearance was determined to be bilateral normal if the size of the nodule or thickness of adrenal gland was less than 10 mm on both sides.

Measurements

We collected data on age, sex, smoking habits, drinking habits, hypertension duration, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), proportion taking oral K^+, and various blood tests (K^+, creatinine, estimated glomerular filtration rate (eGFR), aldosterone-to-renin ratio (ARR), PAC, PRA, fasting blood sugar (FBS), HbA1c (NGSP), total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL)). eGFR was calculated using the following equation: eGFR (mL/min/1.73m^2) = 194 x serum creatinine$^{-1.094}$ x age$^{-0.287}$ x 0.739 (if female) (16). Low eGFR was determined as eGFR <60 mL/min/1.73m^2. Hypokalemia was considered to be present if serum K^+ was ≤3.5 mEq/L or when the patient was taking a K^+ supplement at the diagnosis of PA. Oral K^+ was administered if hypokalemia was present.

We also investigated the prevalences of clinical complications such as diabetes mellitus (DM), dyslipidemia, proteinuria, and CVD. Diagnoses of DM and dyslipidemia were made at each institution according to those guidelines (17, 18). The prevalence of proteinuria was defined as +, 2+ or 3+ protein in urinalyses. CVD included stroke (cerebral infarction, cerebral hemorrhage and subarachnoid hemorrhage), ischemic heart disease (myocardial infarction and angina pectoris), and heart failure requiring hospitalization for treatment. Stroke was confirmed by neurologists and ischemic heart disease was confirmed by cardiologists.

Assay Methods

PACs were measured using commercially available radioimmunoassays or chemiluminescent enzyme immunoassays. The reference range for PACs with patients in a supine position was 30-159 pg/mL (SPAC-S Aldosterone kits, Fuji Rebio, Co., Ltd, Tokyo, Japan) at 37 centers and 30-159 pg/mL (Accuraseed Aldosterone, FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) at 3 centers. PRA was measured using a radioimmunoassay or enzyme immunoassay. The reference range for PRA with patients in a supine position was 0.3-2.9 ng/mL/h (PRA-FR RIA kits, Fuji Rebio, Co., Ltd, Tokyo, Japan) at 23 centers, 0.2-2.3 ng/mL/h (PRA EIA kits, Yamasa, Co., Ltd, Choshi, Japan) at 13 centers, and 0.2-2.7 ng/mL/h (PRA RIA kits, Yamasa, Co., Ltd, Choshi, Japan) at 3 centers. Plasma ARCs were measured using a chemiluminescent enzyme immunoassay (Accuraseed Renin (ARC), FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) at 1 center. The reference range for ARCs with patients in a supine position was 2.5-21.4 pg/mL.

Statistical Analysis

Stata/SE ver. 14 software developed by StataCorp® was used for statistical analyses. All data are expressed as the mean ± SD for normally distributed variables and as the median (25th to 75th percentile) for variables not normally distributed. Sex, smoking habits, drinking habits, proportion taking oral K^+, hypokalemia, proteinuria, and histories of DM, dyslipidemia, and CVD were considered to be binary variables. Student's *t* test or the Mann-Whitney U test was used to compare quantitative variables. Pearson's χ^2 test was used for categorical parameters. Cuzick's non-parametric test was used for trends across ordered groups. Values of P <0.05 were considered to indicate significant differences. Logistic regression analysis was performed to determine which parameters correlated with unilateral PA after adjusting for the patients' backgrounds. Odds ratios (ORs) for unilateral PA were expressed as OR ± 95% confidence interval. In the logistic regression analysis, we included parameters that significantly differed between unilateral and bilateral PA patients in the univariate analyses and did not significantly correlate with each other. We also checked the correlations among these parameters using the Spearman rank correlation test to avoid multicollinearity. We considered that multicollinearity could occur when the Pearson's or Spearman's correlation coefficient r >0.4 and P <0.05. In the analysis of accuracy of unilateral PA diagnosis, the optimal receiver operating characteristic (ROC) curve cutoff point for each parameter was determined using the maximum Youden index. The Youden index was calculated by subtracting 1 from the sum of the sensitivity and specificity.

Ethics

The study was conducted in accordance with Declaration of Helsinki Guidelines and the guidelines for clinical studies published by the Ministry of Health and Labor, Japan, and was approved by the ethics committee of the National Hospital Organization Kyoto Medical Center as the project-leading center and by the institutional ethics committees of the participating centers. This observational study was registered as UMIN ID 18756. This study was performed using an opt-out methodology. The opt out option was presented on the website and as a notice in a prominent place at each center.

RESULTS

Comparison of Bilateral and Unilateral PA Cases

Among the 1,586 PA patients without apparent ATs enrolled in the JPAS study, 200 were diagnosed with unilateral PA and 1386 were diagnosed with bilateral PA (**Figure 1**; **Table 1**). There were more women in the bilateral PA group than in the unilateral PA group. In addition, significant differences were found between the two groups with respect to age, drinking habits, hypertension duration, serum creatinine level, TC, HDL and the rate of proteinuria. The prevalences of low eGFR and CVD were higher among unilateral than bilateral PA patients. SBP, DBP, history of smoking, TG, LDL, FBS, HbA1c, eGFR, and the rates of DM and dyslipidemia were similar between the two groups. Among the aldosterone-related parameters, PAC, ARR, and the rate of hypokalemia were significantly higher and the serum K^+

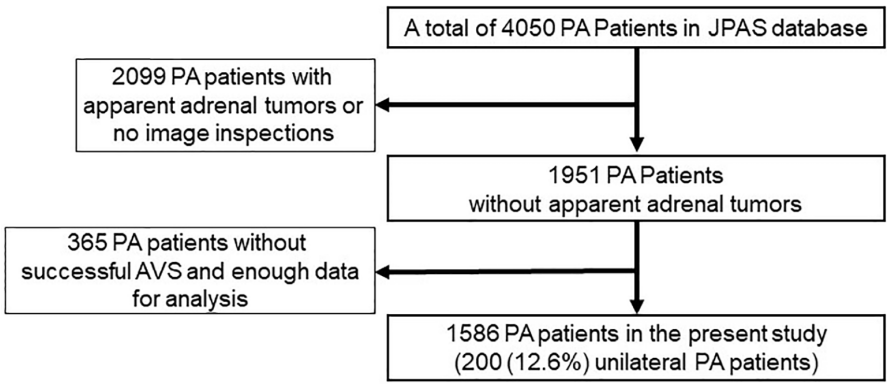

FIGURE 1 | Patient selection criteria for inclusion in this study.

TABLE 1 | Backgrounds and clinical complications of patients with bilateral and unilateral PA without apparent adrenal tumors.

Parameter	Bilateral PA patients n = 1386	Unilateral PA patients n = 200	P
Age, years	51.8 ± 10.9	53.7 ± 11.3	0.022*
Sex, male, %	43.4	71.5	<0.001*
Body mass index, kg/m^2	25.2 ± 4.1	25.3 ± 4.1	0.784
History of Smoking, %	32.1	38.2	0.094
History of Drinking, %	52.0	61.3	0.017*
Systolic blood pressure, mmHg	142.1 ± 17.3	141.8 ± 18.3	0.792
Diastolic blood pressure, mmHg	87.9 ± 13.1	87.5 ± 13.0	0.676
Hypertension duration, years	3 (1-10)	10 (4-18)	<0.001*
Creatinine, mg/dl	0.73 ± 0.22	0.82 ± 0.23	<0.001*
EGFR, ml/min/1.73m^2	79.4 ± 17.2	77.0 ± 21.1	0.087
Low eGFR, %	11.1	16.0	0.043*
Proteinuria, %	7.4	17.0	<0.001*
Total cholesterol, mg/dl	198 ± 33	188 ± 33	<0.001*
Triglyceride, mg/dl	132 ± 82	123 ± 66	0.184
LDL, mg/dl	116 ± 29	112 ± 29	0.112
HDL, mg/dl	57 ± 17	54 ± 15	0.024*
Dyslipidemia, %	27.9	34.1	0.089
Fasting blood sugar, mg/dl	106 ± 30	108 ± 29	0.446
HbA1c (NGSP), %	5.9 ± 1.0	5.8 ± 1.0	0.575
Diabetes mellitus, %	18.9	20.7	0.552
Serum K^+, mEq/l	3.9 ± 0.4	3.5 ± 0.5	<0.001*
Hypokalemia, %	16.9	62.0	<0.001*
ARR, pg/ml per ng/ml/h	420 (290-660)	772 (427-1529)	<0.001*
PAC, pg/ml	156 (118-213)	228 (164-338)	<0.001*
PRA, ng/ml/h	0.4 (0.2-0.6)	0.3 (0.2-0.5)	<0.001*
Cardiovascular disease, %	6.2	10.0	0.045*

Student's t test was used to compare parametric variables. The Mann-Whitney U test was used to compare nonparametric variables. Pearson's χ^2 test was used for categorical parameters. Asterisks (*) indicate significant differences (P <0.05). Hypokalemia was considered to be present if K^+ was ≤3.5 mEq/L or when a patient was taking a potassium supplement. Low eGFR was defined as eGFR <60 ml/min/1.73m^2. ARR, aldosterone-to-renin ratio; EGFR, estimated glomerular filtration rate; HDL, high density

level and PRA were significantly lower among unilateral than bilateral PA patients.

Accuracy of Unilateral PA Diagnosis With Each Parameter

We next assessed the rates of unilateral PA, taking into consideration age, hypertension duration, serum creatinine level, serum K^+ level, ARR, and PAC (**Table 2**). Cuzick's non-parametric test for trend demonstrated that the prevalence of unilateral PA patients significantly increased as age, serum creatinine level, ARR or PAC increased or serum K^+ decreased. We also used ROC curve analysis to calculate the optimal thresholds for these parameters. The optimal cutoff value in each parameter and its sensitivity, specificity, and likelihood ratio (LR) for distinguishing unilateral from bilateral PA are shown in **Table 3**. Serum K^+, ARR and PAC had higher positive LRs than age, hypertension duration or serum creatinine level. Among categorical parameters, male and hypokalemia had low negative LRs, while hypokalemia, in particular, had the highest positive LR among these parameters.

We also performed logistic regression analysis to determine which parameter could contribute to a diagnosis of unilateral PA after adjusting for variables showing significant differences in the univariate analyses (**Table 4**). All parameters were independent each other; however, because ARR potentially correlates with PAC and PRA, we selected age, sex, drinking habits, creatinine, hypokalemia, proteinuria, history of CVD, and ARR as independent variables. In this logistic regression analysis, male, long hypertension duration, hypokalemia, and high ARR significantly increased the odds of having unilateral PA; that means female, short hypertension duration, normokalemia, and low ARR were independently more common among bilateral PA patients than unilateral PA patients.

Efficiency of Diagnosing Unilateral PA Based on Patients' Backgrounds

We considered whether we could efficiently distinguish unilateral PA patients without ATs from other patients based on the results of the logistic regression analysis (**Figure 2**). For this analysis, we

TABLE 2 | Rates of unilateral PA patients without apparent adrenal tumors in each parameter classified by quartile.

Parameter		Rates of Unilateral PA (%)	P
Age, years	<43	10.9	0.022*
	44-51	9.6	
	52-60	15.2	
	≥61	14.7	
HT duration, years	<1	5.5	<0.001*
	1-3	7.0	
	4-9	12.3	
	≥10	23.7	
Creatinine, mg/dL	<0.6	8.1	<0.001*
	0.6-0.70	8.2	
	0.71-0.83	12.3	
	≥0.84	21.6	
Serum K^+, mEq/l	<3.6	34.2	<0.001*
	3.6-3.8	10.7	
	3.9-4.0	7.4	
	≥4.1	5.1	
ARR, pg/ml per ng/ml/h	<298	6.0	<0.001*
	298-442	7.1	
	443-742	11.2	
	≥743	26.2	
PAC, pg/ml	<123	2.6	<0.001*
	123-161	9.5	
	162-225	12.6	
	≥226	25.4	

Cuzick's nonparametric test was performed to assess trends across ordered groups. Asterisks (*) indicate significant differences (P <0.05). ARR, aldosterone-to-renin ratio; HT, hypertension; PA, primary aldosteronism; and PAC, Plasma aldosterone concentration.

did not select hypertension duration because it was unlikely that the reported duration was sufficiently accurate due to memory bias. When a PA patient without apparent ATs was female and did not exhibit hypokalemia, her rate of unilateral PA was only 3.3%. Moreover, the rate decreased to only 1.1% when her ARR was less than 560 pg/ml per ng/ml/h, which was the optimal cut-off with the maximum Youden index calculated from the ROC curve. In fact, only a single female unilateral PA patient presented with no apparent ATs and laboratory tests showing normokalemia and ARR less than 300 pg/ml per ng/ml/h. On the other, when a PA patient without apparent ATs was male and exhibited hypokalemia, his rate of unilateral PA was 44.3%, which increased 54.3% under the condition of ARR ≥560 pg/mL per ng/ml/h.

TABLE 4 | Odds ratios and 95% confidence intervals for each parameter to distinguish unilateral PA patients without apparent adrenal tumors from bilateral PA patients.

Parameter	Odds ratio	Standard error	P	95% Coefficient Interval
Age, years	0.992	0.010	0.414	0.974-1.011
Sex, male	2.899	0.752	<0.001*	1.743-4.822
History of Drinking	1.147	0.237	0.506	0.765-1.720
Hypertension duration, years	1.045	0.012	<0.001*	1.022-1.069
Creatinine, mg/dL	1.092	0.648	0.882	0.342-3.491
Hypokalemia	5.028	0.994	<0.001*	3.413-7.407
ARR, pg/ml per ng/ml/h	1.001	0.0001	<0.001*	1.0005-1.001
Proteinuria	1.278	0.374	0.403	0.720-2.269
Cardiovascular disease	1.035	0.335	0.916	0.548-1.953

Asterisks (*) indicate significant differences (P <0.05). For the odds ratios, numerators are the odds in the unilateral PA group; denominators are the odds in the bilateral PA group. ARR, aldosterone-to-renin ratio and PA, primary aldosteronism.

Clinical Outcomes in Unilateral PA Patients Without Apparent ATs

Adrenalectomy was performed in 123 of the 200 PA patients without apparent ATs. After adrenalectomy, unilateral PA patients without apparent ATs who had postoperative data showed significant improvement in systolic BP (142.2 ± 17.5 to 130.2 ± 15.1, P<0.001), diastolic BP (87.7 ± 11.4 to 81.1 ± 10.7, P<0.001), serum K^+ (3.4 ± 0.5 to 4.3 ± 0.3, P<0.001), and the number of antihypertensive drugs (1.35 ± 0.78 to 0.99 ± 0.80, P<0.001) after 6 to 12 months of follow-up. The pathological diagnoses in these 123 patients were as follows: 98 adenoma, 13 hyperplasia, 8 unable to determine whether adenoma or hyperplasia, 3 normal, and 1 unknown. On the other, unilateral PA patients treated with mineralocorticoid blockers also showed significant improvement in systolic BP (145.2 ± 19.3 to 136.4 ± 15.4, P=0.006) and serum K^+ (3.6 ± 0.4 to 4.2 ± 0.5, P<0.001), but not in diastolic BP (87.2 ± 13.6 to 86.2 ± 11.5, P=0.590) after 6 to 12 months of follow-up.

TABLE 3 | Optimal cutoff values for each parameter and its sensitivity, specificity and likelihood ratio distinguishing unilateral PA patients without apparent adrenal tumors from bilateral PA patients.

Parameters	Optimal Cutoff Value	Sensitivity	Specificity	Positive Likelihood Ratio	Negative Likelihood Ratio
Age, years	55	51.5	60.1	1.29	0.81
HT duration, years	8	93.7	15.2	1.10	0.42
Creatinine, mg/dL	0.74	64.5	58.5	1.55	0.61
Serum K^+, mEq/L	3.7	78.9	61.5	2.05	0.34
ARR, pg/ml per ng/ml/h	559	68.0	66.7	2.04	0.48
PAC, pg/ml	223	53.0	77.9	2.39	0.60
Sex, male	–	71.5	56.6	1.65	0.50
Low eGFR	–	16.0	88.9	1.44	0.94
Proteinuria	–	17.0	92.6	2.30	0.90
Hypokalemia	–	62.0	83.1	3.67	0.46
CVD	–	10.0	93.8	1.61	0.96

For continuous variables, the optimal cutoff value was determined using the maximum Youden index calculated from the ROC curve. Hypokalemia was considered to be present if K^+ was ≤3.5 mEq/L or when a patient was taking a potassium supplement. Low eGFR was defined as eGFR <60 ml/min/1.73m^2. ARR, aldosterone-to-renin ratio; CVD, cardiovascular disease; EGFR, estimated glomerular filtration rate; HT, hypertension; PA, primary aldosteronism; and PAC, plasma aldosterone concentration.

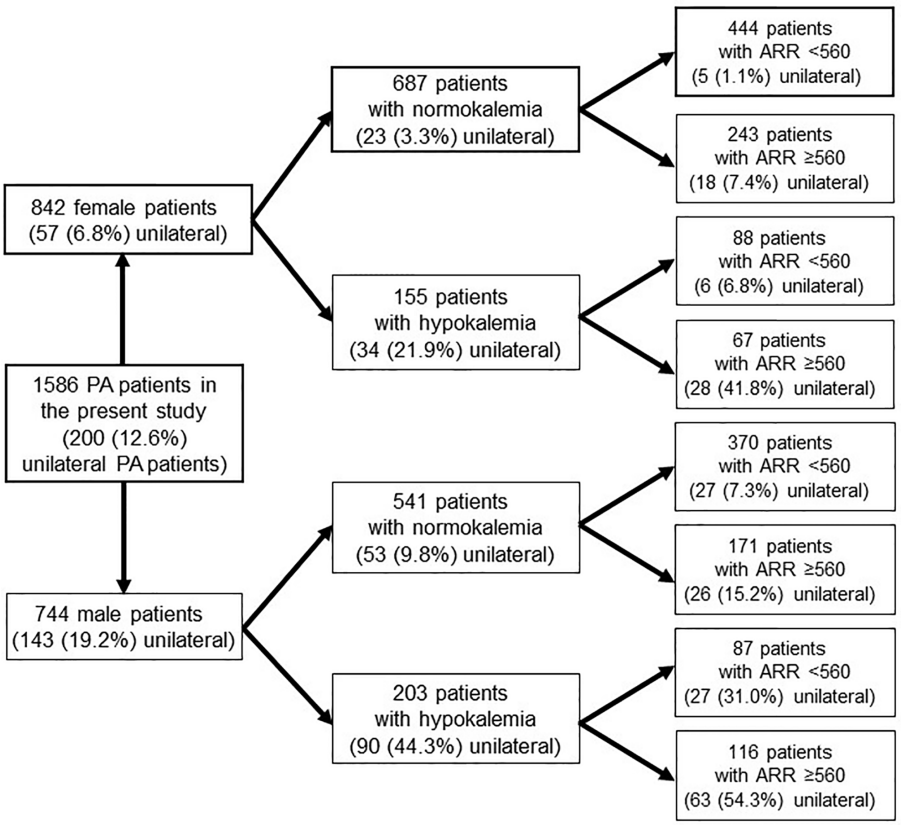

FIGURE 2 | The prevalence of PA patients in each condition.

DISCUSSION

In the present study, only 200 (12.6%) of the 1586 PA patients without apparent ATs were diagnosed with unilateral PA. Being young and female with short hypertension duration, normokalemia, a low creatinine level, low PAC, and low ARR was significantly more common in bilateral than unilateral PA patients. In particular, the rate of female normokalemic unilateral PA patients without apparent ATs and with ARRs less than 560 pg/ml per ng/ml/h was only 1.1%, and only a single patient (0.2%) had an ARR less than 300 pg/ml per ng/ml/h. Actually, hypokalemia, high PAC and high ARR were more common in unilateral than bilateral PA. This was especially true when PA patients without apparent ATs were in the highest quartile for PAC or ARR or in the lowest quartile for serum K^+; for that group the rate of unilateral PA was close to 30%. In previous reports, sex, serum K^+ level, eGFR, PAC, ARR and PAC after a captopril-challenge test or saline-infusion test, and CT findings for the adrenal glands were used to predict PA subtype (19–24). For example, studies by Umakoshi et al. and Kobayashi et al. emphasized normokalemic PA patients with bilateral normal results on CT were highly likely to be bilateral PA patients (23, 24). These findings suggest AVS may not be necessary to detect unilateral PA patients in all cases if we consider the patients' backgrounds. In our study, the sensitivities and specificities of each parameter suggested sex, the serum K^+ level, PAC and ARR could be helpful indicators when assessing the need for AVS in PA patients without apparent ATs. Therefore, among PA patients without apparent ATs, AVS is needed for male hypokalemic PA patients with a high ARR or PAC, given the high rates of the unilateral PA subtype. Conversely, AVS may be unnecessary for female normokalemic PA patients without high ARR due to the low rate of the unilateral subtype among that group.

A meta-analysis of PA patients has shown that, in Japan, men comprise a smaller proportion than do women. However, in other countries, men tend to comprise a higher proportion of PA patients than do women (25). Genetic mutations associated with development of aldosterone-producing adenomas have been reported (26). In particular, ATP1A1 and CACNA1D mutations have been found in men with small adenomas. In a recent study reported by Sam et al., about 90% of men with PA did not have apparent ATs (27). Therefore, in our study, the proportion of men could have appeared larger because we only investigated patients with unilateral PA patients without apparent ATs.

It has been reported that the unilateral PA subtype significantly increases the adjusted odds ratios for CVD, and the incidence of CVD among unilateral PA patients is significantly reduced by

adrenalectomy as compared to medical treatment (4, 28). Furthermore, adrenalectomy was reported to lower blood pressure in PA patients, improve their quality of life, and reduce the incidences of DM and end stage renal disease as compared to medical treatment (29–32). It may therefore be desirable to treat unilateral PA patients with adrenalectomy, especially patients at high risk of CVD. In this study, we found that adrenalectomy was performed in 123 of the 200 PA patients without apparent ATs. In other words, 77 of the 200 unilateral PA patients without apparent ATs did not receive adrenalectomy, despite being diagnosed with the unilateral subtype. In Adrenal Venous Sampling Stats in Primary Aldosteronism (AVSTAT) study, about 70% of reasons for not undergoing adrenalectomy in unilateral PA patients depended on patient's decision (33). In addition, some clinicians decided against adrenalectomy in patients with unilateral PA subtype because their blood pressure was well controlled, they were normokalemic, and they did not have definite adrenal tumors, most of these factors being known prior to AVS. In a recent study, 40 of 70 patients with AVS-confirmed unilateral PA and normal findings on adrenal imaging underwent adrenalectomy; this proportion is not high and similar to that found in our study (27). Even when their condition has been adequately explained, some patients choose not to undergo adrenalectomy after AVS. Another possible explanation for this low proportion is that improvements in systolic BP and hypokalemia were achieved by administering mineralocorticoid blockers to some unilateral PA patients without apparent ATs. However, the fact that adrenalectomy was not performed in about 40% of them suggests that for a substantial number of clinicians, there are barriers to surgically treating patients without apparent ATs unless patients have severe symptoms, like hypokalemia. In the present study, adrenalectomy resulted in significant improvement in hypertension and hypokalemia in unilateral PA patients, even those without apparent ATs. Sam et al. reported that 31 of 36 (86%) PA patients without apparent ATs had complete or partial responses (27). Therefore, adrenalectomy may be indicated in unilateral PA patients without apparent ATs.

Our study has several limitations. This is a retrospective cross-sectional study, which means the incidence of CVD among PA patients is unknown. We only evaluated the data from ACTH-stimulated AVS in this study, which means that bilateral or unilateral diagnostic results may differ in some cases when using the data from non-stimulated AVS. In this study, we defined adrenal lesions greater than or equal to 10mm as apparent nodules because it is difficult to detect lesions smaller than 10mm in some cases. Therefore, it cannot be ruled out that some patients in this study may have micro adenomas. In addition, imaging was not performed in all of our PA patients, which could introduce selection bias.

CONCLUSION

The percentage of unilateral PA patients without apparent ATs was 12.6% and adrenalectomy was not performed in 38.5% of those patients, which suggests AVS is not necessary for all PA patients without apparent ATs. Especially in female PA patients without apparent ATs, AVS could be skipped if they do not have hypokalemia and high ARR. On the other hand, male PA patients with hypokalemia or a high PAC or ARR likely need AVS, given the high prevalence of the unilateral PA subtype among that group.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because the representative of this study is not the corresponding author. Requests to access the datasets should be directed to YO, bpm4567@kuhp.kyoto-u.ac.jp.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The ethics committee of the National Hospital Organization Kyoto Medical Center. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

KOka, MN, MS, NI, and YO were involved in study design and data interpretation. KOka and YO were involved in the data analysis. AT contributed to create the calculable data. MT, TI, and TY especially collected a large amount of data and did data entry. KOki and KT checked the validity of statistical methods. HK, NW, and SI discussed the result and commented on the manuscript. MN, MS, and NI supervised the findings of this work. All authors contributed to the article and approved the submitted version.

FUNDING

This study was conducted as a part of the JPAS (Japan PA Study) and JRAS (Japan Rare Adrenal Diseases Study) supported by research grants from the Japan Agency for Medical Research and Development (AMED) under grant numbers JP17ek0109122 (JPAS) and JP20ek0109352 (JRAS), and the National Center for Global Health and Medicine, Japan (27-1402, 30-1008). This study was also supported by grants from the Ministry of Health, Labor, and Welfare, Japan (Nanbyo-Ippan-046) and the Japan Smoking Foundation.

ACKNOWLEDGMENTS

We thank the JPAS and JRAS members for collecting the clinical data. We also thank Maki Kashiwabara and Keiko Umegaki for their technical and secretarial assistance.

REFERENCES

1. Käyser SC, Dekkers T, Groenewoud HJ, van der Wilt GJ, Carel Bakx J, van der Wel MC, et al. Study Heterogeneity and Estimation of Prevalence of Primary Aldosteronism: A Systematic Review and Meta-Regression Analysis. *J Clin Endocrinol Metab* (2016) 101(7):2826–35. doi: 10.1210/jc.2016-1472
2. Chao CT, Wu VC, Kuo CC, Lin YH, Chang CC, Chueh SJ, et al. Diagnosis and management of primary aldosteronism: an updated review. *Ann Med* (2013) 45(4):375–83. doi: 10.3109/07853890.2013.785234
3. Brown NJ. Contribution of aldosterone to cardiovascular and renal inflammation and fibrosis. *Nat Rev Nephrol* (2013) 9(8):459–69. doi: 10.1038/nrneph.2013.110
4. Ohno Y, Sone M, Inagaki N, Yamasaki T, Ogawa O, Takeda Y, et al. Prevalence of Cardiovascular Disease and Its Risk Factors in Primary Aldosteronism: A Multicenter Study in Japan. *Hypertension* (2018) 71 (3):530–7. doi: 10.1161/HYPERTENSIONAHA.117.10263
5. Kempers MJ, Lenders JW, van Outheusden L, van der Wilt GJ, Schultze Kool LJ, Hermus AR, et al. Systematic review: diagnostic procedures to differentiate unilateral from bilateral adrenal abnormality in primary aldosteronism. *Ann Intern Med* (2009) 151(5):329–37. doi: 10.7326/0003-4819-151-5-200909010-00007
6. Young WF, Stanson AW, Thompson GB, Grant CS, Farley DR, van Heerden JA. Role for adrenal venous sampling in primary aldosteronism. *Surgery* (2004) 136(6):1227–35. doi: 10.1016/j.surg.2004.06.051
7. Rossi GP, Barisa M, Allolio B, Auchus RJ, Amar L, Cohen D, et al. The Adrenal Vein Sampling International Study (AVIS) for identifying the major subtypes of primary aldosteronism. *J Clin Endocrinol Metab* (2012) 97 (5):1606–14. doi: 10.1210/jc.2011-2830
8. Monticone S, Satoh F, Dietz AS, Goupil R, Lang K, Pizzolo F, et al. Clinical Management and Outcomes of Adrenal Hemorrhage Following Adrenal Vein Sampling in Primary Aldosteronism. *Hypertension* (2016) 67(1):146–52. doi: 10.1161/HYPERTENSIONAHA.115.06305
9. Lubitz CC, Economopoulos KP, Sy S, Johanson C, Kunzel HE, Reincke M, et al. Cost-Effectiveness of Screening for Primary Aldosteronism and Subtype Diagnosis in the Resistant Hypertensive Patients. *Circ Cardiovasc Qual Outcomes* (2015) 8(6):621–30. doi: 10.1161/CIRCOUTCOMES.115.002002
10. Fuss CT, Treitl M, Rayes N, Podrabsky P, Fenske WK, Heinrich DA, et al. Radiation exposure of adrenal vein sampling: a German Multicenter Study. *Eur J Endocrinol* (2018) 179(4):261–7. doi: 10.1530/EJE-18-0328
11. Fujii Y, Takeda Y, Kurihara I, Itoh H, Katabami T, Ichijo T, et al. Historical changes and between-facility differences in adrenal venous sampling for primary aldosteronism in Japan. *J Hum Hypertens* (2020) 34(1):34–42. doi: 10.1038/s41371-019-0229-4
12. Nishikawa T, Omura M, Satoh F, Shibata H, Takahashi K, Tamura N, et al. Guidelines for the diagnosis and treatment of primary aldosteronism–the Japan Endocrine Society 2009. *Endocr J* (2011) 58(9):711–21. doi: 10.1507/endocrj.EJ11-0133
13. Shimamoto K, Ando K, Fujita T, Hasebe N, Higaki J, Horiuchi M, et al. The Japanese Society of Hypertension Guidelines for the Management of Hypertension (JSH 2014). *Hypertens Res* (2014) 37(4):253–390. doi: 10.1038/hr.2014.20
14. Umakoshi H, Wada N, Ichijo T, Kamemura K, Matsuda Y, Fuji Y, et al. Optimum position of left adrenal vein sampling for subtype diagnosis in primary aldosteronism. *Clin Endocrinol (Oxf)* (2015) 83(6):768–73. doi: 10.1111/cen.12847
15. Sherlock M, Scarsbrook A, Abbas A, Fraser S, Limumpornpetch P, Dineen R, et al. Adrenal Incidentaloma. *Endocr Rev* (2020) 41(6):775–820. doi: 10.1210/endrev/bnaa008
16. Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K, et al. Revised equations for estimated GFR from serum creatinine in Japan. *Am J Kidney Dis* (2009) 53(6):982–92. doi: 10.1053/j.ajkd.2008.12.034
17. Teramoto T, Sasaki J, Ishibashi S, Birou S, Daida H, Dohi S, et al. Diagnostic criteria for dyslipidemia. *Diagn Criteria Dyslipidemia J Atheroscler Thromb* (2013) 20(8):655–60. doi: 10.5551/jat.17152
18. Seino Y, Nanjo K, Tajima N, Kadowaki T, Kashiwagi A, Araki E, et al. Report of the committee on the classification and diagnostic criteria of diabetes mellitus. *J Diabetes Investig* (2010) 1(5):212–28. doi: 10.1111/j.2040-1124.2010.00074.x
19. Kupers EM, Amar L, Raynaud A, Plouin PF, Steichen O. A clinical prediction score to diagnose unilateral primary aldosteronism. *J Clin Endocrinol Metab* (2012) 97(10):3530–7. doi: 10.1210/jc.2012-1917
20. Nanba K, Tsuiki M, Nakao K, Nanba A, Usui T, Tagami T, et al. A subtype prediction score for primary aldosteronism. *J Hum Hypertens* (2014) 28 (12):716–20. doi: 10.1038/jhh.2014.20
21. Kocjan T, Janez A, Stankovic M, Vidmar G, Jensterle M. A new clinical prediction criterion accurately determines a subset of patients with bilateral primary aldosteronism before adrenal venous sampling. *Endocr Pract* (2016) 22(5):587–94. doi: 10.4158/EP15982.OR
22. Kamemura K, Wada N, Ichijo T, Matsuda Y, Fujii Y, Kai T, et al. Significance of adrenal computed tomography in predicting laterality and indicating adrenal vein sampling in primary aldosteronism. *J Hum Hypertens* (2017) 31(3):195–9. doi: 10.1038/jhh.2016.61
23. Kobayashi H, Abe M, Soma M, Takeda Y, Kurihara I, Itoh H, et al. Development and validation of subtype prediction scores for the workup of primary aldosteronism. *J Hypertens* (2018) 36(11):2269–76. doi: 10.1097/HJH.0000000000001855
24. Umakoshi H, Tsuiki M, Takeda Y, Kurihara I, Itoh H, Katabami T, et al. Significance of Computed Tomography and Serum Potassium in Predicting Subtype Diagnosis of Primary Aldosteronism. *J Clin Endocrinol Metab* (2018) 103(3):900–8. doi: 10.1210/jc.2017-01774
25. Zhou Y, Wang D, Jiang L, Ran F, Chen S, Zhou P, et al. Diagnostic accuracy of adrenal imaging for subtype diagnosis in primary aldosteronism: systematic review and meta-analysis. *BMJ Open* (2020) 10(12):e038489. doi: 10.1136/bmjopen-2020-038489
26. Dutta RK, Söderkvist P, Gimm O. Genetics of primary hyperaldosteronism. *Endocr Relat Cancer* (2016) 23(10):R437–54. doi: 10.1530/ERC-16-0055
27. Sam D, Kline GA, So B, Pasieka JL, Harvey A, Chin A, et al. Surgical Outcomes Among Primary Aldosteronism Patients Without Visible Adrenal Lesions. *J Clin Endocrinol Metab* (2021) 106(2):e824–35. doi: 10.1210/clinem/dgaa821
28. Hundemer GL, Curhan GC, Yozamp N, Wang M, Vaidya A. Cardiometabolic outcomes and mortality in medically treated primary aldosteronism: a retrospective cohort study. *Lancet Diabetes Endocrinol* (2018) 6(1):51–9. doi: 10.1016/S2213-8587(17)30367-4
29. Wu VC, Chueh SJ, Chen L, Chang CH, Hu YH, Lin YH, et al. Risk of new-onset diabetes mellitus in primary aldosteronism: a population study over 5 years. *J Hypertens* (2017) 35(8):1698–708. doi: 10.1097/HJH.0000000000001361
30. Velema M, Dekkers T, Hermus A, Timmers H, Lenders J, Groenewoud H, et al. Quality of Life in Primary Aldosteronism: A Comparative Effectiveness Study of Adrenalectomy and Medical Treatment. *J Clin Endocrinol Metab* (2018) 103(1):16–24. doi: 10.1210/jc.2017-01442
31. Katabami T, Fukuda H, Tsukiyama H, Tanaka Y, Takeda Y, Kurihara I, et al. Clinical and biochemical outcomes after adrenalectomy and medical treatment in patients with unilateral primary aldosteronism. *J Hypertens* (2019) 37(7):1513–20. doi: 10.1097/HJH.0000000000002070
32. Chen YY, Lin YH, Huang WC, Chueh E, Chen L, Yang SY, et al. Adrenalectomy Improves the Long-Term Risk of End-Stage Renal Disease and Mortality of Primary Aldosteronism. *J Endocr Soc* (2019) 3(6):1110–26. doi: 10.1210/js.2019-00019
33. Ohno Y, Naruse M, Beuschlein F, Schreiner F, Parasiliti-Caprino M, Deinum J, et al. Adrenal venous sampling guided adrenalectomy rates in primary aldosteronism: results of an international cohort (AVSTAT). *J Clin Endocrinol Metab* (2020) 106(3):e1400-7. doi: 10.1210/clinem/dgaa706

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

ORIGINAL RESEARCH
published: 19 April 2021
doi: 10.3389/fendo.2021.646097

Hemodynamic and Non-Hemodynamic Components of Cardiac Remodeling in Primary Aldosteronism

Chien-Ting Pan[1,2,3], *Xue-Ming Wu*[4], *Cheng-Hsuan Tsai*[2,3,5], *Yi-Yao Chang*[2,3,6,7], *Zheng-Wei Chen*[1,2,3], *Chin-Chen Chang*[8], *Bo-Ching Lee*[8], *Che-Wei Liao*[2,3,9], *Ya-Li Chen*[2,3], *Lung-Chun Lin*[2,3], *Yi-Ru Chang*[2,3], *Chi-Sheng Hung*[2,3] *and Yen-Hung Lin*[2,3]*

[1] Department of Internal Medicine, National Taiwan University Hospital Yun-Lin Branch, Yun-Lin, Taiwan, [2] Department of Internal Medicine, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan, [3] Cardiovascular Center, National Taiwan University Hospital, Taipei, Taiwan, [4] Department of Internal Medicine, Taoyuan General Hospital, Taoyuan, Taiwan, [5] Department of Internal Medicine, National Taiwan University Hospital, JinShan Branch, New Taipei, Taiwan, [6] Cardiology Division of Cardiovascular Medical Center, Far Eastern Memorial Hospital, New Taipei City, Taiwan, [7] Center of General Education, Chihlee University of Technology, New Taipei City, Taiwan, [8] Department of Medical Imaging, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan, [9] Department of Internal Medicine, National Taiwan University Hospital Hsin-Chu Branch, Hsin-Chu, Taiwan

OPEN ACCESS

Edited by:
Qiang Wei,
Sichuan University, China

Reviewed by:
Rasa Zarnegar,
Cornell University, United States
Cristiana Catena,
University of Udine, Italy

***Correspondence:**
Yen-Hung Lin
austinr34@gmail.com

Specialty section:
This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Endocrinology

Received: *25 December 2020*
Accepted: *24 March 2021*
Published: *19 April 2021*

Citation:
Pan C-T, Wu X-M, Tsai C-H, Chang Y-Y, Chen Z-W, Chang C-C, Lee B-C, Liao C-W, Chen Y-L, Lin L-C, Chang Y-R, Hung C-S and Lin Y-H (2021) Hemodynamic and Non-Hemodynamic Components of Cardiac Remodeling in Primary Aldosteronism. Front. Endocrinol. 12:646097. doi: 10.3389/fendo.2021.646097

Objectives: Patients with primary aldosteronism (PA) have cardiac remodeling due to hemodynamic and non-hemodynamic causes. However, component analysis of cardiac remodeling and reversal in PA patients is lacking. We investigated components of cardiac remodeling and reversal after adrenalectomy in patients with aldosterone-producing adenoma (APA).

Methods: This study prospectively enrolled 304 APA patients who received adrenalectomy and 271 with essential hypertension (EH). Clinical, biochemical and echocardiographic data were collected in both groups and 1 year after surgery in the APA patients. The hemodynamic and non-hemodynamic components of left ventricular (LV) remodeling were represented by predicted left ventricular mass index (LVMI) (pLVMI) and inappropriately excessive LVMI (ieLVMI, defined as LVMI-pLVMI).

Results: After propensity score matching, 213 APA and 213 EH patients were selected. APA patients had higher hemodynamic (pLVMI) and non-hemodynamic (ieLVMI) components of LV remodeling than EH patients. In multivariate analysis, baseline pLVMI was correlated with systolic blood pressure (SBP) and serum potassium, whereas ieLVMI was correlated with log plasma aldosterone concentration but not blood pressure. Post-operative echocardiography was available in 207 patents and showed significant decreases in both pLVMI and ieLVMI after adrenalectomy. In multivariate analysis, ΔpLVMI was correlated with SBP, ΔSBP, and pre-operative pLVMI, whereas ΔieLVMI was correlated with Δlog aldosterone-to-renin ratio (ARR) and pre-operative ieLVMI.

Conclusions: This study concluded that extensive cardiac remodeling in APA patients occurs through hemodynamic and non-hemodynamic causes. Adrenalectomy can improve both hemodynamic and non-hemodynamic components of LV remodeling. Regressions of pLVMI and ieLVMI were correlated with decreases in blood pressure and ARR, respectively.

Keywords: primary aldosteronism, aldosterone producing adenomas, aldosterone (ALDO), cardiac remodeling, left ventricular hypertrophy (LVH), inappropriately excessive left ventricular mass

HIGHLIGHTS

We investigated cardiac remodeling and reversal after adrenalectomy in 304 patients with aldosterone-producing adenoma (APA) with 271 essential hypertension (EH) through hemodynamic and non-hemodynamic components as predicted left ventricular mass index (LVMI) (pLVMI) and inappropriately excessive LVMI (ieLVMI). APA patients had higher pLVMI and ieLVMI correlated with blood pressure and aldosterone. 207 APA patents showed decreased pLVMI and ieLVMI after adrenalectomy with ΔpLVMI correlated with blood pressure and ΔieLVMI correlated with Δlog aldosterone-to-renin ratio. This study demonstrated improved cardiac remodeling in APA patients through hemodynamic and non-hemodynamic causes after adrenalectomy, which correlated with decreases in blood pressure and ARR, respectively.

INTRODUCTION

Primary aldosteronism (PA) is a disease featuring excessive endogenous aldosterone (1, 2). Previous studies and a recent meta-analysis have reported an increased risk of cardiovascular diseases and greater cardiac remodeling in PA patients compared to those with essential hypertension (EH) (3, 4).

Cardiac remodeling is prognostic and linked to heart failure progression (5). It manifests clinically as changes in size, shape, and function of the heart, and is influenced by hemodynamic load, neurohormonal activation and other factors (5). Left ventricular hypertrophy (LVH) with increased cardiac mass is the most common and well-studied type of cardiac remodeling, and it has been clinically shown to be closely correlated to systolic and diastolic function, and to further predict long-term outcomes (6, 7).

Hypertension plays a key role in the hemostasis of hemodynamics, is a well-known risk factor for LVH (8), and is a major cause of LVH in patients with PA. However, PA with elevated blood pressure has been shown to cause a higher degree of LVH than hypertension itself, and an increased prevalence of LVH has been reported in patients with PA compared to those with EH after adjusting for blood pressure in previous retrospective cohorts (3). In addition, small prospective studies have demonstrated a higher incidence of LVH in PA patients than in blood pressure-matched EH controls (9). Excessive endogenous aldosterone has been reported to be another major cause of LVH, however these studies have included confounders such as the influence of blood pressure. Furthermore, the treatment of PA with unilateral adrenalectomy effectively decreases blood pressure and relieves aldosterone overproduction. Regression of LVH has been observed in previous studies in association with decreases in blood pressure and aldosterone or aldosterone-to-renin ratio (ARR) (9–11), however which component contributes most has yet to be clarified.

Inappropriate left ventricular mass is defined as the ratio between measured and predicted left ventricular mass taking sex, body size, and cardiac workload into account. It represents a non-hemodynamic or neurohormonal cause of LVH, and it has been reported to offer additional prognostic value in patients with LVH (12, 13). A previous study reported different distributions of inappropriate left ventricular mass among PA and EH groups (14). Inappropriately excessive left ventricular mass as a novel and promising parameter, the difference between measured and predicted left ventricular mass, may provide further information.

In this study, we compared measured, predicted, and inappropriately excessive left ventricular mass between a prospective cohort of patients with aldosterone-producing adenoma (APA) and EH controls, and evaluated their associations with cardiac remodeling and treatment response after unilateral adrenalectomy. We further investigated the pathogenesis of LVH due to hemodynamic and non-hemodynamic causes.

MATERIALS AND METHODS

Participant Enrollment, Physiological and Laboratory Measurements

We enrolled patients with PA and EH from October 2006 to January 2016 from National Taiwan University Hospital. The PA patients were all registered in the Taiwan Primary Aldosteronism Investigation (TAIPAI) database (15). Detailed medical histories were collected from every patient including demographic characteristics and medications. EH was diagnosed according to standard algorithms after a thorough survey of the medical history and laboratory tests to exclude possible secondary hypertension. Comprehensive evaluations including physiological and laboratory studies and echocardiography were performed on enrollment (baseline), and follow-up evaluations were performed 12 months later after adrenalectomy in the APA patients. Clinical outcome of APA patients was classified as clinical cure and clinical

non-cure. Clinical Cure refers to clinical complete success with normalization of BP with no antihypertensive medication use which is identical to the definition of "completely clinically cured" in the Primary Aldosteronism Surgical Outcomes (PASO) Classification System (16). Clinical non-cure refers clinical partial success and absent according to Primary Aldosteronism Surgical Outcome (PASO) Classification System (16).

Physiological assessments of systolic blood pressure (SBP) and diastolic blood pressure (DBP) were obtained using a sphygmomanometer according to clinical guidelines. Plasma aldosterone concentration (PAC) was measured using a radioimmunoassay with a commercial kit (Aldosterone Maia Kit; Adaltis Italia S.P.A., Bologna, Italy), and plasma renin activity (PRA) was measured as the generation of angiotensin-I *in vitro* using a commercially available radioimmunoassay kit (Cisbio, Bedford, Massachusetts, USA). All antihypertensive medications were discontinued for at least 21 days before measuring plasma PRA and PAC as suggested in clinical guidelines. Diltiazem and/or doxazosin were administered to lower elevated blood pressure when clinically indicated.

This study was conducted in compliance with the Declaration of Helsinki, and it was approved by the Institutional Review Board of National Taiwan University Hospital (Taipei, Taiwan). Informed consent was obtained from all individuals before enrollment.

Diagnostic Criteria for Primary Aldosteronism and Subtype Identification

The confirmation and diagnosis of PA and further identification of APA were made according to previously published protocols and algorithms (2, 17). Patients who met the following three criteria were defined as having PA: (1) autonomous excess aldosterone production with an ARR > 35; (2) a TAIPAI score > 60%; and (3) post-saline loading PAC > 10 ng/dl, or PAC/PRA > 35 (ng/dl)/(ng/ml per h) in a post-captopril test, or PAC > 6 ng/dl in a fludrocortisone suppression test (2). APA was diagnosed in patients with PA and at least one of the following three conditions: (1) adenoma on a computed tomography (CT) scan for preoperative evaluation; (2) lateralization of aldosterone secretion evidenced by adrenal vein sampling or dexamethasone suppression NP-59 single photon emission computed tomography (SPECT)/CT; and (3) pathologically proven adenoma after surgery if the patients received an operation (2). The choice of unilateral adrenalectomy or medical treatment with mineralocorticoid receptor antagonists was discussed with the APA patients, along with a pre-operational assessment. The PA patients were enrolled after consenting to the surgery and if they were physically suitable for adrenalectomy.

Echocardiographic Evaluation and Left Ventricle Geometry Calculation

A standardized echocardiographic ultrasound system (SONOS 5500 HP-Philips or IE33, Philips, Andover, Massachusetts, USA) was used, and transthoracic echocardiographic images were obtained in fundamental imaging modes by experienced and qualified technicians blind to the clinical diagnosis. M-mode measurements, two-dimensional imaging with standard views, and Doppler ultrasonography were acquired in each patient.

Left ventricular (LV) dimensions, septal and posterior wall thickness, and LVEF (M-mode) were measured *via* the parasternal long axis view according to the guidelines of the American Society of Echocardiography (18). Echocardiographic measured LV mass index (LVMI) was calculated according to the method of Devereux and Reichek: [LV mass = 1.04 x [(septal thickness + LV end-diastolic diameter + posterior wall thickness)^3 - (LV end-diastolic diameter)^3]-13.6] (19), then indexed with body mass index. Predicted LVMI (pLVMI) was estimated using a previously derived equation: predicted LVM = 55.37 + 6.64 x height$^{2.7}$ + 0.64 x stroke work - 18.07 x gender (where gender was scored as male=1 and female=2) (20). Left ventricle volume was calculated using Tericholz's formula, and stroke work was calculated as SBP (in mmHg) x stroke volume x 0.0144 (14). Inappropriately excessive LVMI (ieLVMI) was defined as: measured LVMI – predicted LVMI (21). LVH was defined according to Devereux's criteria: LVMI ≥ 134 g/m2 in men and 110 g/m2 in women (22). The cut-off point of increased relative wall thickness (RWT) was set as 0.42, and LV morphology was classified (18). Concentric hypertrophy was defined as the presence of LVH and increased RWT; eccentric hypertrophy was defined as the presence of LVH without increased RWT; concentric remodeling was defined as the absence of LVH with increased RWT; and normal geometry was defined as the absence of LVH without increased RWT (18, 22). In this study, "Δ" denotes the difference between pre-operative and post-operative measurements calculated as the post-operative value – pre-operative value.

Statistical Analysis

All continuous variables were expressed as mean ± SD if normally distributed and as median with interquartile range if non-normally distributed. The PA group and EH controls were matched using propensity score matching adjusted for age, sex, SBP, DBP, number of antihypertensive medication types, hypertension history using the Python-based extensions, FUZZY and PSM, in SPSS. All continuous variables were compared across groups using the Student's t test (normally distributed) or the Wilcoxon rank-sum test (non-normally distributed), while the paired sample t-test was used if two groups were dependent. Categorical variables were presented as counts and percentages and were compared using the McNemar test. Equality of two proportions was assessed using the Pearson chi-square test. Data of PAC, PRA, and ARR were log-transformed due to non-normality as assessed using the Kolmogorov–Smirnov test. Pearson's correlation tests were performed to determine correlations between LVMI/pLVMI/ieLVMI and ΔLVMI/ΔpLVMI/ΔieLVMI and clinical parameters. Significant determinants found in the Pearson's correlation test ($p \leqq 0.05$) were then examined using a multivariate linear regression test with backward subset selection to identify independent factors predicting LVMI/pLVMI/ieLVMI and ΔLVMI/ΔpLVMI/ΔieLVMI. All statistical analyses were performed using SPSS for Windows version 25.0 (SPSS Inc., Chicago, Illinois, USA). All tests were two-tailed, and a p value of ≤ 0.05 was considered to indicate statistical significance.

RESULTS

Patient Characteristics and Demographics

In this study, we enrolled 304 patients with PA, all of whom had APA, and 271 EH patients. The basic clinical characteristics of the patients are listed in **Table 1**. More of the APA group were female. In addition, the APA group had significantly higher SBP and DBP, longer duration of hypertension, used more types of antihypertensive medications, and had lower serum creatinine and potassium levels than the EH group. Moreover, the APA group had significantly higher serum PAC, lower PRA, and higher derived ARR than the EH group, and the differences remained after log-transformation. Other parameters were comparable between the two groups. The APA group used more antihypertensive medications compared to the EH controls, except for vasodilators and diuretics. After propensity-score matching adjusting for age, sex, SBP and DBP, duration of hypertension, and total number of antihypertensive types, we successfully matched 213 APA patients to 213 EH patient (**Table 1**). There were no significant differences among the clinical characteristics of the two groups except for lower PRA, log-transformed PRA, serum potassium and creatinine levels, and higher PAC, log-transformed PAC, ARR, and log-transformed ARR in the APA group. More of the EH group used angiotensin receptor blockers and more of the APA group used alpha-blockers, however there were no

TABLE 1 | Clinical characteristics of patients with primary aldosteronism and essential hypertension.

Unmatched data				Propensity-score matching			
Patient characteristics	**Primary aldosteronism (n = 304)**	**Essential hypertension (n = 271)**	**p value**	**Patient characteristics**	**Primary aldosteronism (n = 213)**	**Essential hypertension (n = 213)**	**p value**
Sex (Male), n (%)	132 (43.4%)	146 (53.9%)	0.012	Sex (Male), n (%)	102 (47.9%)	107 (50.2%)	0.628
Age (years)	50.9 ± 11.3	52.7 ± 14.9	0.109	Age (years)	51.3 ± 11.2	52.0 ± 13.8	0.592
Body height (cm)	162.5 ± 8.5	163.5 ± 9.6	0.163	Body height (cm)	163.1 ± 8.4	163.0 ± 9.9	0.935
Body weight (kg)	67.4 ± 14.0	69.2 ± 14.9	0.152	Body weight (kg)	67.8 ± 13.8	68.9 ± 15.2	0.417
BMI (kg/m^2)	25.4 ± 4.0	25.7 ± 4.3	0.340	BMI (kg/m^2)	25.3 ± 3.8	25.8 ± 4.3	0.258
BSA (m^2)	1.72 ± 0.20	1.75 ± 0.22	0.063	BSA (m^2)	1.73 ± 0.20	1.74 ± 0.22	0.429
HR (bpm)	73.0 ± 12.6	74.0 ± 13.6	0.399	HR (bpm)	72.8 ± 11.9	73.8 ± 13.2	0.448
SBP (mmHg)	154.1 ± 20.3	146.0 ± 21.7	< 0.001	SBP (mmHg)	149.7 ± 18.3	147.6 ± 21.7	0.279
DBP (mmHg)	91.8 ± 13.5	85.7 ± 13.7	< 0.001	DBP (mmHg)	88.5 ± 11.9	86.9 ± 13.5	0.201
Serum creatinine level (mg/dl)	0.92 ± 0.40	1.03 ± 0.57	0.008	Serum creatinine level (mg/dl)	0.91 ± 0.39	1.01 ± 0.58	0.044
Serum potassium level (mmol/dl)	3.52 ± 0.68	4.17 ± 0.41	< 0.001	Serum potassium level (mmol/dl)	3.56 ± 0.68	4.14 ± 0.41	< 0.001
PAC (ng/dl)[a]	44.92 (42.38)	32.46 (26.93)	< 0.001	PAC (ng/dl)[a]	45.18 (42.40)	31.74 (25.20)	< 0.001
PRA (ng/ml per h)[a]	0.21 (0.49)	2.00 (4.66)	< 0.001	PRA (ng/ml per h)[a]	0.21 (0.50)	2.17 (5.30)	< 0.001
ARR[a]	229.15 (745.14)	15.74 (39.65)	< 0.001	ARR[a]	222.00 (741.50)	15.34 (33.00)	< 0.001
Log-transformed PAC	1.68 ± 0.26	1.51 ± 0.25	< 0.001	Log-transformed PAC	1.67 ± 0.26	1.51 ± 0.25	< 0.001
Log-transformed PRA	-0.75 ± 0.71	0.25 ± 0.71	< 0.001	Log-transformed PRA	-0.75 ± 0.69	0.28 ± 0.70	< 0.001
Log-transformed ARR	2.43 ± 0.76	1.25 ± 0.72	< 0.001	Log-transformed ARR	2.42 ± 0.74	1.21 ± 0.72	< 0.001
Number of antihypertensive medication type	2.2 ± 1.3	1.8 ± 1.1	< 0.001	Number of antihypertensive medication type	2.0 ± 1.2	1.9 ± 1.1	0.643
Hypertension history (years)	8.0 ± 7.4	6.4 ± 7.8	0.016	Hypertension history (years)	7.1 ± 6.7	7.2 ± 8.1	0.897
Hypertension medication				Hypertension medication			
ACEI, n (%)	14 (4.6%)	4 (1.5%)	0.031	ACEI, n (%)	10 (4.7%)	4 (1.9%)	0.103
ARB, n (%)	119 (39.1%)	145 (53.5%)	0.001	ARB, n (%)	73 (34.3%)	130 (61.0%)	< 0.001
Alpha-blocker, n (%)	74 (24.3%)	27 (10.0%)	< 0.001	Alpha-blocker, n (%)	48 (22.5%)	25 (11.7%)	0.003
Beta-blocker, n (%)	120 (39.5%)	81 (29.9%)	0.016	Beta-blocker, n (%)	71 (33.3%)	63 (29.6%)	0.404
CCB, n (%)	217 (71.4%)	167 (61.6%)	0.013	CCB, n (%)	146 (68.5%)	142 (66.7%)	0.679
Vasodilator, n (%)	18 (5.9%)	15 (5.5%)	0.843	Vasodilator, n (%)	9 (4.2%)	13 (6.1%)	0.381
Diuretics, n (%)	31 (10.2%)	26 (9.6%)	0.809	Diuretics, n (%)	19 (8.9%)	24 (11.3%)	0.421

Values are expressed as mean ± SD, median (interquartile range), or number (percentage).

ACEI, angiotensin-converting enzyme inhibitor; ARB, AT1 blocker; ARR, aldosterone–renin ratio; CCB, calcium channel blocker; PAC, plasma aldosterone concentration; PRA, plasma renin activity.

[a]*Expressed as median and interquartile range.*

significant differences in other antihypertensive medications between the two groups.

Echocardiographic Comparison Between APA and EH

Echocardiographic parameters were then compared between the APA and EH groups before and after propensity score matching (**Table 2**). Both before and after propensity score matching, the APA group had a significantly higher LVMI, pLVMI, and ieLVMI compared to the EH controls. In addition, the APA group had significantly higher percentages of LVH and concentric hypertrophy before and after matching. Comparisons of LVMI, pLVMI, and ieLVMI between the APA and EH groups after propensity-score matching are shown in **Figure 1**.

Factors Affecting LVMI/pLVMI/ieLVMI in the APA and EH Groups

Regression analysis of factors predicting LVMI/pLVMI/ieLVMI in the study cohort are listed in **Table 3**. In multivariable analysis, sex (β = - 0.158, p = 0.001), SBP (β = 0.151, p = 0.001), serum creatinine level (β = 0.199, p < 0.001), serum potassium level (β = -0.133, p = 0.005), log-transformed PAC (β = 0.141, p = 0.003), and number of antihypertensive medication types (β = 0.134, p = 0.006) were independently associated with LVMI. In addition, sex (β = - 0.270, p < 0.001), SBP (β = 0.644, p < 0.001), DBP (β = -0.256, p < 0.001) and potassium level (β = -0.158, p < 0.001) were independently associated with pLVMI, and the presence of PA (β = 0.131, p = 0.008), body mass index (β = 0.143, p = 0.002), serum creatinine level (β = 0.240, p < 0.001), log-transformed PAC (β = 0.123, p = 0.012), and number of antihypertensive medication types (β = 0.145, p = 0.003) were independently associated with ieLVMI.

Clinical and Echocardiographic Evaluations in the APA Patients Before and After Adrenalectomy

Among the 304 APA patients, echocardiography data 12 months after adrenalectomy were available in 207, and their clinical and echocardiographic data are listed in **Table 4**. Significantly lower SBP and DBP, and significantly higher serum potassium and creatinine levels were noted after adrenalectomy. In addition, a significantly lower number of antihypertensive types was observed, along with significant decreases in PAC, log-transformed PAC, ARR, and log-transformed ARR, and significant increases in PRA and log-transformed PRA after adrenalectomy. Interventricular septal diameter, left ventricular posterior wall diameter and RWT decreased significantly after adrenalectomy, whereas left ventricular end-diastolic diameter, left ventricular end-systolic diameter and left ventricular ejection fraction did not. There were also significant decreases in LVMI, pLVMI, and ieLVMI after adrenalectomy. Changes in LVMI, pLVMI and ieLVMI in the APA patients before and after adrenalectomy are shown in **Figure 2**. The percentage of LVH decreased significantly after adrenalectomy.

TABLE 2 | Echocardiographic features and Doppler-derived indexes of patients with primary aldosteronism and essential hypertension.

Unmatched data				Propensity-score matching			
Echographic parameters	**Primary aldosteronism (n = 304)**	**Essential hypertension (n = 271)**	**p value**	**Echographic parameters**	**Primary aldosteronism (n = 213)**	**Essential hypertension (n = 213)**	**p value**
LVEDD (cm)	4.73 ± 0.48	4.64 ± 0.50	0.037	LVEDD (cm)	4.72 ± 0.49	4.66 ± 0.51	0.177
LVESD (cm)	2.84 ± 0.44	2.83 ± 0.52	0.801	LVESD (cm)	2.86 ± 0.45	2.81 ± 0.51	0.302
IVSD (cm)	1.19 ± 0.22	1.14 ± 0.23	0.012	IVSD (cm)	1.18 ± 0.22	1.14 ± 0.22	0.098
LVPWD (cm)	1.12 ± 0.18	1.08 ± 0.19	0.011	LVPWD (cm)	1.11 ± 0.18	1.07 ± 0.18	0.049
RWT	0.48 ± 0.08	0.47 ± 0.09	0.269	RWT	0.47 ± 0.08	0.46 ± 0.08	0.284
LVEF (%)	70.2 ± 7.6	69.2 ± 7.9	0.118	LVEF (%)	69.7 ± 8.0	69.7 ± 7.6	0.998
Transmitral E velocity (cm/s)	75.72 ± 19.44	76.82 ± 19.30	0.504	Transmitral E velocity (cm/s)	75.73 ± 19.42	76.88 ± 18.57	0.537
Transmitral A velocity (cm/s)	80.25 ± 18.06	80.72 ± 18.46	0.765	Transmitral A velocity (cm/s)	80.00 ± 17.96	80.43 ± 18.36	0.814
E deceleration time (ms)	212.7 ± 44.8	206.7 ± 52.9	0.159	E deceleration time (ms)	210.9 ± 42.8	206.0 ± 51.2	0.298
LVMI (g/m2)	142.04 ± 42.53	125.92 ± 39.00	<0.001	LVMI (g/m2)	139.17 ± 43.01	125.67 ± 36.32	0.001
Predicted LVMI (g/m2)	91.37 ± 17.37	84.22 ± 17.23	<0.001	Predicted LVMI (g/m2)	89.08 ± 15.77	85.47 ± 16.33	0.024
Inappropriately excessive LVMI (g/m2)	50.67 ± 35.89	42.09 ± 35.19	0.006	Inappropriately excessive LVMI (g/m2)	50.09 ± 35.97	39.99 ± 30.91	0.003
LVH, n (%)	199 (68.2%)	114 (44.7%)	<0.001	LVH, n (%)	130 (64.4%)	90 (44.1%)	<0.001
LV morphology, n (%)				LV morphology, n (%)			
Concentric hypertrophy, n (%)	165 (56.5%)	95 (37.3%)	<0.001	Concentric hypertrophy, n (%)	109 (54.0%)	72 (35.3%)	<0.001
Eccentric hypertrophy, n (%)	34 (11.6%)	19 (7.5%)	0.098	Eccentric hypertrophy, n (%)	21 (10.4%)	18 (8.8%)	0.591
Concentric remodeling, n (%)	60 (20.5%)	85 (33.3%)	0.001	Concentric remodeling, n (%)	45 (22.3%)	67 (32.8%)	0.017
Normal geometry, n (%)	33 (11.3%)	56 (22.0%)	0.001	Normal geometry, n (%)	27 (13.4%)	47 (23.0%)	0.012

Values are expressed as mean ± SD or number (percentage). IVSD, interventricular septal end diastole thickness; LAD, left atrial diameter; LADI, left atrial diameter index adjusted for BSA; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; LVESD, left ventricular end-systolic diameter; LVH, left ventricular hypertrophy; LVM, left ventricular mass; LVMI, left ventricular mass index; LVPWD, left ventricular posterior wall end diastole thickness; RWT, relative wall thickness.

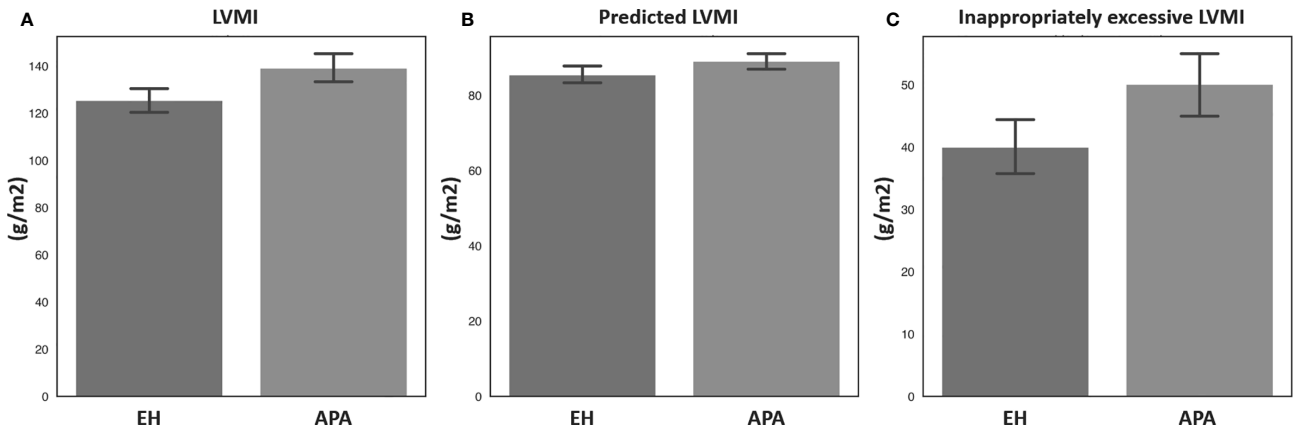

FIGURE 1 | Comparisons of **(A)** LVMI, **(B)** predicted LVMI, and **(C)** inappropriately excessive LVMI between patients with aldosterone-producing adenoma and essential hypertension after propensity score matching. APA, aldosterone-producing adenoma; EH, essential hypertension; LVMI, left ventricular mass index.

Comparison of the changes before and after operation of APA according to clinical cure or clinical non-cure was demonstrated in **Supplements Table 1** and **2**. Patients in clinical cure group had less duration of hypertension and greater antihypertensive medication reduction after adrenalectomy than patients in clinical non-cure group. Patients in both groups had significant reduction of SBP, DBP, PAC, and ARR while patients in clinical cure group had more reduction than patients in clinical non-cure group. Patients in both groups had significant reduction of LVMI pLVMI and ieLVMI expect pLVMI in clinical non-cure group which is borderline significant (p=0.058). The difference of ΔLVMI between the two groups is borderline significant (p = 0.086). Patients in clinical cure group had significantly higher ΔpLVMI than non-cure group (p<0.001). The difference of ΔieLVMI between the two groups is not significant (p = 0.450).

Factors Affecting Changes in LVMI/pLVMI/ieLVMI in the APA Patients

Regression analysis of factors predicting changes in LVMI/pLVMI/ieLVMI after adrenalectomy among the APA group are listed in **Table 5**. In multivariable analysis, SBP (β = -0.244, p = 0.001), ΔSBP (β = 0.268, p < 0.001), Δlog-transformed ARR (β = 0.199, p < 0.001) and pre-operative LVMI (β = 0.687, p < 0.001) were significantly associated with ΔLVMI; SBP (β = -0.411, p < 0.001), ΔSBP (β = 0.655, p < 0.001) and pre-operative pLVMI (β = 0.561, p < 0.001) were significantly associated with ΔpLVMI; and Δlog-transformed ARR (β = 0.155, p = 0.004) and pre-operative ieLVMI (β = 0.664, p < 0.001) were significantly associated with ΔieLVMI.

DISCUSSION

There are five findings in this study. First, after propensity score matching for age, sex, SBP, DBP, hypertension duration, and use of antihypertensive, the APA patients had greater LVMI, pLVMI, and ieLVMI, with more LVH compared to the EH patients. Second, LVMI was independently associated with SBP, number of antihypertensive medication types, and log-transformed PAC, while pLVMI (hemodynamic component of LV remodeling) was associated with SBP, and ieLVMI was associated with log-transformed PAC (non-hemodynamic component of LV remodeling). Third, significant regressions of LVMI, pLVMI and ieLVMI and reversal of LVH were observed after adrenalectomy. Fourth, ΔLVMI was associated with SBP, ΔSBP, and Δlog-transformed ARR, while ΔpLVMI was associated with SBP, and ΔSBP and ΔieLVMI were associated with Δlog-transformed ARR. These findings clearly showed the factors associated with hemodynamic and non-hemodynamic component of LV remodeling in PA patients, and the factors associated with the regression of both components after adrenalectomy. Last, the study demonstrates that inappropriately excessive left ventricular mass (ieLVM) could be a novel and promising parameter evaluating aldosterone-induced non-hemodynamic left ventricular remodeling.

A previous meta-analysis reported that LVH identified by ECG or echocardiography was highly prognostic and an independent risk factor for cardiovascular outcomes (23). In addition, regression of LVH after antihypertensive medication has been associated with a significant reduction in cardiovascular risk compared with persistent or new-onset LVH (24). Besides being an initial step in clinical diseases, LV hypertrophy has also been proposed to be a compensatory process for abnormal loading conditions in studies taking pLVMI with gender, cardiac loading condition, and body size into consideration (8, 20, 23, 25). Blood pressure has also been shown to be a central factor for the onset and progression of LVH (7), however, many nonhemodynamic factors have also been implicated in the pathogenesis of hypertensive LVH (26). For example, LVMI was related to plasma fibrinogen and aldosterone in EH patients after adjusting age, blood pressure, and body mass index (27). In another study, post-saline load plasma aldosterone is positively related to left ventricular mass independent of blood pressure (28).

TABLE 3 | Correlation study between LVMI, pLVMI, and ieLVMI and clinical parameters (after propensity-score matching adjusted for age, sex, SBP, DBP, antihypertensive types, hypertension years) (n = 426).

Variable	LVMI				pLVMI				ieLVMI			
	Univariate regression		Multivariate regression		Univariate regression		Multivariate regression		Univariate regression		Multivariate regression	
	β (95% C.I)	p value	β (95% C.I)	p value	β (95% C.I)	p value	β (95% C.I)	p value	β (95% C.I)	p value	β (95% C.I)	p value
Presence of PA	0.168 (0.071, 0.264)	0.001			0.112 (0.015, 0.209)	0.024			0.149 (0.053, 0.246)	0.003	0.131 (0.035, 0.227)	0.008
Sex (male)	-0.262 (-0.356, -0.168)	< 0.001	-0.158 (-0.252, -0.063)	0.001	-0.300 (-0.393, -0.206)	< 0.001	-0.270 (-0.350, -0.190)	< 0.001	-0.168 (-0.264, -0.071)	0.001		
Age	0.023 (-0.074, 0.121)	0.640			0.025 (-0.073, 0.123)	0.620			0.019 (-0.079, 0.117)	0.698		
BMI	0.095 (-0.002, 0.191)	0.057			-0.069 (-0.167, 0.028)	0.164			0.144 (0.047, 0.241)	0.004	0.143 (0.051, 0.236)	0.002
SBP	0.285 (0.191, 0.379)	< 0.001	0.151 (0.059, 0.241)	0.001	0.545 (0.463, 0.627)	< 0.001	0.644 (0.539, 0.750)	< 0.001	0.083 (-0.014, 0.181)	0.094		
DBP	0.080 (-0.017, 0.177)	0.109			0.253 (0.158, 0.348)	< 0.001	-0.256 (-0.363, -0.149)	< 0.001	-0.024 (-0.122, 0.074)	0.627		
Serum creatinine level	0.284 (0.189, 0.377)	< 0.001	0.199 (0.100, 0.296)	< 0.001	0.162 (0.065, 0.259)	0.001			0.261 (0.167, 0.356)	< 0.001	0.240 (0.144, 0.337)	< 0.001
Serum potassium level	-0.183 (-0.279, -0.086)	< 0.001	-0.133 (-0.225, -0.041)	0.005	-0.204 (-0.300, -0.108)	<0.001	-0.158 (-0.237, -0.079)	< 0.001	-0.123 (-0.220, -0.025)	0.014		
Log-transformed PAC	0.163 (0.066, 0.260)	0.001	0.141 (0.049, 0.232)	0.003	0.101 (0.003, 0.199)	0.043			0.141 (0.044, 0.238)	0.005	0.123 (0.027, 0.218)	0.012
Log-transformed PRA	-0.130 (-0.227, -0.032)	0.009			-0.101 (-0.199, -0.003)	0.043			-0.107 (-0.205, -0.009)	0.032		
Log-transformed ARR	0.142 (0.044, 0.238)	0.004			0.104 (0.007, 0.202)	0.036			0.120 (0.022, 0.217)	0.016		
Number of antihypertensive medication type	0.284 (0.190, 0.378)	< 0.001	0.134 (0.039, 0.228)	0.006	0.173 (0.076, 0.269)	< 0.001			0.259 (0.164, 0.354)	< 0.001	0.145 (0.048, 0.243)	0.003
Hypertension history	0.157 (0.060, 0.253)	0.001			0.125 (0.028, 0.222)	0.012			0.123 (0.026, 0.220)	0.013		

ARR, aldosterone–renin ratio; LVMI, left ventricular mass index; PAC, plasma aldosterone concentration; PRA, plasma renin activity.

An inappropriate increase in LVM, which is referred to as an abnormal (non-compensatory) increase in LVM as opposed to a normal (compensatory) LVM increase, has been reported to be more strongly associated with more metabolic risk factors (29). Previous studies have reported a higher cardiovascular risk in individuals with inappropriate LVM even without traditionally defined LVH (12, 13, 30), and the additional prognostic value of changes in inappropriate LVM has been observed in those with traditionally defined LVH (12, 13). Inappropriate LVM is thus an ideal method to evaluate nonhemodynamic or neurohumoral factors in the pathogenesis of LVH in patients with PA after eliminating the influence of sex, body size, and cardiac workload which is increased in PA because of elevated blood pressure. Muiesan et al. in 2008 were the first to report a prospective cross-sectional study comparing PA patients with EH controls to assess the non-hemodynamic effect of excessive aldosterone on LVM (14). They found a higher prevalence of inappropriate LVM among PA patients with traditionally defined LVH compared with EH controls, and a trend of a higher prevalence of inappropriate LVM among PA patients without traditionally defined LVH. These findings provided evidence of an aldosterone-induced increase in LVM exceeding the amount needed to compensate for hemodynamic load.

Several previous studies have reported differences in LVMI and LVH between PA and EH groups and improvements after surgery or medications. Rossi et al. reported increased LV wall thickness and LVMI with higher percentages of LVH and concentric remodeling in PA patients compared to matched EH controls, and that adrenalectomy markedly reduced LV wall thickness and LVMI in APA patients (9). Catena et al.

TABLE 4 | Clinical characteristics, echocardiographic features, doppler-derived indexes, and change of patients with primary aldosteronism receiving adrenalectomy.

Patient characteristics	Pre-OP	Post-OP	p value
SBP (mmHg)	154.8 ± 20.1	137.7 ± 18.7	<0.001
DBP (mmHg)	92.3 ± 13.9	84.8 ± 11.6	<0.001
Serum creatinine level (mg/dl)	0.91 ± 0.38	1.06 ± 0.66	<0.001
Serum potassium level (mmol/dl)	3.60 ± 0.68	4.32 ± 0.54	<0.001
PAC (ng/dl)[a]	45.60 (43.60)	28.55 (22.00)	<0.001
PRA (ng/ml per h)[a]	0.23 (0.50)	1.76 (4.20)	<0.001
ARR[a]	234.29 (1107.8)	16.88 (35.70)	<0.001
Log-transformed PAC	1.71 ± 0.25	1.47 ± 0.27	<0.001
Log-transformed PRA	-0.76 ± 0.74	0.18 ± 0.63	<0.001
Log-transformed ARR	2.46 ± 0.78	1.29 ± 0.64	<0.001
Number of antihypertensive medication type	2.3 ± 1.3	0.7 ± 1.1	<0.001
Echocardiographic variables	Pre-OP	Post-OP	p value
LVEDD (cm)	4.74 ± 0.46	4.71 ± 0.46	0.186
LVESD (cm)	2.84 ± 0.42	2.83 ± 0.42	0.414
IVSD (cm)	1.19 ± 0.21	1.12 ± 0.19	<0.001
LVPWD (cm)	1.13 ± 0.18	1.05 ± 0.15	<0.001
RWT	0.48 ± 0.08	0.45 ± 0.08	<0.001
LVEF (%)	70.2 ± 7.1	70.1 ± 7.0	0.867
Transmitral E velocity (cm/s)	75.2 ± 18.8	73.8 ± 17.5	0.256
Transmitral A velocity (cm/s)	80.5 ± 17.6	79.5 ± 18.3	0.364
E deceleration time (ms)	212.7 ± 44.9	223.0 ± 71.2	0.059
LVMI (g/m2)	142.07 ± 40.60	127.39 ± 34.61	<0.001
Predicted LVMI (g/m2)	90.89 ± 16.23	83.89 ± 16.19	<0.001
Inappropriately excessive LVMI (g/m2)	50.89 ± 33.86	42.90 ± 26.92	<0.001
LVH, n (%) (n = 207)	140 (67.6%)	116 (56.0%)	0.002

Values are expressed as mean ± SD, median (interquartile range), or number (percentage).
ARR, aldosterone–renin ratio; PAC, plasma aldosterone concentration; PRA, plasma renin activity.
[a]*Expressed as median and interquartile range.*
(n = 207).

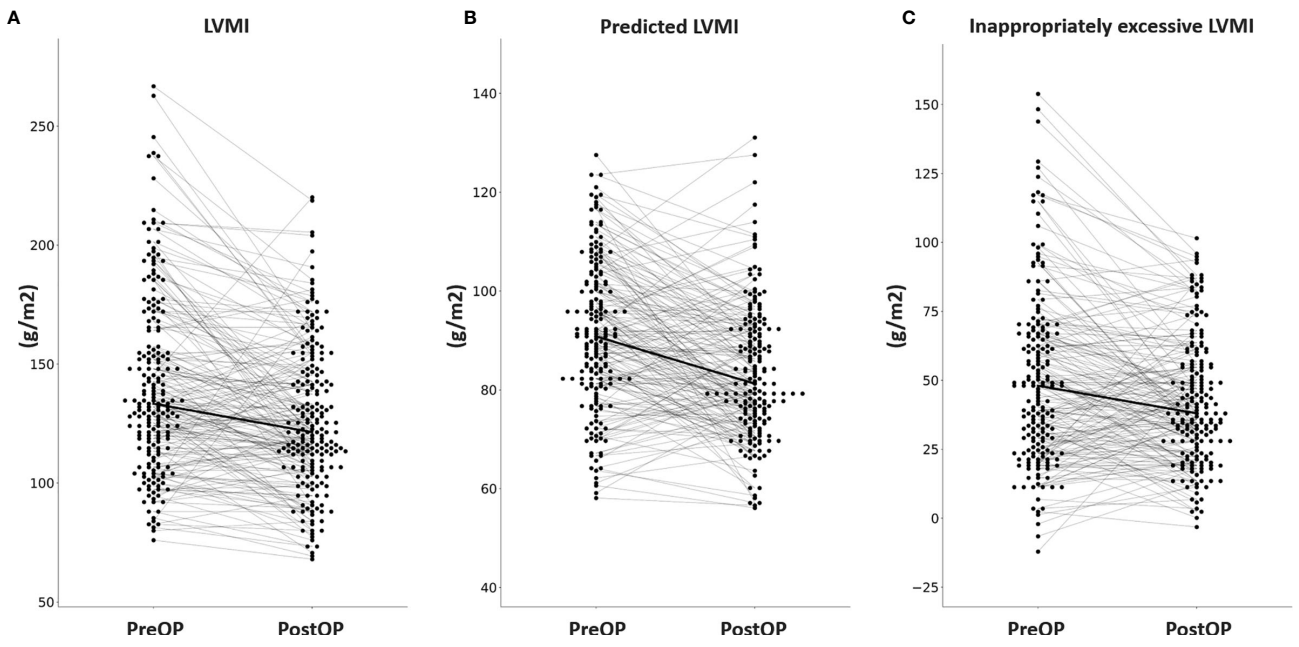

FIGURE 2 | Changes in **(A)** LVMI, **(B)** predicted LVMI, **(C)** and inappropriately excessive LVMI in aldosterone-producing adenoma patients before and after adrenalectomy. LVMI, left ventricular mass index; PreOP, before adrenalectomy; PostOP, after adrenalectomy.

TABLE 5 | Correlation study between ΔLVMI, ΔpLVMI, ΔieLVMI and clinical parameters (unmatched).

Variable	ΔLVMI				ΔpLVMI				ΔieLVMI			
	Univariate regression		Multivariate regression		Univariate regression		Multivariate regression		Univariate regression		Multivariate regression	
	β (95% C.I)	p value	β (95% C.I)	p value	β (95% C.I)	p value	β (95% C.I)	p value	β (95% C.I)	p value	β (95% C.I)	p value
Sex (male)	-0.036 (-0.173, 0.102)	0.611			-0.102 (-0.243, 0.039)	0.155			-0.033 (-0.175, 0.109)	0.648		
Age	-0.150 (-0.286, -0.014)	0.031			-0.101 (-0.242, 0.041)	0.162			-0.066 (-0.208, 0.075)	0.356		
BMI	0.024 (-0.113, 0.161)	0.733			0.015 (-0.127, 0.157)	0.835			0.045 (-0.097, 0.187)	0.531		
SBP	0.177 (0.041, 0.312)	0.011	-0.244 (-0.390, -0.099)	0.001	0.350 (0.217, 0.483)	< 0.001	-0.411 (-0.562, -0.260)	< 0.001	-0.008 (-0.150, 0.134)	0.913		
ΔSBP	0.251 (0.113, 0.388)	< 0.001	0.268 (0.129, 0.406)	< 0.001	0.613 (0.501, 0.725)	< 0.001	0.655 (0.530, 0.780)	< 0.001	-0.077 (-0.219, 0.064)	0.282		
DBP	0.117 (-0.019, 0.254)	0.092			0.209 (0.070, 0.348)	0.003			-0.004 (-0.146, 0.138)	0.955		
ΔDBP	0.153 (0.012, 0.293)	0.033			0.370 (0.238, 0.502)	< 0.001			-0.045 (-0.187, 0.097)	0.535		
Serum creatinine level	-0.068 (-0.205, 0.069)	0.332			0.029 (-0.113, 0.171)	0.690			-0.031 (-0.173, 0.111)	0.667		
ΔSerum creatinine level	-0.136 (-0.278, 0.006)	0.062			-0.193 (-0.336, 0.050)	0.009			-0.036 (-0.182, 0.110)	0.625		
Serum potassium level	-0.176 (-0.311, -0.040)	0.011			-0.219 (-0.358, -0.081)	0.002			-0.119 (-0.260, 0.022)	0.097		
ΔSerum potassium level	-0.187 (-0.326, -0.047)	0.009			-0.206 (-0.346, -0.065)	0.004			-0.106 (-0.249, 0.037)	0.144		
Log-transformed PAC	0.165 (0.029, 0.300)	0.018			0.126 (-0.015, 0.267)	0.079			0.083 (-0.059, 0.224)	0.251		
ΔLog-transformed PAC	0.205 (0.065, 0.344)	0.004			0.187 (0.045, 0.329)	0.010			0.127 (-0.016, 0.271)	0.082		
Log-transformed PRA	-0.073 (-0.210, 0.063)	0.293			-0.067 (-0.208, 0.075)	0.355			-0.060 (-0.201, 0.082)	0.407		
ΔLog-transformed PRA	-0.189 (-0.329, -0.049)	0.008			-0.168 (-0.311, -0.025)	0.021			-0.117 (-0.261, 0.027)	0.111		
Log-transformed ARR	0.121 (-0.015, 0.257)	0.082			0.103 (-0.038, 0.244)	0.151			0.083 (-0.059, 0.224)	0.250		
ΔLog-transformed ARR	0.288 (0.150, 0.425)	< 0.001	0.199 (0.092, 0.306)	< 0.001	0.242 (0.100, 0.383)	0.001			0.182 (0.038, 0.325)	0.013	0.155 (0.049, 0.261)	0.004
Number of antihypertensive medication type	0.019 (-0.118, 0.156)	0.786			0.036 (-0.105, 0.178)	0.613			0.002 (-0.140, 0.143)	0.983		
Change of number of antihypertensive medication type	0.038 (-0.102, 0.178)	0.598			0.076 (-0.068, 0.221)	0.298			0.012 (-0.133, 0.157)	0.869		

(Continued)

TABLE 5 | Continued

Variable	ΔLVMI				ΔpLVMI				ΔieLVMI			
	Univariate regression		Multivariate regression		Univariate regression		Multivariate regression		Univariate regression		Multivariate regression	
	β (95% C.I)	p value	β (95% C.I)	p value	β (95% C.I)	p value	β (95% C.I)	p value	β (95% C.I)	p value	β (95% C.I)	p value
Hypertension history	-0.025 (-0.162, 0.112)	0.722			0.047 (-0.095, 0.189)	0.513			-0.048 (-0.189, 0.094)	0.509		
Clinical success to off medication	0.078 (-0.061, 0.218)	0.272			0.106 (-0.038, 0.250)	0.148			0.055 (-0.090, 0.200)	0.454		
pre-OP LVMI	0.577 (0.465, 0.690)	< 0.001	0.687 (0.574, 0.800)	< 0.001	0.254 (0.116, 0.391)	< 0.001			0.538 (0.418, 0.657)	< 0.001		
pre-OP pLVMI	0.283 (0.151, 0.416)	< 0.001			0.515 (0.393, 0.637)	< 0.001	0.561 (0.433, 0.689)	< 0.001	0.048 (-0.094, 0.190)	0.505		
pre-OP ieLVMI	0.555 (0.441, 0.670)	< 0.001			0.061 (-0.081, 0.202)	0.401			0.628 (0.518, 0.739)	< 0.001	0.664 (0.558, 0.771)	< 0.001

Correlation analysis, univariate, and multivariate linear regression analysis for changes in LVMI among total PA patients (n = 207).
ARR, aldosterone–renin ratio; LVMI, left ventricular mass index; PAC, plasma aldosterone concentration; PRA, plasma renin activity.

reported a greater LVM and more LVH among PA patients compared to EH controls with improvements after treatment with adrenalectomy or spironolactone (31). In our previous study of 30 APA patients, we also found a decreased LVMI in patients with LVH after adrenalectomy, and a decreased LVMI was correlated with preoperative LVMI, and postoperative changes in SBP and potassium level (10, 11, 32). We also found significant correlations among 24-hour urinary aldosterone, sodium, glomerular hyperfiltration and LVMI and ieLVMI among PA patients, as well as diastolic dysfunction (21, 33–35).

To the best of our knowledge, this prospective cohort study includes the largest sample size to date, with 304 PA patients (all of whom had APA) and 271 EH controls, and 213 matched pairs. The cases were matched using propensity score matching for sex, age, SBP, DBP, number of antihypertensive medication types, and hypertension history, with the aim of eliminating as many confounding factors as possible. The use of ieLVMI is different from previous studies and demonstrated the effect of excessive aldosterone on LVH in addition to adaptation to compensate for hemodynamic load. Our findings may help to improve the prediction of cardiovascular outcomes after eliminating the influence of hypertension. The utilization of LVMI, pLVMI and ieLVMI provided further insight into the pathogenesis of increased LVMI, a process involving mixed etiologies, in which pLVMI represented the hemodynamic component and ieLVMI the non-hemodynamic component. Correlation studies of LVMI/pLVMI/ieLVMI between the patients with APA and EH identified the factors associated with increased LVMI, while correlation studies of ΔLVMI/ΔpLVMI/ΔieLVMI after adrenalectomy identified the factors associated with changes in LV morphology and treatment effect of adrenalectomy. Of note, ΔLVMI was independently correlated with ΔSBP and ΔlogARR. Furthermore, ΔpLVMI was independently correlated with ΔSBP, while ΔieLVMI was correlated with ΔlogARR. These findings emphasize the treatment benefits of adrenalectomy, which both reduces blood pressure and aldosterone by removing the aldosterone secreting source in the adrenal gland, thereby resulting in LVH regression in both hemodynamic and non-hemodynamic pathways. The use of ieLVMI has the advantage of excluding the influence of blood pressure on the left structure. In addition, the exclusive association between ΔieLVMI and ΔlogARR consolidates the pathogenesis of aldosterone-induced neurohormonal LVH, which is independent of the influence of hypertension. Moreover, this shows that the treatment effect of adrenalectomy was not just from treating hypertension.

POSSIBLE FUTURE STUDY AND STUDY LIMITATIONS

There are several limitations to this study. First, this clinical study showed the association between preoperative and postoperative endocrinological and cardiac structural changes, however the study design cannot elucidate the actual causative role of aldosterone in LV remodeling. Second, our follow-up period was 1 year, and longer follow-up periods are warranted to examine whether there is any further late-onset effect of adrenalectomy. Third, we did not perform the saline load test with plasma aldosterone measurement and fibrinogen level on all the APA patients before and after the operation. Fourth, this study only investigated excess aldosterone and showed that elevated ARR increased LVMI, but the effect on cardiovascular mortality or morbidity was not investigated. Further long-term follow-up studies are needed to investigate the clinical impact of aldosterone excess on cardiovascular outcomes.

CONCLUSION

Extensive cardiac remodeling through hemodynamic and non-hemodynamic causes occurs in APA patients. Adrenalectomy improved both hemodynamic and non-hemodynamic components of LV remodeling. The regression of pLVMI and ieLVMI were correlated with decreases in blood pressure and ARR, respectively. These findings provide further evidence of aldosterone-induced hemodynamic and non-hemodynamic LV remodeling in patients with PA, and the effect of adrenalectomy.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by National Taiwan University Hospital (NTUH) Research Ethics Committee (REC). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

C-TP: experimental design and manuscript writing. X-MW, C-HT, Y-YC and ZW-C: patient enrollment and experimental design. C-CC and B-CL: diagnosis helping, AVS, and report. C-WL and Y-LC: patient case management and experimental design. L-CL and Y-RC: diagnosis helping, echocardiography, experimental design. C-SH and Y-HL: patient enrollment, experimental design, funding raising, manuscript editing. All authors contributed to the article and approved the submitted version.

FUNDING

This study was partially supported by grants from the Ministry of Science and Technology (MOST 106-2314-B-002 -048 –MY3) and National Taiwan University Hospital (NTUH 109-A141). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

ACKNOWLEDGMENTS

Taiwan Primary Aldosteronism Investigation (TAIPAI) Study Group: Vin-Cent Wu, MD, PhD; Y-HL; Che-Hsiung Wu; Yi-Luwn Ho, MD, PhD; Hung-Wei Chang, MD, PhD; Lian-Yu Lin MD, PhD; Fu-Chang Hu, MS, ScD; Kao-Lang Liu, MD; Shuo-Meng Wang, MD; Kuo-How Huang, MD; Yung-Ming Chen, MD; Chin-Chi Kuo; MD, C-CC; Shih-Chieh Chueh, MD, PhD; Ching-Chu Lu, MD; Shih-Cheng Liao, MD; Ruoh-Fang Yen, MD, PhD; and Kwan-Dun Wu, MD, PhD. The authors would like to thank the staff of the Second Core Lab in the Department of Medical Research in National Taiwan University Hospital for technical assistance.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.646097/full#supplementary-material

REFERENCES

1. Funder JW, Carey RM, Mantero F, Murad MH, Reincke M, Shibata H, et al. The Management of Primary Aldosteronism: Case Detection, Diagnosis, and Treatment: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab* (2016) 101:1889–916. doi: 10.1210/jc.2015-4061
2. Wu VC, Hu YH, Er LK, Yen RF, Chang CH, Chang YL, et al. Case detection and diagnosis of primary aldosteronism - The consensus of Taiwan Society of Aldosteronism. *J Formos Med Assoc* (2017) 116:993–1005. doi: 10.1016/j.jfma.2017.06.004
3. 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) 45:1243–8. doi: 10.1016/j.jacc.2005.01.015
4. Monticone S, D'Ascenzo F, Moretti C, Williams TA, Veglio F, Gaita F, et al. Cardiovascular events and target organ damage in primary aldosteronism compared with essential hypertension: a systematic review and meta-analysis. *Lancet Diabetes Endocrinol* (2018) 6:41–50. doi: 10.1016/S2213-8587(17)30319-4
5. Cohn JN, Ferrari R, Sharpe N. Cardiac remodeling–concepts and clinical implications: a consensus paper from an international forum on cardiac remodeling. Behalf of an International Forum on Cardiac Remodeling. *J Am Coll Cardiol* (2000) 35:569–82. doi: 10.1016/S0735-1097(99)00630-0
6. de Simone G, Kitzman DW, Palmieri V, Liu JE, Oberman A, Hopkins PN, et al. Association of inappropriate left ventricular mass with systolic and diastolic dysfunction: the HyperGEN study. *Am J Hypertens* (2004) 17:828–33. doi: 10.1016/S0895-7061(04)00818-0
7. Ruilope LM, Schmieder RE. Left ventricular hypertrophy and clinical outcomes in hypertensive patients. *Am J Hypertens* (2008) 21:500–8. doi: 10.1038/ajh.2008.16
8. Agabiti-Rosei E, Muiesan ML. Hypertensive left ventricular hypertrophy: pathophysiological and clinical issues. *Blood Press* (2001) 10:288–98. doi: 10.1080/080370501753400593
9. Rossi GP, Sacchetto A, Visentin P, Canali C, Graniero GR, Palatini P, et al. Changes in left ventricular anatomy and function in hypertension and primary aldosteronism. *Hypertension* (1996) 27:1039–45. doi: 10.1161/01.HYP.27.5.1039
10. Lin YH, Huang KH, Lee JK, Wang SM, Yen RF, Wu VC, et al. Factors influencing left ventricular mass regression in patients with primary aldosteronism post adrenalectomy. *J Renin Angiotensin Aldosterone Syst* (2011) 12:48–53. doi: 10.1177/1470320310376424
11. Liao CW, Chen A, Lin YT, Chang YY, Wang SM, Wu VC, et al. The Relation Between the Degree of Left Ventricular Mass Regression and Serum Potassium Level Change in Patients With Primary Aldosteronism After Adrenalectomy. *J Investig Med* (2015) 63:816–20. doi: 10.1097/JIM.0000000000000215

12. de Simone G, Palmieri V, Koren MJ, Mensah GA, . Roman MJ, Devereux RB. Prognostic implications of the compensatory nature of left ventricular mass in arterial hypertension. *J Hypertens 19* (2001) 19(1):119–25. doi: 10.1097/00004872-200101000-00016
13. Muiesan ML, Salvetti M, Paini A, Monteduro C, Galbassini G, Bonzi B, et al. Inappropriate left ventricular mass changes during treatment adversely affects cardiovascular prognosis in hypertensive patients. *Hypertension* (2007) 49:1077–83. doi: 10.1161/HYPERTENSIONAHA.107.087320
14. Muiesan ML, Salvetti M, Paini A, Agabiti-Rosei C, Monteduro C, Galbassini G, et al. Inappropriate left ventricular mass in patients with primary aldosteronism. *Hypertension* (2008) 52:529–34. doi: 10.1161/HYPERTENSIONAHA.108.114140
15. Lin YH, Lee HH, Liu KL, Lee JK, Shih SR, Chueh SC, et al. Reversal of myocardial fibrosis in patients with unilateral hyperaldosteronism receiving adrenalectomy. *Surgery* (2011) 150:526–33. doi: 10.1016/j.surg.2011.02.006
16. Miller BS, Turcu AF, Nanba AT, Hughes DT, Cohen MS, . Gauger PG, et al. Refining the Definitions of Biochemical and Clinical Cure for Primary Aldosteronism Using the Primary Aldosteronism Surgical Outcome (PASO) Classification System. *World J Surg* (2018) 42:453–63. doi: 10.1007/s00268-017-4311-1
17. Chao CT, Wu VC, Kuo CC, Lin YH, Chang CC, Chueh SJ, et al. Diagnosis and management of primary aldosteronism: an updated review. *Ann Med* (2013) 45:375–83. doi: 10.3109/07853890.2013.785234
18. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr* (2015) 28:1–39.e14. doi: 10.1016/j.echo.2014.10.003
19. Devereux RB, Reichek N. Echocardiographic determination of left ventricular mass in man. *Anatomic Validation Method Circ* (1977) 55:613–8. doi: 10.1161/01.CIR.55.4.613
20. de Simone G, Devereux RB, Kimball TR, Mureddu GF, Roman MJ, Contaldo F, et al. Interaction between body size and cardiac workload: influence on left ventricular mass during body growth and adulthood. *Hypertension* (1998) 31:1077–82. doi: 10.1161/01.HYP.31.5.1077
21. Hung CS, Ho YL, Chang YY, Wu VC, Wu XM, Lee JK, et al. Twenty-four-hour urinary aldosterone predicts inappropriate left ventricular mass index in patients with primary aldosteronism. *Sci World J* (2013) 2013:294594. doi: 10.1155/2013/294594
22. Devereux RB. Detection of left ventricular hypertrophy by M-mode echocardiography. *Anatomic Validation Standardization Comparison Other Methods Hypertension* (1987) 9:II19–26. doi: 10.1161/01.HYP.9.2_Pt_2.II19
23. Vakili BA, . Okin PM, Devereux RB. Prognostic implications of left ventricular hypertrophy. *Am Heart J* (2001) 141:334–41. doi: 10.1067/mhj.2001.113218
24. Verdecchia P, Angeli F, Borgioni C, Gattobigio R, de Simone G, . Devereux RB, et al. Changes in cardiovascular risk by reduction of left ventricular mass in hypertension: a meta-analysis. *Am J Hypertens* (2003) 16:895–9. doi: 10.1016/S0895-7061(03)01018-5
25. Devereux RB, Wachtell K, Gerdts E, Boman K, Nieminen MS, Papademetriou V, et al. Prognostic significance of left ventricular mass change during treatment of hypertension. *JAMA* (2004) 292:2350–6. doi: 10.1001/jama.292.19.2350
26. Schmieder RE. The role of non-haemodynamic factors of the genesis of LVH. *Nephrol Dial Transplant* (2005) 20:2610–2. doi: 10.1093/ndt/gfi190
27. Catena C, Colussi G, Valeri M, Sechi LA. Association of aldosterone with left ventricular mass in hypertension: interaction with plasma fibrinogen levels. *Am J Hypertens* (2013) 26:111–7. doi: 10.1093/ajh/hps006
28. Catena C, Verheyen ND, Url-Michitsch M, Kraigher-Krainer E, Colussi G, Pilz S, et al. Association of Post-Saline Load Plasma Aldosterone Levels With Left Ventricular Hypertrophy in Primary Hypertension. *Am J Hypertens* (2016) 29:303–10. doi: 10.1093/ajh/hpv104
29. Palmieri V, de Simone G, Roman MJ, Schwartz JE, Pickering TG, Devereux RB. Ambulatory blood pressure and metabolic abnormalities in hypertensive subjects with inappropriately high left ventricular mass. *Hypertension* (1999) 34:1032–40. doi: 10.1161/01.HYP.34.5.1032
30. de Simone G, Verdecchia P, Pede S, Gorini M, Maggioni AP. Prognosis of inappropriate left ventricular mass in hypertension: the MAVI Study. *Hypertension* (2002) 40:470–6. doi: 10.1161/01.HYP.0000034740.99323.8A
31. Catena C, Colussi G, Lapenna R, Nadalini E, Chiuch A, Gianfagna P, et al. Long-term cardiac effects of adrenalectomy or mineralocorticoid antagonists in patients with primary aldosteronism. *Hypertension* (2007) 50:911–8. doi: 10.1161/HYPERTENSIONAHA.107.095448
32. Lin YH, Wang SM, Wu VC, Lee JK, Kuo CC, Yen RF, et al. The association of serum potassium level with left ventricular mass in patients with primary aldosteronism. *Eur J Clin Invest* (2011) 41:743–50. doi: 10.1111/j.1365-2362.2010.02462.x
33. Chang YY, Lee HH, Hung CS, Wu XM, Lee JK, Wang SM, et al. Association between urine aldosterone and diastolic function in patients with primary aldosteronism and essential hypertension. *Clin Biochem* (2014) 47:1329–32. doi: 10.1016/j.clinbiochem.2014.05.062
34. Hung CS, Wu XM, Chen CW, Chen YH, Wu VC, Liao CW, et al. The relationship among cardiac structure, dietary salt and aldosterone in patients with primary aldosteronism. *Oncotarget* (2017) 8:73187–97. doi: 10.18632/oncotarget.17505
35. Liao MT, Wu XM, Chang CC, Liao CW, Chen YH, Lu CC, et al. The Association between Glomerular Hyperfiltration and Left Ventricular Structure and Function in Patients with Primary Aldosteronism. *Int J Med Sci* (2015) 12:369–77. doi: 10.7150/ijms.10975

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

ORIGINAL RESEARCH
published: 27 April 2021
doi: 10.3389/fendo.2021.665912

A Bibliometric Analysis of Primary Aldosteronism Research From 2000 to 2020

Chengyuan Wang[1†], Hongwei Jing[1†], Zuyu Sun[1†], Jiaxi Yao[1], Xinyu Zhang[2], Tao Liu[1] and Ying Wu[3*]*

[1] Department of Urology, The First Hospital of China Medical University, Shenyang, China, [2] Department of Pulmonary Critical Care Medicine, The First Hospital of China Medical University, Shenyang, China, [3] Phase I Clinical Trails Center, The First Hospital of China Medical University, Shenyang, China

OPEN ACCESS

Edited by:
Qiang Wei,
West China Hospital,
Sichuan University, China

Reviewed by:
Qifu Li,
First Affiliated Hospital of Chongqing Medical University, China
Tao Chen,
West China Hospital of Sichuan University, China

****Correspondence:***
Ying Wu
wuying@cmu.edu.cn
Tao Liu
cmuliu2016@163.com

[†]These authors have contributed equally to this work and share first authorship

Specialty section:
This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Endocrinology

Received: *09 February 2021*
Accepted: *06 April 2021*
Published: *27 April 2021*

Citation:
Wang C, Jing H, Sun Z, Yao J, Zhang X, Liu T and Wu Y (2021) A Bibliometric Analysis of Primary Aldosteronism Research From 2000 to 2020. Front. Endocrinol. 12:665912. doi: 10.3389/fendo.2021.665912

Thousands of papers on primary aldosteronism (PA) have been published in the last two decades. This study aimed to evaluate the research hotspots and future trends in PA research using bibliometric analysis. A total of 2,365 PA research papers between 2000 and 2020 were included. The dominant position of the United States in global PA research throughout this 20-year period was evident, and it was also the country most frequently involved in international cooperation. The University of Padua was the most productive institution and a leader in research collaboration. The Journal of Clinical Endocrinology & Metabolism was the most productive journal in terms of the number of publications on PA. Further, Mulatero P, Reincke M, Beuschlein F and Wu VC all made significant contributions to PA research. Five hotspots have been identified: (1) metabolic syndrome associated with PA; (2) molecular mechanisms of PA; (3) adrenal adenoma and adrenal cortex; (4) hypertension associated with PA; and (5) clinical monitoring parameters and diagnosis in patients with PA. Our results suggest that the molecular mechanisms of PA will remain research hotspots in the future. International collaboration is also expected to widen and deepen in the field of PA research.

Keywords: primary aldosteronism, bibliometric analysis, VOSviewer, bibliographic item co-occurrence matrix builder, research hotspots

INTRODUCTION

Primary aldosteronism (PA), characterized by an increased aldosterone production, is the most frequent form of secondary hypertension (1, 2). PA patients have an increased risk of stroke, heart failure, coronary artery disease, atrial fibrillation (1, 3–5), renal damage (6, 7), diabetes (8, 9), metabolic syndrome (9, 10). PA is associated with reduced quality of life and an increased prevalence of mental fatigue, anxiety and depression (11–13). PA was first described by Jerome Conn in 1955 in a patient (14). During the past 65 years, especially in the last two decades, great progress has been made

Abbreviations: PA, Primary aldosteronism; WoSCC, Web of Science Core Collection; BICOMB, Bibliographic Item Co-Occurrence Matrix Builder; IF, impact factor; H-index, Hirsch index; JCR, Journal Citation Reports; AVS, Adrenal vein sampling.

in the field of PA research regarding genetic and genomic mechanisms, pathophysiology, diagnostics, and therapeutics (2, 15, 16). However, there is still a lack of comprehensive reports that can assist researchers in obtaining an intuitive overview and reveal research trends in the PA research field.

Bibliometric analysis is a novel scientific method used to evaluate contributions to a research field, including those by countries, institutions, authors, and journals. Further, bibliometric analysis can predict the hotspots and trends within a certain research area through information visualization (17–19). However, few bibliometric studies have been performed in the field of PA research.

In the present study, we performed a comprehensive bibliometric analysis of PA research literature from 2000 to 2020, taking into account the number of annual publications, countries, international cooperation, institutions, journals, authors, and keyword co-occurrence visualization analysis. Furthermore, perspectives on progress in the field of PA research over the past two decades were considered. Overlay visualization maps of co-occurring keywords and double-clustering analysis were also performed in order to confirm the trends and hotspots in PA research. We hope that this study will provide new perspectives and a basis for future PA research.

MATERIALS AND METHODS

Data Sources and Search Strategy

The Web of Science is one of the most influential databases of scientific literature. In this study, all data were retrieved from the Web of Science Core Collection (WoSCC) *via* the China Medical University library website. The retrieval strategy was TS = primary aldosteronism.

Screening Criteria and Data Downloads

The publication period in the present study was limited to the period from 2000 to 2020. Non-English language, non-article, and non-review publications were excluded. WoSCC data including titles, author information, abstracts, keywords, journals, and references were downloaded in.txt format. To avoid the bias caused by frequent database updates, all literature retrieval and data downloads were completed on the same day (February 1, 2021). Two investigators (CW and JY) independently performed the search and had an agreement of 95% (kappa = (P0 − Pe)/(n − Pe) = 0.95 > 0.75), showing significant consistency (20).

Statistical Analysis

In the present study, a comprehensive description of various publishing characteristics is provided, including authors, institutions, countries, journals, keywords, impact factor (IF), and Hirsch index (h-index). IFs were obtained from the 2019 Journal Citation Reports (JCR) to assess the scientific value of research (21). The value of the h-index was defined as the number of papers with citation number ≥ h and is considered to be an important indicator for assessing both the productivity and impact of the published work of scientists, journals, or countries (22). The filtered data from WoSCC was imported into the online analysis platform of literature metrology (http://bibliometric.com/) and VOSviewer 1.6.15 (Leiden University, Leiden, The Netherlands) for bibliometric analysis. Apache ECharts (https://echarts.apache.org/), a JavaScript-based data visualization tool, was used to visualize the annual number of publications and the number of cumulative publications in different countries/regions. The online bibliometric analysis platform was used to visualize international collaboration between countries. VOSviewer was used for analysis and visualization of bibliometric networks such as authors, institutions, journals, co-citations, and the keywords used in the articles (23). Network visualization maps and overlay visualization maps were generated using VOSviewer. The online bibliometric analysis platform and Microsoft Excel 2016 were used to assess the impact of authors, institutions, and journals. The filtered data from WoSCC were imported into Bibliographic Item Co-occurrence Matrix Builder (BICOMB) to construct a keyword-article binary matrix (24). The rows of the matrix represented publications, while the columns represented highly frequent keywords. Additionally, gCLUTO software 1.0 was used to perform double-clustering analysis, and to build mountain maps and heat maps based on the results of the clustering analysis (24).

RESULTS

Trends and Annual Publications

As shown in **Figure 1**, a total of 3,459 papers were identified, and 2,365 papers (1,929 articles and 436 reviews) from 2000 to 2020 were ultimately included according to the screening criteria. **Figure 2** shows the growth trend of the annual publications related to PA, from 48 in 2000 to 250 in 2020. Based on the WoSCC database, the 2,365 papers were cited 65,149 times, and each paper was cited an average of 27.55 times.

Contribution of Countries and Institutions

According to the WoSCC database, 69 countries or regions contributed to publications on PA between 2000 and 2020. The top 24 countries or regions in terms of the number of publications (n > 10) on PA are presented on a world map in **Figure 3A**, and the top 10 are presented as numbers in **Table 1**.

TABLE 1 | The top 10 countries or regions contributing to publications in PA research.

Rank	Country/Region	Records	Percentage. (N/2365), %
1	USA	602	25.455
2	Japan	419	17.717
3	Italy	384	16.237
4	China	293	12.389
5	Germany	291	12.304
6	Australia	151	6.385
7	France	126	5.328
8	Netherlands	100	4.228
9	Canada	96	4.059
10	England	95	4.017

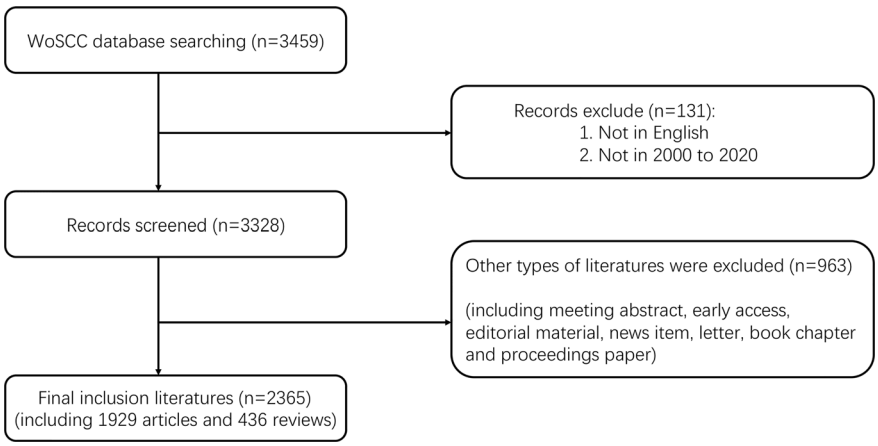

FIGURE 1 | Flowchart of data filtration processing and excluding publications.

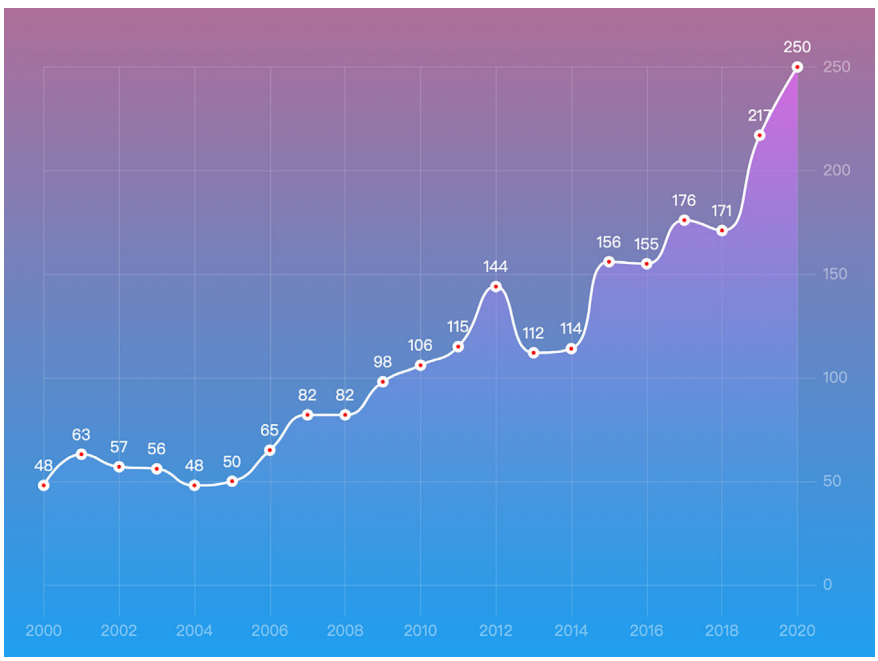

FIGURE 2 | Annual number of the published publications in PA research from 2000 to 2020.

The United States was the largest contributor, with 602 papers published, followed by Japan (n = 419), Italy (n = 384), China (n = 293), Germany (n = 291), Australia (n = 151), France (n = 126), Netherlands (n = 100), Canada (n = 96), and England (n = 95). The United States and Japan contributed many more papers to the number of publications on PA than other countries or regions (**Figure 3A** and **Table 1**). Within the survey period, close cooperation between countries or regions around the world was extremely common. International cooperation analysis indicated that the United States was the country most frequently involved in international cooperation (**Figure 3B**).

The most productive institutions were also evaluated in our study. As shown in **Table 2**, with 140 papers published, the University of Padua was the most productive institution and was followed by National Taiwan University Hospital (n = 102), University of Turin (n = 96), Tohoku University (n = 86), University of Queensland (n = 82), University of Mississippi (n = 67), National Taiwan University (n = 63), University of Michigan (n = 63), University of Paris 5 (n = 63), and the Georges Pompidou European Hospital (n = 62). The collaboration network was generated using VOSviewer software, and the threshold was set to 20 as the minimum

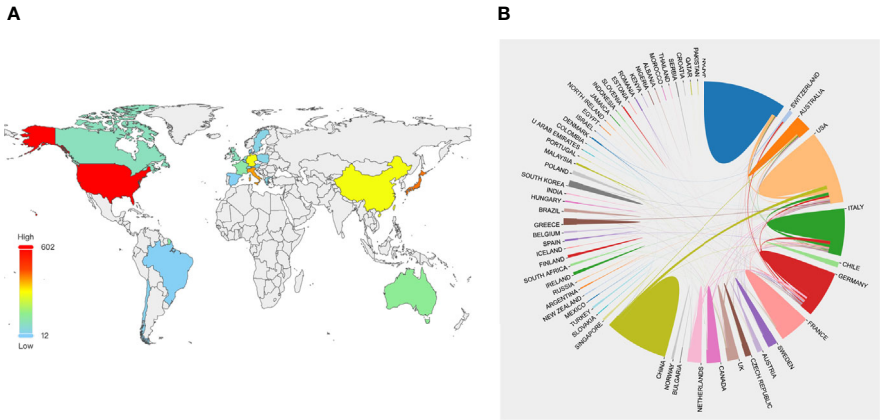

FIGURE 3 | The distribution of countries or regions in PA research. **(A)** Distribution of PA literatures in the world map. The color of each country or region on the world map represents the amount of literature published, according to the color gradient in the lower left corner. **(B)** The network map of cooperation between countries or regions. Different colors represent different countries or regions, the area of each color represents the amount of literature published in each country or regions, and the thickness of the connecting line indicates the cooperation frequency.

TABLE 2 | The top 10 most productive institutions in PA research.

Rank	Country/Region	Records	Percentage. (N/2365), %
1	Univ Padua	140	5.920
2	Natl Taiwan Univ Hosp	102	4.313
3	Univ Turin	96	4.059
4	Tohoku Univ	86	3.636
5	Univ Queensland	82	3.467
6	Univ Mississippi	67	2.833
7	Natl Taiwan Univ	63	2.664
7	Univ Michigan	63	2.664
7	Univ Paris 05	63	2.664
10	Hop Europeen Georges Pompidou	62	2.622

number of documents of an institution, while 1,000 was set as the minimum number of citations of an institution. Finally, 22 out of the 1,942 institutions were identified. During these two decades, University of Padua cooperated with almost all influential scientific institutions in studies on PA (**Figure 4**).

Contribution of Journals

In the present study, a comprehensive analysis of the contribution of journals with journal characteristics was provided, including journal titles, article counts, total citations, citations per article, IF (2019), quartile in category (2019), and h-index. The top 10 most productive journals in the field of PA research are listed in **Table 3**; in total, these journals published 809 papers, accounting for 34.21% of the total publications. *Journal of Clinical Endocrinology & Metabolism* (n = 165), *Hypertension* (n = 142), and *Journal of Hypertension* (n = 111) were the top three journals in terms of the number of publications on PA (**Table 3**). These three journals were the top three journals in terms of the highest total number of citations (6,399 *vs.* 4,394 *vs.* 2,146 citations, respectively), and they were also the top three journals with the highest average number of citations per paper (38.78 *vs.* 30.94 *vs.* 19.33 times, respectively). *Hypertension*, *Journal of Clinical Endocrinology & Metabolism*, and *European Journal of Endocrinology* had the highest IFs of any journals in 2019 (7.713 *vs.* 5.399 *vs.* 5.308, respectively). The highest h-index was 35, belonged to *Hypertension*. Among the top 10 most productive journals, *Journal of Clinical Endocrinology & Metabolism*, *Hypertension*, *Journal of Hypertension* and *European Journal of Endocrinology* were classified as Q1 according to the JCR 2019 standards (**Table 3**). The top 10 most highly cited publications are listed in **Table 4**.

Contributions of Authors

The top 10 most productive authors in the field of PA research are presented in **Table 5**. Among them, Reincke M from the Ludwig-Maximilians-Universität München in Germany ranked first (n = 129). Mulatero P from the Department of Medical Sciences, University of Torino in Italy and Wu VC from the Department of Internal Medicine, National Taiwan University Hospital in Taiwan were the second most productive authors (n = 97). Furthermore, Stowasser M, Mulatero P, and Rossi GP were the top three authors with the highest total number of citations (3,885 *vs.* 3,421 *vs.* 3,392 times, respectively, **Table 5**). A co-authorship overlay visualization map was generated using VOSviewer software, and the threshold for the minimum number of documents by an author was set to 20. Finally, 55 authors who met the threshold were identified, and Mulatero P, Reincke M, Beuschlein F and Wu VC were shown to have cooperated closely (**Figure 5A**). A citation overlay visualization map was also generated using VOSviewer software, and the threshold for the minimum number of citations of an author was set to 1,000. Finally, 55 authors who met the threshold were identified, and it could be seen that Reincke M, Mulatero P and Wu VC had made significant contributions to the field of PA research (**Figure 5B** and **Table 5**).

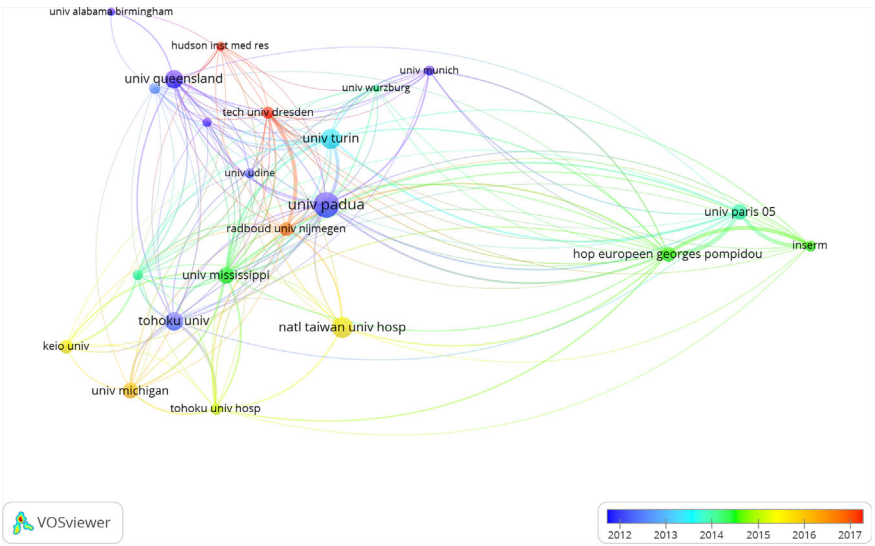

FIGURE 4 | Co-authorship overlay visualization map of institutions. The color of each circle corresponds to the average publication year, the size of a circle is proportional to the number of literatures, and the thickness of the connecting line indicates the cooperation frequency.

TABLE 3 | The top 10 most active journals that published articles in PA research.

Rank	Journal title	Article counts	Total number of citations	Average number of citations	IF(2019)	Quartile in category (2019)	H-index
1	Journal of Clinical Endocrinology & Metabolism	165	6,399	38.78	5.399	Q1	33
2	Hypertension	142	4,394	30.94	7.713	Q1	35
3	Journal of Hypertension	111	2,146	19.33	4.171	Q1	21
4	Hormone and Metabolic Research	88	940	10.68	2.562	Q3	14
5	European Journal of Endocrinology	66	997	15.11	5.308	Q1	23
6	Clinical Endocrinology	58	783	13.5	3.38	Q2	17
7	Journal of Human Hypertension	49	626	12.78	2.26	Q3	11
8	Hypertension Research	46	532	11.57	2.941	Q2	12
9	American Journal of Hypertension	45	696	15.47	2.669	Q3	13
10	Endocrine Journal	39	500	12.82	1.952	Q4	12

Analysis of Research Hotspots

With an appearance of more than 20 times, 34 of the most frequent keywords were extracted from the included publications and are displayed in **Table 6**. Five clusters were sorted through double-clustering using gCLUTO. The relationship between publications and high-frequency keywords was visualized using a volcano map and matrix map (**Figure 6**). The matrix map is shown in **Figure 6A**, in which column labels represent articles, while row labels represent keywords. To combine similar rows in a single cluster, the rows of the initial matrix were reset and each cluster was partitioned by black horizontal lines. In the matrix map, the upper dendrogram represents article associations, while the left represents high-frequency keyword associations. The results of the volcano map in **Figure 6B** directly show the high-dimensional character of the data. In this three-dimensional image, five different mountains represent five different clusters, numbered from 0 to 4.

The above 34 high-frequency keywords were divided into five clusters. All representative articles involved in each cluster were mined to further summarize hotspots in the field of PA Finally, five hotspots were identified using BICOMB and gCLUTO software packages:

Cluster 0: Metabolic syndrome associated with PA.

Cluster 1: Molecular mechanisms of PA.

Cluster 2: Adrenal adenoma and adrenal cortex.

Cluster 3: Hypertension associated with PA.

Cluster 4: Clinical monitoring parameters and diagnosis in patients with PA.

To explore the changes of hotspots over a period of time, a network visualization map of keyword co-occurrence was generated using VOSviewer software, and the results showed that the keywords “KCNJ5”, “K(+) channel mutations”, “somatic

TABLE 4 | The top 10 high-cited papers in PA research during 2000 to 2020.

Rank	Title	Authors	Year	Journal	Total citations
1	Case detection, diagnosis, and treatment of patients with primary aldosteronism: An endocrine society clinical practice guideline	Funder, John W. et al.	2008	Journal of Clinical Endocrinology & Metabolism	1073
2	Resistant hypertension: Diagnosis, evaluation, and treatment—A Scientific Statement from the American Heart Association Professional Education Committee of the Council for High Blood Pressure Research	Calhoun, David A. et al.	2008	Hypertension	998
3	Evidence for an increased rate of cardiovascular events in patients with primary aldosteronism	Milliez, P. et al.	2005	Journal of The American College Of Cardiology	960
4	A prospective study of the prevalence of primary aldosteronism in 1,125 hypertensive patients	Rossi, Gian Paolo. et al.	2006	Journal of The American College Of Cardiology	859
5	The Management of Primary Aldosteronism: Case Detection, Diagnosis, and Treatment: An Endocrine Society Clinical Practice Guideline	Funder, John W. et al.	2016	Journal of Clinical Endocrinology & Metabolism	777
6	A survey on adrenal incidentaloma in Italy	Mantero, F. et al.	2000	Journal of Clinical Endocrinology & Metabolism	636
7	Extensive personal experience—Increased diagnosis of primary aldosteronism, including surgically correctable forms, in centers from five continents	Mulatero, P. et al.	2004	Journal of Clinical Endocrinology & Metabolism	614
8	The incidentally discovered adrenal mass	Young, William F. et al.	2007	New England Journal of Medicine	602
9	Hyperaldosteronism among with resistant black and white subjects hypertension	Calhoun, DA. et al.	2002	Hypertension	445
10	Effect of spironolactone on blood pressure in subjects with resistant hypertension	Chapman, Neil. et al.	2007	Hypertension	428

TABLE 5 | The top 10 most productive authors in PA research.

Rank	Author	Article counts	Total number of citations	Average number of citations	First author counts	First author citation counts	Corresponding author counts	Corresponding author citation counts
1	Reincke, M	129	3,241	25.12	4	261	65.25	40
2	Mulatero, P	97	3,421	35.27	26	1,614	62.08	45
3	Wu, VC	97	1,137	11.72	18	453	25.17	19
4	Rossi, GP	93	3,392	36.47	45	2,656	59.02	81
5	Beuschlein, F	87	2,251	25.87	8	173	21.63	18
6	Stowasser, M	76	3,885	51.12	24	858	35.75	51
7	Wu, KD	70	1,142	16.31	2	31	15.5	16
8	Lin, YH	70	890	12.71	7	148	21.14	20
9	Veglio, F	66	2,763	41.86	0	0	0	1
10	Williams, TA	65	1,673	25.74	12	402	33.5	18

mutations", and "KCNJ5 mutations" began to appear in the last 5 years (**Figure 7**).

DISCUSSION

In the era of the information explosion, bibliometric analysis can help scientific researchers to manage their knowledge and visualize knowledge structures more intuitively. By presenting visual results, bibliometric analysis can help new researchers in a specific field to grasp the overall trends in the field being investigated. It can also reveal milestone manuscripts, the most productive authors and institutions, and current research hotspots, as well as future trends (25–27). In our study, a comprehensive bibliometric analysis of global scientific publications in the field of PA research from 2000 to 2020 was performed.

The number of publications in a particular research field can reflect the productivity and developments in the field over time (28). In the present study, a total of 2,365 publications, including 250 literature in 2020, were included (**Figures 1**, **2**). The results showed that the number of publications in the field of PA was

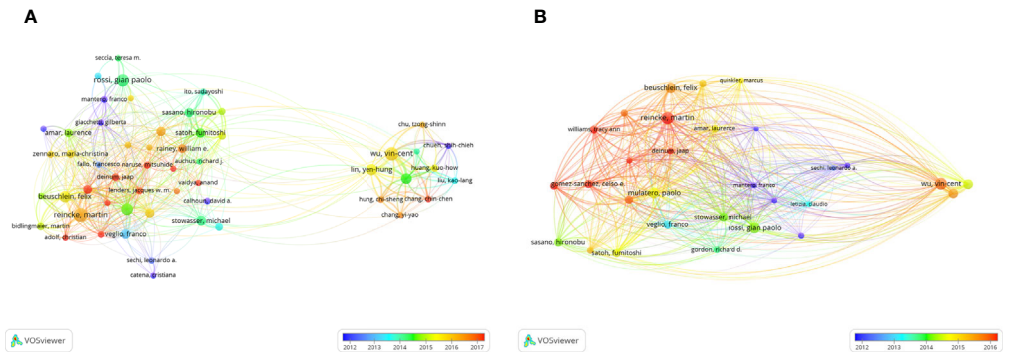

FIGURE 5 | The distribution of authors in PA research. **(A)** Author co-authorship overlay visualization map. The color of each circle corresponds to the average publication year of the author, the size of a circle is proportional to the number of literatures published by the author, and the thickness of the connecting line indicates the cooperation frequency. **(B)** Author co-citation overlay visualization map. The color of each circle corresponds to the average publication year of the author, the size of a circle is proportional to the total number of citations of the author, and the thickness of the connecting line indicates the strength of the co-citation link.

TABLE 6 | Keywords of PA research hotspots.

Rank	Keywords	Frequency	Percentage (%)
1	primary aldosteronism	668	8.4653
2	aldosterone	421	5.3352
3	hypertension	356	4.5115
4	hyperaldosteronism	165	2.0910
5	adrenal vein sampling	146	1.8502
6	adrenalectomy	133	1.6855
7	aldosterone-producing adenoma	119	1.5080
8	renin	90	1.1405
9	aldosterone-producing adenoma	73	0.9251
10	primary hyperaldosteronism	71	0.8998
11	pheochromocytoma	66	0.8364
12	Cushing's syndrome	63	0.7984
13	blood pressure	63	0.7984
14	secondary hypertension	59	0.7477
15	resistant hypertension	57	0.7223
16	adrenal gland	57	0.7223
17	spironolactone	55	0.6970
18	aldosteronism	51	0.6463
19	hypokalemia	46	0.5829
20	adrenal	45	0.5703
21	adrenal adenoma	38	0.4816
22	diagnosis	37	0.4689
23	Conn's syndrome	36	0.4562
24	adrenal incidentaloma	31	0.3929
25	CYP11B2	29	0.3675
26	adrenal cortex	28	0.3548
27	KCNJ5	28	0.3548
28	adenoma	25	0.3168
29	essential hypertension	24	0.3041
30	endocrine hypertension	23	0.2915
31	prevalence	22	0.2788
32	eplerenone	22	0.2788
33	cortisol	21	0.2661
34	mineralocorticoid receptor	20	0.2535

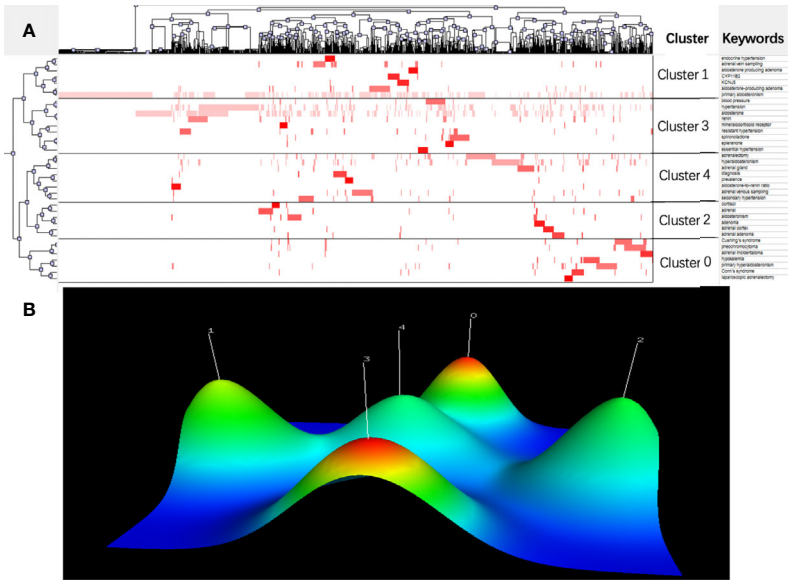

FIGURE 6 | Research hotspots in the field of PA. **(A)** Visualized matrix of biclustering of highly frequent keywords in the research field of PA. Color of each blot represented the frequency of occurrence of keywords in all literatures. **(B)** Mountain visualization of biclustering of highly frequent keywords in the research field of PA. The height and color of the mountain are proportional to internal similarity and standard deviation of cluster (Blue: high deviation; Red: low deviation).

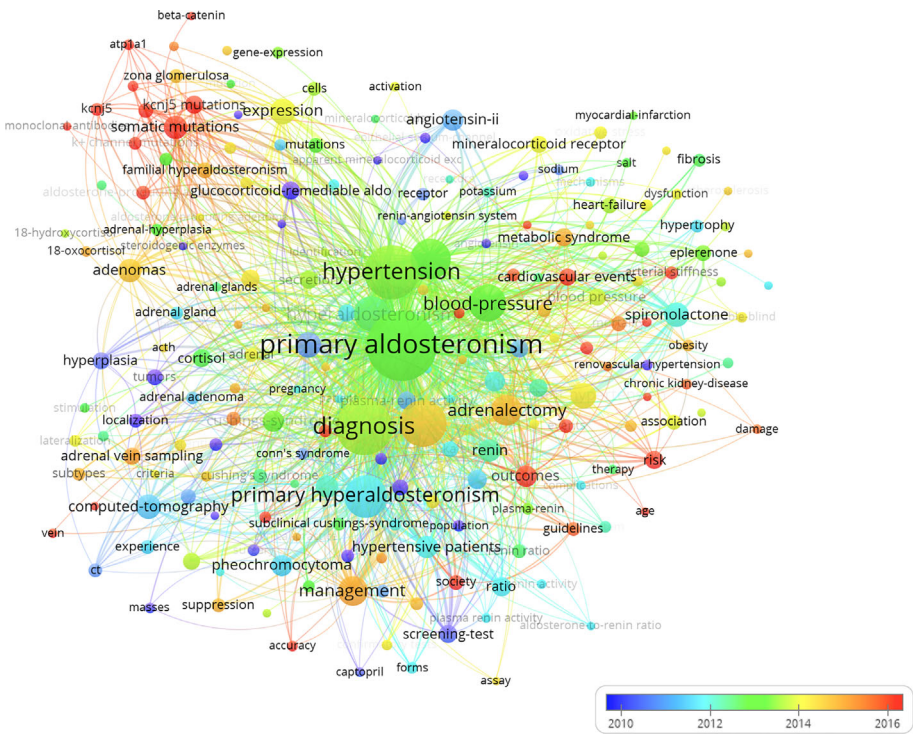

FIGURE 7 | Keywords co-occurrence overlay visualization map. The color of each circle corresponds to the average publication year. The size of a circle is proportional to the frequency of occurrence of the keyword, and the thickness of the connecting line indicates the strength of the keywords co-occurrence link.

maintained at a substantial level in the two decades from 2000 to 2020, which suggests that PA remains a hot research field, and more and more scholars may participate in PA research.

The number of publications in a research field is an important indicator for evaluating the scientific research level of a country or institution (25, 28, 29). Our study showed that the United States and Japan were the two largest contributors to the number of publications on PA (**Figure 3A** and **Table 1**), highlighting their impact in the PA research field. The value of international collaboration in supporting innovation and addressing unmet challenges is well recognized worldwide (30). From 2000 to 2020, many countries or regions around the world collaborated on studies in the research field of PA. Furthermore, our results demonstrated that the United States had the highest collaboration performance, especially with China and Japan (**Figure 3B**). Meanwhile, University of Padua was identified as the most productive institution during the 20-year period (**Table 2**) and cooperated with almost all influential scientific institutions in the PA research field, including National Taiwan University Hospital, University of Turin, Tohoku University, and University of Queensland (**Figure 4**). These results showed that highly collaborative countries or institutions generally had a high academic level, suggesting that international cooperation will remain a future trend in the field of PA research.

Journal indicators obtained from bibliometric analysis can provide a reliable reference for researchers to search documents or submit manuscripts (31, 32). All the top 10 journals publishing literature on PA were included in the category of "Internal medicine" or "Endocrinology". Our results showed that *Journal of Clinical Endocrinology & Metabolism* published the highest number of PA-related papers and had the highest number of total citations (**Table 3**). Our results also showed that the most frequently cited publication was a clinical practice guideline written by John W Funder and colleagues from Prince Henry's Institute of Medical Research in Australia, and that "Case detection, diagnosis, and treatment of patients with primary aldosteronism: An endocrine society clinical practice guideline", was a milestone in the PA research field in the two decades from 2000 to 2020 (33) (**Table 4**). These results suggest that these active journals and highly cited papers can provide a reliable reference for scholars concerned with the progress of PA research, and PA plays an important role in the fields of endocrinology and internal medicine.

Based on the WoSCC database, Reincke M published the highest number of PA-related papers, while Stowasser M had the highest number of total citations (**Table 5**). Furthermore, Mulatero P, Reincke M, Beuschlein F and Wu VC cooperated closely and published a considerable number of highly cited publications, as evidenced in the co-authorship overlay network visualization map and citation overlay visualization map (**Figure 5A**, **B**). Therefore, they can be regarded as the leaders in the PA research field.

Because of the heterogeneity of the PA research field, we divided the keywords in our study into five clusters *via* double-clustering analysis (**Figure 6**). Cluster 0 is related to PA-related metabolic syndrome. The abnormal glucose metabolism caused by insulin resistance is related to the excessive production of aldosterone, which is the main cause of metabolic dysfunction in patients with PA (34). Cluster 1 is related to molecular mechanisms of PA. In recent years, somatic mutations are identified in genes associated with PA, KCNJ5, CACNA1D, ATP1A1 and ATP2B3 (35–37). In general, the emergence of Next-generation sequencing (NGS) technology has driven researchers to understand the pathogenic and molecular mechanisms of PA (38). Cluster 2 is related to adrenal adenoma and adrenal cortex. PA results from excessive production of aldosterone by the adrenal cortex. Adrenal adenoma is considered a benign neoplasm of the adrenal cortex. In addition, genetic studies have helped to understand the relationship between benign aldosterone-producing adrenocortical proliferation and ion channel mutations (2, 39, 40). Cluster 3 is related to hypertension associated with PA. PA accounts for 5–10% of all hypertension patients and exist in 20% of those with resistant hypertension. Our understanding of PA-related hypertension has increased tremendously during the last two decades and exploring how PA leads to hypertension is the key to improve the outcome of long-term diseases and improve the quality of life of PA patients (2, 6, 41). Cluster 4 is related to clinical monitoring parameters and diagnosis in PA patients. Adrenal vein sampling (AVS), the most recommended procedure for lateralization in PA, has many limitations such as required technical expertise, increased costs, and potential complications (42). As a consequence, the development of new non-invasive imaging techniques and monitoring parameters is conducive to timely diagnosis, provides appropriate treatment, and prevents deleterious cardiovascular outcomes of PA, which is also a challenge for doctors and researchers in the future (2, 43).

Keyword co-occurrence network visualization analysis is a widely accepted method for determining research hotspots and predicting research trends (44). Our results indicated that the keywords such as "KCNJ5", "K(+) channel mutations", "somatic mutations", and "KCNJ5 mutations" appeared frequently in the last 5 years, suggesting that the study of the genomics and mechanisms of PA will remain research hotspots over the next few years. Multicenter studies have reported that the most frequent genetic abnormalities are KCNJ5 somatic mutations, which were found in approximately 40% of aldosterone-producing adenoma (APA), a subtype of primary aldosteronism (35–37). Somatic mutations in KCNJ5, which encodes the Gprotein-coupled inward rectifier K+ channel, have been considered as a cause of PA (37, 40). Our analysis also suggests that scientists are still trying to gain a comprehensive understanding of PA, and we expect scientists to make breakthroughs in the pathogenesis and management of this condition in the near future.

However, there were some limitations in our study. Firstly, the WoSCC database is updated continuously and dynamically. Therefore, our results are temporary in nature. Secondly, non-

English publications were excluded. Hence, a discrepancy may exist between our results and the real publication characteristics.

In conclusion, the annual number of publications on PA grew in the two decades between 2000 and 2020. The United States was the leading country in this research field, while the University of Padua also achieved important research results and played a certain role in promoting the development of PA research. Furthermore, Mulatero P, Reincke M, Beuschlein F and Wu VC made significant contributions to this research field. Research hotspot analyses suggest that the molecular mechanisms of PA will remain research hotspots in the future. International collaboration was also prevalent, and it is expected to widen and deepen in the future. These results provide new perspectives for the study of PA and may have a beneficial effect on further study regarding the etiology, diagnosis, and treatment of this condition.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

YW and TL conceived the study. CW, HJ, ZS, JY, and XZ participated in statistical analysis. CW wrote the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

The study received funding from The Education Department of Liaoning Province (FWZR2020003).

REFERENCES

1. Byrd JB, Turcu AF, Auchus RJ. Primary Aldosteronism: Practical Approach to Diagnosis and Management. *Circulation* (2018) 138(8):823–35. doi: 10.1161/CIRCULATIONAHA.118.033597
2. Zennaro MC, Boulkroun S, Fernandes-Rosa FL. Pathogenesis and Treatment of Primary Aldosteronism. *Nat Rev Endocrinol* (2020) 16(10):578–89. doi: 10.1038/s41574-020-0382-4
3. Savard S, Amar L, Plouin PF, Steichen O. Cardiovascular Complications Associated With Primary Aldosteronism: A Controlled Cross-Sectional Study. *Hypertension* (2013) 62(2):331–6. doi: 10.1161/HYPERTENSIONAHA.113.01060
4. Monticone S, Burrello J, Tizzani D, Bertello C, Viola A, Buffolo F, et al. Rabbia F *Et Al*: Prevalence and Clinical Manifestations of Primary Aldosteronism Encountered in Primary Care Practice. *J Am Coll Cardiol* (2017) 69(14):1811–20. doi: 10.1016/j.jacc.2017.01.052
5. Monticone S, D'Ascenzo F, Moretti C, Williams TA, Veglio F, Gaita F, et al. Cardiovascular Events and Target Organ Damage in Primary Aldosteronism Compared With Essential Hypertension: A Systematic Review and Meta-Analysis. *Lancet Diabetes Endocrinol* (2018) 6(1):41–50. doi: 10.1016/S2213-8587(17)30319-4
6. Sechi LA, Novello M, Lapenna R, Baroselli S, Nadalini E, Colussi GL, et al. Long-Term Renal Outcomes in Patients With Primary Aldosteronism. *JAMA* (2006) 295(22):2638–45. doi: 10.1001/jama.295.22.2638
7. Wu VC, Kuo CC, Wang SM, Liu KL, Huang KH, Lin YH, et al. Tsai CT *Et Al*: Primary Aldosteronism: Changes in Cystatin C-Based Kidney Filtration, Proteinuria, and Renal Duplex Indices With Treatment. *J Hypertens* (2011) 29(9):1778–86. doi: 10.1097/HJH.0b013e3283495cbb
8. Akehi Y, Yanase T, Motonaga R, Umakoshi H, Tsuiki M, Takeda Y, et al. Katabami T *Et Al*: High Prevalence of Diabetes in Patients With Primary Aldosteronism (PA) Associated With Subclinical Hypercortisolism and Prediabetes More Prevalent in Bilateral Than Unilateral PA: A Large, Multicenter Cohort Study in Japan. *Diabetes Care* (2019) 42(5):938–45. doi: 10.1210/js.2019-SUN-144
9. Colussi G, Catena C, Lapenna R, Nadalini E, Chiuch A, Sechi LA. Insulin Resistance and Hyperinsulinemia are Related to Plasma Aldosterone Levels in Hypertensive Patients. *Diabetes Care* (2007) 30(9):2349–54. doi: 10.2337/dc07-0525
10. Rossi GP, Sechi LA, Giacchetti G, Ronconi V, Strazzullo P, Funder JW. Primary Aldosteronism: Cardiovascular, Renal and Metabolic Implications. *Trends Endocrinol Metab* (2008) 19(3):88–90. doi: 10.1016/j.tem.2008.01.006
11. Reincke M. Anxiety, Depression, and Impaired Quality of Life in Primary Aldosteronism: Why We Shouldn't Ignore it! *J Clin Endocrinol Metab* (2018) 103(1):1–4. doi: 10.1210/jc.2017-02141
12. Citton M, Viel G, Torresan F, Rossi GP, Iacobone M. Effect of Unilateral Adrenalectomy on the Quality of Life of Patients With Lateralized Primary Aldosteronism. *BMC Surg* (2019) 18(Suppl 1):105. doi: 10.1186/s12893-018-0432-1
13. Velema MS, de Nooijer A, Hermus A, Timmers H, Lenders JWM, Husson O, et al. A Disease-Specific Quality of Life Questionnaire for Primary Aldosteronism. *Endocr Connect* (2019) 8(4):389–97. doi: 10.1530/EC-19-0026
14. Conn JW. Presidential Address. I. Painting Background. II. Primary Aldosteronism, a New Clinical Syndrome. *J Lab Clin Med* (1955) 45(1):3–17.
15. Stowasser M. Primary Aldosteronism in 2011: Towards a Better Understanding of Causation and Consequences. *Nat Rev Endocrinol* (2011) 8(2):70–2. doi: 10.1038/nrendo.2011.223
16. Fernandes-Rosa FL, Boulkroun S, Zennaro MC. Genetic and Genomic Mechanisms of Primary Aldosteronism. *Trends Mol Med* (2020) 26(9):819–32. doi: 10.1016/j.molmed.2020.05.005
17. Cooper ID. Bibliometrics Basics. *J Med Libr Assoc* (2015) 103(4):217–8. doi: 10.3163/1536-5050.103.4.013
18. Devos P, Menard J. Trends in Worldwide Research in Hypertension Over the Period 1999-2018: A Bibliometric Study. *Hypertension* (2020) 2020: HYPERTENSIONAHA12015711. doi: 10.1161/HYPERTENSIONAHA.120.15711
19. Wang Y, Zhao N, Zhang X, Li Z, Liang Z, Yang J, et al. Gao Y *Et Al*: Bibliometrics Analysis of Butyrophilins as Immune Regulators [1992-2019] and Implications for Cancer Prognosis. *Front Immunol* (2020) 11:1187. doi: 10.3389/fimmu.2020.01187
20. Landis JR, Koch GG. The Measurement of Observer Agreement for Categorical Data. *Biometrics* (1977) 33(1):159–74. doi: 10.2307/2529310
21. Eyre-Walker A, Stoletzki N. The Assessment of Science: The Relative Merits of Post-Publication Review, the Impact Factor, and the Number of Citations. *PLoS Biol* (2013) 11(10):e1001675. doi: 10.1371/journal.pbio.1001675
22. Hirsch JE. An Index to Quantify an Individual's Scientific Research Output. *Proc Natl Acad Sci U S A* (2005) 102(46):16569–72. doi: 10.1073/pnas.0507655102
23. van Eck NJ, Waltman L. Citation-Based Clustering of Publications Using Citnetexplorer and Vosviewer. *Scientometrics* (2017) 111(2):1053–70. doi: 10.1007/s11192-017-2300-7
24. Li F, Li M, Guan P, Ma S, Cui L. Mapping Publication Trends and Identifying Hot Spots of Research on Internet Health Information Seeking Behavior: A Quantitative and Co-Word Biclustering Analysis. *J Med Internet Res* (2015) 17 (3):e81. doi: 10.2196/jmir.3326
25. Glanville J, Kendrick T, McNally R, Campbell J, Hobbs FD. Research Output on Primary Care in Australia, Canada, Germany, the Netherlands, the United Kingdom, and the United States: Bibliometric Analysis. *BMJ* (2011) 342: d1028. doi: 10.1136/bmj.d1028
26. Boudry C, Baudouin C, Mouriaux F. International Publication Trends in Dry Eye Disease Research: A Bibliometric Analysis. *Ocul Surf* (2018) 16(1):173–9. doi: 10.1016/j.jtos.2017.10.002
27. Stout NL, Alfano CM, Belter CW, Nitkin R, Cernich A, Lohmann Siegel K, et al. A Bibliometric Analysis of the Landscape of Cancer Rehabilitation Research (1992-2016). *J Natl Cancer Inst* (2018) 110(8):815–24. doi: 10.1093/jnci/djy108

28. Durieux V, Gevenois PA. Bibliometric Indicators: Quality Measurements of Scientific Publication. *Radiology* (2010) 255(2):342–51. doi: 10.1148/radiol.09090626
29. Soteriades ES, Falagas ME. Comparison of Amount of Biomedical Research Originating From the European Union and the United States. *BMJ* (2005) 331 (7510):192–4. doi: 10.1136/bmj.331.7510.192
30. Gal D, Glanzel W, Sipido KR. Mapping Cross-Border Collaboration and Communication in Cardiovascular Research From 1992 to 2012. *Eur Heart J* (2017) 38(16):1249–58. doi: 10.1093/eurheartj/ehw459
31. Oelrich B, Peters R, Jung K. A Bibliometric Evaluation of Publications in Urological Journals Among European Union Countries Between 2000-2005. *Eur Urol* (2007) 52(4):1238–48. doi: 10.1016/j.eururo.2007.06.050
32. Garfield E. Journal Impact Factor: A Brief Review. *CMAJ* (1999) 161(8):979–80.
33. Force ADT, Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, et al. Acute Respiratory Distress Syndrome: The Berlin Definition. *JAMA* (2012) 307(23):2526–33. doi: 10.1001/jama.2012.5669
34. Fallo F, Pilon C, Urbanet R. Primary Aldosteronism and Metabolic Syndrome. *Horm Metab Res* (2012) 44(3):208–14. doi: 10.1055/s-0031-1295412
35. Azizan EA, Poulsen H, Tuluc P, Zhou J, Clausen MV, Lieb A, et al. Somatic Mutations in ATP1A1 and CACNA1D Underlie a Common Subtype of Adrenal Hypertension. *Nat Genet* (2013) 45(9):1055–60. doi: 10.1038/ng.2716
36. Beuschlein F, Boulkroun S, Osswald A, Wieland T, Nielsen HN, Lichtenauer UD, et al. Somatic Mutations in ATP1A1 and ATP2B3 Lead to Aldosterone-Producing Adenomas and Secondary Hypertension. *Nat Genet* (2013) 45 (4):440–4. 444e441-442. doi: 10.1038/ng.2550
37. Mulatero P, Monticone S, Rainey WE, Veglio F, Williams TA. Role of KCNJ5 in Familial and Sporadic Primary Aldosteronism. *Nat Rev Endocrinol* (2013) 9 (2):104–12. doi: 10.1038/nrendo.2012.230
38. Ono Y, Yamazaki Y, Omata K, Else T, Tomlins SA, Rhayem Y, et al. Histological Characterization of Aldosterone-Producing Adrenocortical Adenomas With Different Somatic Mutations. *J Clin Endocrinol Metab* (2020) 105(3):E282–9. doi: 10.1210/clinem/dgz235
39. Vaidya A, Mulatero P, Baudrand R, Adler GK. The Expanding Spectrum of Primary Aldosteronism: Implications for Diagnosis, Pathogenesis, and Treatment. *Endocr Rev* (2018) 39(6):1057–88. doi: 10.1210/er.2018-00139
40. Choi M, Scholl UI, Yue P, Bjorklund P, Zhao B, Nelson-Williams C, et al. K+ Channel Mutations in Adrenal Aldosterone-Producing Adenomas and Hereditary Hypertension. *Science* (2011) 331(6018):768–72. doi: 10.1126/science.1198785
41. Grassi G, Calhoun DA, Mancia G, Carey RM. Resistant Hypertension Management: Comparison of the 2017 American and 2018 European High Blood Pressure Guidelines. *Curr Hypertens Rep* (2019) 21(9):67. doi: 10.1007/s11906-019-0974-3
42. Chen Cardenas SM, Santhanam P. (11)C-Metomidate PET in the Diagnosis of Adrenal Masses and Primary Aldosteronism: A Review of the Literature. *Endocrine* (2020) 70(3):479–87. doi: 10.1007/s12020-020-02474-3
43. Rossi GP, Cesari M, Lenzini L, Seccia TM. Disease Monitoring of Primary Aldosteronism. *Best Pract Res Clin Endocrinol Metab* (2020) 34(2):101417. doi: 10.1016/j.beem.2020.101417
44. Chen C. Searching for Intellectual Turning Points: Progressive Knowledge Domain Visualization. *Proc Natl Acad Sci U S A* (2004) 101:5303–10. doi: 10.1073/pnas.0307513100

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

ORIGINAL RESEARCH
published: 28 April 2021
doi: 10.3389/fendo.2021.644927

NP-59 Adrenal Scintigraphy as an Imaging Biomarker to Predict *KCNJ5* Mutation in Primary Aldosteronism Patients

Ching-Chu Lu[1,2], *Ruoh-Fang Yen*[1], *Kang-Yung Peng*[3], *Jei-Yie Huang*[1,2], *Kwan-Dun Wu*[3], *Jeff S. Chueh*[4] *and Wan-Yu Lin*[2,5*]

[1] Department of Nuclear Medicine, National Taiwan University Hospital, Taipei, Taiwan, [2] Institute of Epidemiology and Preventive Medicine, College of Public Health, National Taiwan University, Taipei, Taiwan, [3] Division of Nephrology, Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan, [4] Glickman Urological and Kidney Institute, Cleveland Clinic Lerner College of Medicine, Cleveland Clinic, Cleveland, OH, United States, [5] Department of Public Health, College of Public Health, National Taiwan University, Taipei, Taiwan

OPEN ACCESS

Edited by:
Ben Nephew, Worcester Polytechnic Institute, United States

Reviewed by:
Jinbo Hu, First Affiliated Hospital of Chongqing Medical University, China
Yuh-Feng Wang, Dalin Tzu Chi Hospital, Taiwan

***Correspondence:**
Wan-Yu Lin
linwy@ntu.edu.tw

Specialty section:
This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Endocrinology

Received: *22 December 2020*
Accepted: *06 April 2021*
Published: *28 April 2021*

Citation:
Lu C-C, Yen R-F, Peng K-Y, Huang J-Y, Wu K-D, Chueh JS and Lin W-Y (2021) NP-59 Adrenal Scintigraphy as an Imaging Biomarker to Predict KCNJ5 Mutation in Primary Aldosteronism Patients. Front. Endocrinol. 12:644927. doi: 10.3389/fendo.2021.644927

Purpose: Somatic *KCNJ5* mutation occurs in half of unilateral primary aldosteronism (PA) and is associated with more severe phenotype. Mutation status can only be identified by tissue sample from adrenalectomy. NP-59 adrenal scintigraphy is a noninvasive functional study for disease activity assessment. This study aimed to evaluate the predictive value of NP-59 adrenal scintigraphy in somatic *KCNJ5* mutation among PA patients who received adrenalectomy.

Methods: Sixty-two PA patients who had NP-59 adrenal scintigraphy before adrenalectomy with available *KCNJ5* mutation status were included. Two semiquantitative parameters, adrenal to liver ratio (ALR) and lesion to contralateral ratio of bilateral adrenal glands (CON) derived from NP-59 adrenal scintigraphy, of mutated and wild-type patients were compared. Cutoff values calculated by receiver-operating characteristic (ROC) analysis were used as a predictor of *KCNJ5* mutation.

Results: Twenty patients had *KCNJ5* mutation and 42 patients were wild type. Patients harboring *KCNJ5* mutation had both higher ALR and CON ($p = 0.0031$ and 0.0833, respectively) than wild-type patients. With ALR and CON cutoff of 2.10 and 1.95, the sensitivity and specificity to predict *KCNJ5* mutation were 85%, 57% and 45%, 93%, respectively. Among 20 patients with *KCNJ5* mutation, 16 showed G151R point mutation (*KCNJ5*- G151R) and 4 showed L168R point mutation (*KCNJ5*-L168R), which former one had significantly lower ALR ($p=0.0471$).

Conclusion: PA patients harboring somatic *KCNJ5* mutation had significantly higher NP-59 uptake regarding to ALR and CON than those without mutation. APAs with *KCNJ5*-L168R point mutation showed significantly higher ALR than those with *KCNJ5*-G151R point mutation.

Keywords: primary aldosteronism, NP-59 adrenal scintigraphy, *KCNJ5*, semiquantification, mutation prediction

INTRODUCTION

Primary aldosteronism (PA) is the most common cause of secondary hypertension which is characterized by overproduction of aldosterone, leading to hypertension and sometimes hypokalemia (1). There are three concerns making PA an important issue. First, the prevalence is underestimated. Due to highly heterogeneity of each study, lack of uniform screening tests and cutoff values, it is difficult to conclude a definite prevalence. Roughly 10% is a commonly acceptable prevalence among hypertensive populations (1). However, prevalence of 30% has been reported, which may occur in severe hypertensive populations at tertiary referral center (2). Second, PA patients had significantly higher risk of cardiovascular events, diabetes and metabolic syndrome as compared with patients with essential hypertension (3). Third, PA is controllable and even curable through proper treatment, by means of mineralocorticoid antagonist for bilateral disease and adrenalectomy for unilateral disease (4). John W Funder has stated that PA is a public health issue on the basis of prior mentioned concerns and underlying great impact on medical care resources (5).

The major advance of understanding PA pathophysiology is the identification of *KCNJ5* mutation in aldosterone-producing adenomas (APAs) (6). Mutated *KCNJ5* leads to persistent cell depolarization turning out to aldosterone overproduction. Approximately 40% APAs harbored *KCNJ5* mutation, while eastern countries had much lower mutation rate than Asian countries. *KCNJ5* mutation is also associated with clinical phenotype. Younger age, higher plasma aldosterone, larger tumor, and female were more commonly seen with mutation (7). However, mutation status is only available by surgically resected specimen, which the mutation rate among PA patients will not be truly revealed.

NP-59 adrenal scintigraphy is a molecular imaging evaluating adrenal cortical function based on the activity of cholesterol uptake and transfer. It is able to correctly differentiate unilateral disease from bilateral disease and with excellent predictive value of postsurgical outcome (8, 9). Although adrenal venous sampling (AVS) is currently the gold standard for lateralization, NP-59 adrenal scintigraphy is an alternative method since AVS is technically dependent and invasive. Considering that more severe disease brings higher cholesterol demand to produce aldosterone, and *KCNJ5* mutation is associated with higher plasma aldosterone, we assume that NP-59 adrenal scintigraphy may be an imaging biomarker to predict *KCNJ5* mutation.

MATERIALS AND METHODS

Patients

The study protocol was approved by the Institutional Review Board of National Taiwan University Hospital (approval No. 201002002R and 200912003R). Patients were retrospectively recruited from the Taiwan Primary Aldosteronism Investigation (TAIPAI) database with the following inclusion criteria: (1) clinically confirmed PA by either saline loading test or captopril test (a positive saline loading test is defined as post-test PAS higher than 10 ng/dl and a positive captopril test is defined as PAC suppression less than 30% of the baseline level concurrent with suppressed PRA or ARR > 35 ng/dl per ng/ml/h), (2) had NP-59 adrenal scintigraphy with single photon emission computed tomography (SPECT) and computed tomography (CT) before surgery, (3) underwent adrenalectomy within 1 year after NP-59 scintigraphy, and (4) available *KCNJ5* mutation status from surgical specimen. The only exclusion criteria was known malignancy with adrenal gland involvement. Adrenalectomy was determined by a successful non-stimulated AVS (which is defined as a selective index greater than 2) with a lateralization index than 2. Clinical and biochemical profiles were acquired at initial evaluation and 1 year after adrenalectomy.

Protocol and Interpretation of NP-59 Adrenal Scintigraphy

Medications may alter NP-59 uptake such as glucocorticoids, diuretics, spironolactone, beta-blockers, alpha-blockers, and calcium channel blockers were postponed or switched to alternative medications (10). Oral dexamethasone suppression (8 mg daily) was carried out throughout the study for 8 days. One mCi NP-59 was slowly injected intravenously on the 4th day, and SPECT/CT was performed on the 96th hour after NP-59 injection. Patients were also given 1 ml of diluted Lugol's solution daily to protect the thyroid from free ^{131}I uptake.

Two semiquantitative parameters were used to evaluated adrenal cortical function as previously reported (9). Maximal count of the adrenal gland with lesion (defined as adrenal gland having adrenalectomy) divided by the mean count of the liver resulted in adrenal to liver ratio (ALR). Maximal count of adrenal gland with lesion to maximal count of contralateral adrenal gland is defined as CON.

KCNJ5 Sequencing

The specimens of APAs after adrenalectomy were collected and stored at −72°C until analysis. Genomic DNA were isolated from APAs using the QIAamp DNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The DNA regions containing the most frequently occurred point mutations of *KCNJ5*, p.Gly151Arg (G151R) and p.Leu168Arg (L168R), were amplified and sequenced using gene-specific primers (forward 5′-CTTCATTTGGTGGCTCATTGC-′3, reverse 5′-GGGACTTGAT GAGCTTGGC-′3) as previously reported (11). The annealing temperature was 58°C. Direct sequencing of PCR products was performed using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, USA) with a 3730 DNA Analyzer (Applied Biosystems, Foster City, USA). Sequences were analyzed using DNAStar Lasergene SeqMan Pro 7.1.0 software. Standard protocol of sequencing in TAIPAI followed that which had been previously reported (12).

Statistical Analysis

Descriptive statistics were used for patients' characteristics. Continues data were expressed as median with 25th percentile and 75th percentile. Mann-Whitney U test was used for data comparison. Difference of ALR and CON between mutated and wild-type patients were compared by Mann-Whitney U test.

Receiver-operating characteristic (ROC) curves were plotted and areas under the curve (AUC) were calculated for ALR and CON. The sensitivity and specificity of ALR and CON to predict *KCNJ5* mutation were calculated according to the optimal cutoff value selected by Youden's index. A p value of less than 0.05 was deemed statistically significant. All statistical analyses were performed using MedCalc Statistical Software version 17.9.2 (MedCalc Software bvba, Ostend, Belgium).

RESULTS

Patient Characteristics

From October 2007 to October 2018, 64 patients were enrolled in this study. Two patients were excluded due to insufficient imaging information (one with missing data and the other with only SPECT imaging). Finally, 62 patients were enrolled for analysis, including 35 male (56%) and 27 female (44%), with a median age of 53 years (45–63). All patients received unilateral adrenalectomy and the median time interval between NP-59 adrenal scintigraphy and adrenalectomy was 134 days (75–249). The median ALR was 2.205 (1.69–2.98) and CON was 1.325 (1.14–1.83). The patients' characteristics were listed in **Table 1**.

Correlation Between Clinical/Biochemical Profiles and *KCNJ5* Mutation

Mutated patients had significantly higher preoperative plasma aldosterone concentration (PAC, 60.505 ng/dl [43.465–79.525] *versus* 43.2 ng/dl [26.6–56.44], p = 0.0130) and borderline higher portion of presence of hypokalemia before surgery (60% *versus* 13%, p = 0.0546). There were no differences regarding to age, gender, hypertension history, tumor size, preoperative antihypertensive medications, preoperative systolic blood pressure (BP), preoperative diastolic BP, preoperative plasma renin activity (PRA), and preoperative aldosterone to renin ratio (ARR).

There were significant differences regarding to postoperative clinical and biochemical profiles between mutated and wild-type patients. Significant lower postoperative systolic BP (125.5 mmHg [120.5–140.5] versus 139.5 mmHg [126–156], p = 0.02), diastolic BP (78.5 mmHg [72–86] *versus* 83 mmHg [80–97], p = 0.02), higher PRA (3.98 ng/ml/h [1.115–6.8456] *versus* 0.99 ng/ml/h [0.44–4], p = 0.02) and borderline lower ARR (9.515 ng/dl per ng/ml/h [5.94–31.875] *versus* 25.175 ng/dl per ng/ml/h [10.545–71.72], p = 0.06) were noted in mutated patients. Normalization of hypokalemia was seen in most patients except one mutated and two wild-type patients.

Correlation Between NP-59 Adrenal Scintigraphy and *KCNJ5* Mutation

ALR were significantly higher in mutated patients than in wild-type patients (2.815 [2.13–3.54] *versus* 2.005 [1.56–2.55], p = 0.0031; **Figure 1**), while CON showed borderline higher in mutated patients (1.315 [1.14–1.49] *versus* 1.69 [1.20–2.456], p = 0.0833; **Figure 1**). ROC analysis showed that an ALR cutoff of 2.10 and a CON cutoff of 1.95 were the best values to predict *KCNJ5* mutation. By ALR cutoff of 2.10, the sensitivity, specificity, positive predictive value, negative predictive value, and accuracy were 85%, 57%, 49%, 89% and 66%, respectively. By CON cutoff of 1.95, the sensitivity, specificity, positive predictive value, negative predictive value, and accuracy were 45%, 93%, 75%, 78%, and 77%, respectively. Among 12 patients with both ALR greater than 2.1 and CON greater than 1.95, 10 harbored *KCNJ5* mutation (83%). Twenty-four of 27 patients (89%) having ALR less than 2.10 and CON less than 1.95 had wild-type *KCNJ5*. For patients with CON greater than 1.95, ALR was greater than 2.10 without exception. Representative SPECT/CT images of two patients with and without *KCNJ5* mutations were shown in **Figure 2**.

G151R and L168R Point Mutations

Among 20 patients harboring *KCNJ5* mutation, 16 of them had G151R point mutation and 4 of them had L168R point mutation. There were no differences regarding to age, gender, hypertension

TABLE 1 | Association between patients' characteristics and *KCNJ5* mutation.

	Wild-type KCNJ5 (n = 42)	Mutated KCNJ5 (n = 20)	p
Age	54 (45–63)	53 (42–58)	0.2583
Gender	Male=24, Female=18	Male=11, Female=9	0.9164
Hypertension history (year)	5.5 (2–10)	4 (2.5–14)	0.8144
Size (cm)	1.55 (0.9–1.9)	1.55 (1.2–1.8)	0.5350
Preoperative antihypertensive medications	2 (2–3)	3 (1.5–3)	0.1158
Preoperative systolic BP (mm Hg)	156.5 (140–167)	142 (135.5–155)	0.0627
Preoperative diastolic BP (mm Hg)	95 (80–101)	86 (81–95)	0.1523
Preoperative PAC (ng/dl)	43.2 (26.6–56.44)	60.505 (43.465–79.525)	0.0130
Preoperative PRA (ng/ml/h)	0.275 (0.1–0.55)	0.34 (0.125–0.7)	0.1000
Preoperative ARR (ng/dl per ng/ml/h)	149.62 (60.04–577.88)	258.44 (79.97–578.875)	0.1944
Presence of hypokalemia before surgery	13 (31%)	12 (60%)	0.0546
Postoperative systolic BP (mm Hg)	139.5 (126–156)	125.5 (120.5–140.5)	0.0177
Postoperative diastolic BP (mm Hg)	83 (80–97)	78.5 (72–86)	0.0222
Postoperative PAC (ng/dl)	30.49 (23.635–54.11)	35.91 (27.15–42.475)	0.8351
Postoperative PRA (ng/ml/h)	0.99 (0.44–4)	3.98 (1.115–6.845)	0.0154
Postoperative ARR (ng/dl per ng/ml/h)	25.175 (10.545–71.72)	9.515 (5.94–31.875)	0.0557
Presence of hypokalemia after surgery	2 (5%)	1 (5%)	0.9682
ALR	2.005 (1.56–2.55)	2.815 (2.13–3.54)	0.0031
CON	1.69 (1.20–2.465)	1.315 (1.14–1.49)	0.0833

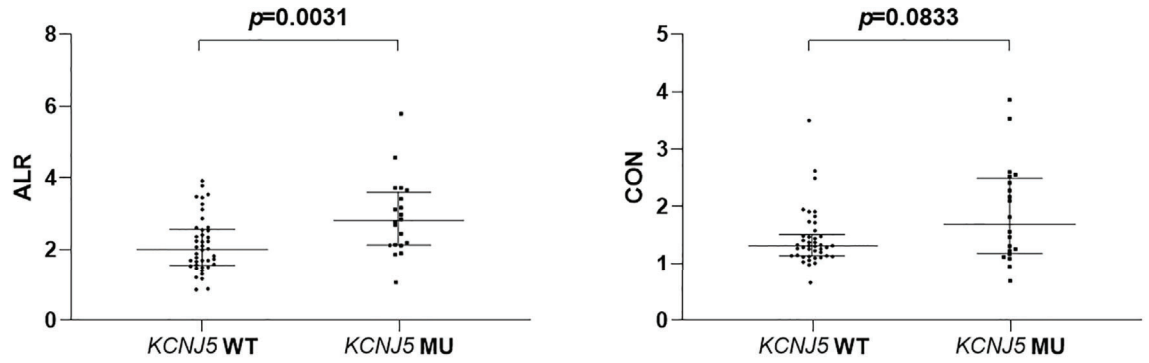

FIGURE 1 | Comparison of semiquantitative parameters of NP-59 adrenal scintigraphy according to *KCNJ5* mutation status.

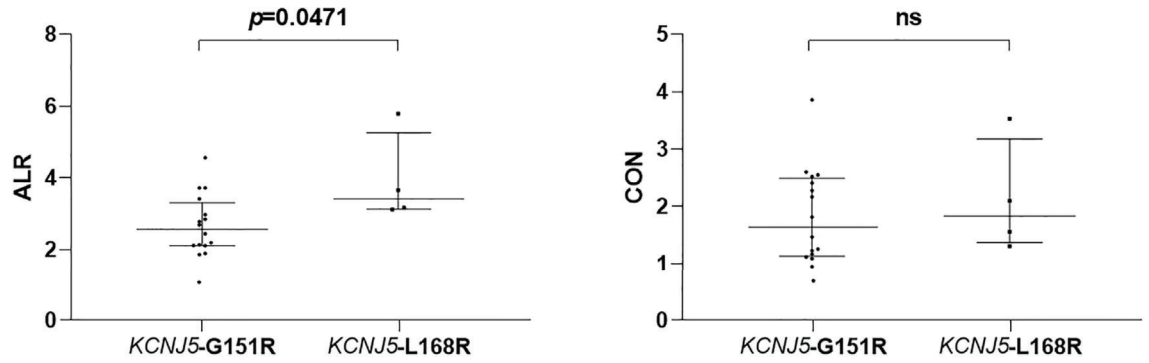

FIGURE 2 | Comparison of semiquantitative parameters of NP-59 adrenal scintigraphy according to different point mutations of *KCNJ5*. ns, non-significant.

history, tumor size, preoperative antihypertensive medications, preoperative systolic BP, preoperative diastolic BP, preoperative PAC, preoperative PRA and preoperative ARR. Postoperative clinical and biochemical profiles were similar except borderline lower PAC in patients with *KCNJ5*-L168R point mutation (20.05 ng/dl [12.24–32.15] *versus* 37.27 ng/dl [31.75–42.72], p = 0.07). ALR of *KCNJ5*-G151R was significantly lower than of *KCNJ5*-L168R point mutation (2.565 [2.115–3.20] *versus* 3.415 [3.145–4.725], p = 0.0471; **Figure 3**). There was no difference between these point mutations regarding to CON (1.645 [1.145–2.465] *versus* 1.83 [1.435–2.815], p = 0.5536; **Figure 3**). ROC analysis showed that with cutoff of 2.98, ALR had best ability to differentiate *KCNJ5*-G151R from *KCNJ5*-L168R point mutation. The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy were 75%, 100%, 100%, 50%, 80%, respectively. The patients' characteristics were listed in **Table 2**.

DISCUSSION

In the present study, we found significant correlation between NP-59 uptake and *KCNJ5* mutation. Adrenal glands with tumor harboring *KCNJ5* mutation had higher NP-59 uptake than contralateral adrenal glands both by direct comparison (CON) and corrected by liver background (ALR). Furthermore, tumors with *KCNJ5*-G151R point mutation had significantly higher ALR than those with *KCNJ5*-L168R point mutation. To our knowledge, this is the first study analyzing SPECT/CT imaging to predict genetic mutation.

Five decades have passed since the first adrenal cortical imaging agent was developed (13). Basic concept is that cholesterol is the key component of hormones released from adrenal cortex, and radiolabeled cholesterol should be able to lead the way to imaging the factory. The original compound was ^{125}I-19-iodocholesterol (14, 15) followed by first-in-human study by Beierwaltes et al. (16). Subsequent studies using ^{131}I-19-iodocholesterol were applied to variety of adrenal diseases such as Cushing's syndrome and primary aldosteronism (17–19). A chemical impurity ^{131}I-6β-iodomethyl-19-norcholesterol (NP-59) was found during the synthesis of 19-iodocholesterol which showed three to five times higher adrenal uptake (20).

Criticism to NP-59 adrenal scintigraphy in PA lateralization also arose with long history, mainly based on limited imaging resolution resulting in poor ability to detect small APAs (21). Guideline by expert consensus has addressed that NP-59 adrenal scintigraphy has no role in subtype evaluation of PA (22). Great

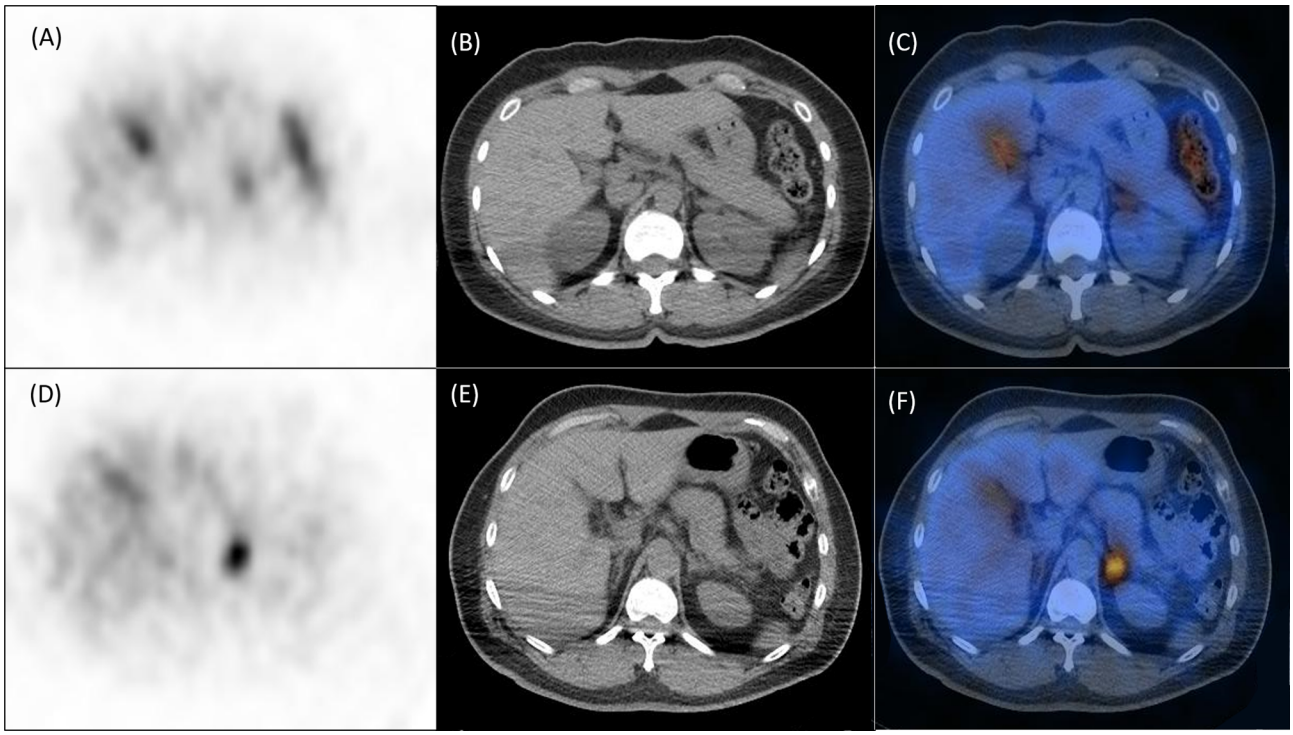

FIGURE 3 | Representative NP-59 adrenal SPECT/CT and *KCNJ5* mutation status. Upper panel, findings of a 40-year-old female without *KCNJ5* mutation. SPECT **(A)**, CT **(B)** and fusion SPECT/CT **(C)** showed moderate NP-59 uptake at left adrenal gland with ALR of 2.62 and CON of 1.38. Lower panel, findings of a 54-year-old male with *KCNJ5* mutation. SPECT **(D)**, CT **(E)** and fusion SPECT/CT **(F)** showed significant NP-59 uptake at left adrenal gland with ALR of 3.72 and CON of 2.41.

TABLE 2 | Characteristics of clinical and NP-59 adrenal scintigraphy regarding to different point mutation of *KCNJ5*.

	***KCNJ5*-G151R (n = 16)**	***KCNJ5*-L168R (n = 4)**	**p**
Age	53 (44–59)	48 (37–57)	0.5700
Gender	Male = 9, Female = 7	Male=2, Female=2	0.8676
Hypertension history (year)	6 (3–14)	3 (1.25–9.5)	0.3652
Size (cm)	1.5 (1.15–1.8)	2.05 (1.45–2.5)	0.1550
Preoperative antihypertensive medications	3 (1.5–3)	4 (2–5.5)	0.2624
Preoperative systolic BP (mm Hg)	141 (135.5–151)	156 (133.5–167)	0.2981
Preoperative diastolic BP (mm Hg)	86 (81–94)	92 (80–104.5)	0.5072
Preoperative PAC (ng/dl)	68.56 (43.465–79.525)	50.095 (37.5–103.195)	0.7768
Preoperative PRA (ng/ml/h)	0.39 (0.14–0.70)	0.12 (0.055–0.725)	0.3940
Preoperative ARR (ng/dl per ng/ml/h)	257.47 (79.97–416.565)	815.665 (292.2–2030.715)	0.3445
Presence of hypokalemia before surgery	9 (56%)	2 (50%)	0.8802
Postoperative systolic BP (mm Hg)	124.5 (119–140.5)	123.5 (115.5–126.5)	0.5082
Postoperative diastolic BP (mmHg)	80.5 (72–87)	76.5 (72–79)	0.3937
Postoperative PAC (ng/dl)	37.27 (31.75–42.72)	20.05 (12.24–32.15)	0.0725
Postoperative PRA (ng/ml/h)	3.98 (1.115–6.59)	4.055 (1.035–15.5)	0.8501
Postoperative ARR (ng/dl per ng/ml/h)	9.515 (6.33–32.025)	15.255 (1.04–31.875)	0.4497
Presence of hypokalemia after surgery	0	0	N/A
ALR	2.565 (2.115–3.20)	3.415 (3.145–4.725)	0.0471
CON	1.645 (1.145–2.465)	1.83 (1.435–2.815)	0.5536

effort has been put on improving imaging quality by SPECT to illustrate adrenal lesions more clearly (23–25). We have previously reported the most comprehensive study using SPECT/CT to differentiate APA from idiopathic bilateral adrenal hyperplasia with sensitivity and specificity of 82% and 67%, respectively (8). Semiquantitative analysis could be easily performed from SPECT/CT imaging. We furthermore applied two semiquantitative parameters, ALR and CON, to predict postsurgical outcome which found that ALR and CON greater than the cutoff were significantly correlated with improvement of

postsurgical outcome (9). In Taiwan mini-frontier of primary aldosteronism we suggested that NP-59 adrenal scintigraphy is an alternative method for lateralization when AVS is not available based on the above mentioned evidence (26).

Recognition of *KCNJ5* mutation in APAs by Choi et al. in 2011 is the most tremendous progression to understand the pathophysiology of PA. *KCNJ5* mutation altered the selectivity of encoding potassium channel which lead to cell membrane depolarization, influx of calcium ion and subsequent aldosterone production (6). *KCNJ5* is the most frequent genetic mutations in APAs with overall prevalence of 43%, ranging from 12% to 80% and it is widely studied for the correlation with phenotype, mainly in female, with younger age, larger tumor and higher PAC (7). Moreover, *KCNJ5* mutations were associated with postsurgical outcome. Kitamoto et al. found that patients with *KCNJ5* mutation had higher rate of hypertension resolution and decreased left ventricular hypertrophy after adrenalectomy (27). Vilela et al. also represented that *KCNJ5* mutation is the only independent predictor of hypertension remission (28). Change et al. recently demonstrated that mutation carriers had higher greater decrease in left ventricular mass index (LVMI) and inappropriately LVMI in a prospective cohort (29).

The importance of *KCNJ5* mutation arises from its high prevalence, phenotype association and postsurgical outcome correlation. However, *KCNJ5* mutation is only available with surgical specimen. Therefore, we aimed to utilize NP-59 adrenal scintigraphy as an imaging biomarker to predict *KCNJ5* mutation. In fact, it is common to use nuclear medicine imaging to predict disease biomarkers since it is noninvasive and easily to manipulate. Radiomics extracts quantitative imaging data and associates these features to relevant clinical profiles. Several studies have addressed the correlation between mutations and semiquantitative parameters of nuclear medicine imaging and the most relevant example is to predict *EGFR* mutation in lung cancer by 18F-fluorodeoxyglucose (FDG) positron emission tomography (PET). Maximal standard uptake value (SUVmax), which is the most commonly used semiquantitative parameter in PET, is associated with non-small cell lung cancer (NSCLC) with *EGFR* mutation and ALK positivity showing higher value (30). Radiomic features and conventional parameters (metabolic tumor volume and SUVmax) were proved to predictive of *EGFR* mutation in NSCLC (31). SUVmax is also shown to be correlated with *KRAS* mutation in colorectal cancer, $BRAF^{V600E}$ mutation in thyroid cancer and HER2 expression in gastric cancer (32–34).

In the present study we found that APA with *KCNJ5* mutation expressed significantly higher NP-59 uptake than those without *KCNJ5* mutation, in terms of ALR and CON. This correlation could be explained by the straightforward mechanism. Mutated channels alter the permeability of sodium which lead to depolarization of cell membrane and subsequent autonomous aldosterone production. Aldosterone production requires cholesterol as synthesis material and radiolabeled cholesterol, NP-59, is delivered to the overproduction side of adrenal gland. Mutated APAs produce more aldosterone than wild-type APAs which lead to more NP-59 uptake. In our cohort this genetic difference reflects both on the level of phenotype, preoperative PAC, and of molecular imaging, NP-59 adrenal scintigraphy.

AVS is currently the gold standard for lateralization and some studies have addressed the impact of *KCNJ5* mutation on lateralization index (LI). In 170 PA patients Seccia et al. evaluated 40 of them with selective index of AVS greater than 2.0 at both side, and found that LI was significantly higher in APA with *KCNJ5* mutations those without mutations ($p = 0.02$, detail value of LI not specified in the study) (35). Zheng et al. analyzed a larger Chinese cohort with 162 PA patients which revealed borderline higher LI in APA with *KCNJ5* mutations than those without mutations (10.9 [7.5–22.7] *versus* 6.4 [3.0–10.6], $p = 0.053$) (36). In contrast, Oßwald et al. found 19 PA patients harboring *KCNJ5* mutation had similar LI compared to 32 patients without *KCNJ5* mutation (20.5, interquartile range 30.3 *versus* 16.0, interquartile range 41.9) (37). The conflict and nonreplicated results not only attract attention of necessity of larger study, but also face up the contentious issues of AVS such as unstandardized protocol, different interpretation criteria and variable failed rate. NP-59 adrenal scintigraphy is an ancient nuclear medicine study with well-established protocol and sufficient evidence for both interpretation, lateralization and prognosis. Considering noninvasive nature and beneficial both for lateralization and mutation prediction, it is reasonable to have NP-59 adrenal scintigraphy before further treatment.

It is important to conduct the correlation between genotype and imaging findings into clinical practice, mainly referring to treatment. Predicting *EGFR* mutation by SUVmax of PET could identify proper candidate who may be beneficial from target therapy. In the present study we expect to use NP-59 adrenal scintigraphy selecting patients who may beneficial from medical treatment other than mineralocorticoid receptor antagonist (MRA). MRA is the drug of choice for bilateral disease as well as for patients who have contraindications to surgery or are not willing to receive surgery (which is common in Eastern countries) (22). The first line MRA is spironolactone acting by direct antagonizing the receptors which effectively lowers PAC. Side effects come along with its affinity to androgen receptor leading to gynecomastia and erectile dysfunction in male and to progesterone receptor leading to menstrual irregularity in female (38). In the SPARTACUS trial 57% of the patients developed these side effects from spironolactone compared with 1% of the adrenaletomy patients. This dose-dependent side effect has indeed limited the clinical application (39). Eplerenone is an alternative MRA when spironolactone is not tolerated or optimal BP is not achieved (22). It is selective for mineralocorticoid receptor without significant interaction with androgen or progesterone receptor and therefore it has much lower side effect compared to spironolactone (40). However, Eplerenone has less affinity to mineralocorticoid receptor and is only approved for PA use in Japan and USA, not in Europe, Australia and Taiwan (41, 42). Moreover, the price of eplerenone is 26 times compared to spironolactone in Taiwan. Epithelial sodium channel antagonists, such as amiloride, are the second-line choice for medical treatment of PA. It works as potassium-sparing diuretic which improves hypertension and hypokalemia. Although less effective than spironolactone,

amiloride is generally well-tolerated due to lack of androgenic effect (43, 44). Calcium channel blockers (CCBs) could be a choice for PA treatment with variable mechanisms and effects. Dihydropyridine CCBs reduced BP by competing aldosterone binding to mineralocorticoid receptor (45). However, the concentration of most dihydropyridine CCBs is not able to block mineralocorticoid receptor at regular doses in treating hypertension (46). Several studies have been published to illustrate the pharmacological effects of above mentioned alternative medications regarding to *KCNJ5* mutation. Tauber et al. reported that amiloride and its analog EIPA blocked with Na^+-permeable mutated *KCNJ5* cells with L168R point mutation. Although nondihydropyridine CCBs have no effect on mineralocorticoid receptor, verapamil and diltiazem showed potent inhibition of *KCNJ5*-L168R cells (47). Physicians are not able to know the effects of these alternative medications before prescription. In our cohort, patients harboring *KCNJ5*-L168R mutation had significantly higher ALR compared to those with *KCNJ5*-G151R mutation. It is of great novelty that amiloride and verapamil/diltiazem may work on patients with stronger potency and less side effects by the mutation status provided by NP-59 adrenal scintigraphy.

Recently macrolide antibiotics is proved to inhibit HEK293T cells transfected with mutated *KCNJ5* but not to wild-type cells without antibiotic activity (48). Thereafter Caroccia et al. proceeded the study which demonstrated that clarithromycin lowered CYP11B2 gene expression and aldosterone secretion in CD56+ cells ex vivo from *KCNJ5* mutated APAs, but not in CD56+ cells from wild-type APAs (49). NP-59 adrenal scintigraphy provides noninvasive method to predict *KCNJ5* mutation and may be able to identify patients beneficial to macrolide treatment. For patients intolerable to the dose-dependent side effect of spironolactone who may harboring *KCNJ5* mutation predicted by NP-59 adrenal scintigraphy, macrolide could be a choice to treatment regimen in the future, and the key point is to identify *KCNJ5* mutation in advance.

There are some limitations in our study. First, the retrospective, single-center design with relatively small sample size may lead to selection bias. Second, the patients in the present study were all Taiwanese. There is significant difference among races regarding to *KCNJ5* mutation rate. Higher *KCNJ5* mutation rate was noted in Eastern Asians (70%) compared to Caucasians (38%) (50). Validation of our results to general population requires more comprehensive survey. Third, other gene mutations such as *ATP1A1*, *ATP2B3*, *CACNA1D*, and *CTNNB1* account for a certain portion of somatic mutations in APA (51). Although these mutations were not found in the present cohort, it is not negligible and further study should be conducted.

In conclusion, our study suggested that semiqualitative parameters from NP-59 adrenal scintigraphy could predict *KCNJ5* mutations in PA patients. APAs harboring *KCNJ5* mutations had significantly higher ALR and borderline higher CON than those without *KCNJ5* mutations. Furthermore, APAs with *KCNJ5*-L168R mutation had significantly higher ALR than those with *KCNJ5*-G151R point mutation. Precision medicine, which individualized treatment based on distinct signature of each patient, is the trend of disease management. Our study is a proof-of-concept and the first study applying SPECT to predict mutation status which may be utilized in treatment plan of PA.

DATA AVAILABILITY STATEMENT

The data sets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the institutional review board of National Taiwan University Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

C-CL: data collection, imaging analysis, and manuscript writing. R-FY: imaging analysis. K-YP: genetic analysis. J-YH: statistical consult. K-DW: data collection. JC: data collection. W-YL: study design and correspondence. All authors contributed to the article and approved the submitted version.

FUNDING

The study is partly supported by the grant provided from National Taiwan University Hospital (110-S5009).

REFERENCES

1. Rossi GP, Bernini G, Caliumi C, Desideri G, Fabris B, Ferri C, et al. A Prospective Study of the Prevalence of Primary Aldosteronism in 1,125 Hypertensive Patients. *J Am Coll Cardiol* (2006) 48:2293–300. doi: 10.1016/j.jacc.2006.07.059
2. Kayser SC, Dekkers T, Groenewoud HJ, van der Wilt GJ, Carel Bakx J, van der Wel MC, et al. Study Heterogeneity and Estimation of Prevalence of Primary Aldosteronism: A Systematic Review and Meta-Regression Analysis. *J Clin Endocrinol Metab* (2016) 101:2826–35. doi: 10.1210/jc.2016-1472
3. Monticone S, D'Ascenzo F, Moretti C, Williams TA, Veglio F, Gaita F, et al. Cardiovascular Events and Target Organ Damage in Primary Aldosteronism Compared With Essential Hypertension: A Systematic Review and Meta-Analysis. *Lancet Diabetes Endocrinol* (2018) 6:41–50. doi: 10.1016/S2213-8587(17)30319-4
4. Huang KH, Yu CC, Hu YH, Chang CC, Chan CK, Liao SC, et al. Targeted Treatment of Primary Aldosteronism - The Consensus of Taiwan Society of Aldosteronism. *J Formos Med Assoc* (2019) 118:72–82. doi: 10.1016/j.jfma.2018.01.006
5. Funder JW. Primary Aldosteronism as a Public Health Issue. *Lancet Diabetes Endocrinol* (2016) 4:972–3. doi: 10.1016/S2213-8587(16)30272-8

6. Choi M, Scholl UI, Yue P, Bjorklund P, Zhao B, Nelson-Williams C, et al. K+ Channel Mutations in Adrenal Aldosterone-Producing Adenomas and Hereditary Hypertension. *Science* (2011) 331:768–72. doi: 10.1126/science.1198785
7. Lenzini L, Rossitto G, Maiolino G, Letizia C, Funder JW. Rossi Gp. A Meta-Analysis of Somatic Kcnj5 K(+) Channel Mutations in 1636 Patients With an Aldosterone-Producing Adenoma. *J Clin Endocrinol Metab* (2015) 100:E1089–95. doi: 10.1210/jc.2015-2149
8. Yen RF, Wu VC, Liu KL, Cheng MF, Wu YW, Chueh SC, et al. 131I-6beta-Iodomethyl-19-Norcholesterol SPECT/CT for Primary Aldosteronism Patients With Inconclusive Adrenal Venous Sampling and CT Results. *J Nucl Med* (2009) 50:1631–7. doi: 10.2967/jnumed.109.064873
9. Lu CC, Wu VC, Wu KD, Liu KL, Lin WC, Cheng MF, et al. Prognostic Value of Semiquantification NP-59 SPECT/CT in Primary Aldosteronism Patients After Adrenalectomy. *Eur J Nucl Med Mol Imaging* (2014) 41:1375–84. doi: 10.1007/s00259-014-2692-z
10. Gross MD, Valk TW, Swanson DP, Thrall JH, Grekin RJ, Beirewaltes WH. The Role of Parmacologic Manipulation in Adrenal Cortical Scintigraphy. *Semin Nucl Med* (1981) 11:128–48. doi: 10.1016/S0001-2998(81)80042-6
11. Azizan EA, Murthy M, Stowasser M, Gordon R, Kowalski B, Xu S, et al. Somatic Mutations Affecting the Selectivity Filter of KCNJ5 are Frequent in 2 Large Unselected Collections of Adrenal Aldosteronomas. *Hypertension* (2012) 59:587–91. doi: 10.1161/HYPERTENSIONAHA.111.186239
12. Wu VC, Huang KH, Peng KY, Tsai YC, Wu CH, Wang SM, et al. Prevalence and Clinical Correlates of Somatic Mutation in Aldosterone Producing adenoma-Taiwanese Population. *Sci Rep* (2015) 5:11396. doi: 10.1038/srep11396
13. Nagai T, Solis BA, Koh CS. An Approach to Developing Adrenal-Gland Scanning. *J Nucl Med* (1968) 9:576–81.
14. Counsell RE, Ranade VV, Blair RJ, Beierwaltes WH, Weinhold PA. Tumor Localizing Agents. IX. Radioiodinated Cholesterol. *Steroids* (1970) 16:317–28. doi: 10.1016/S0039-128X(70)80116-7
15. Varma VM, Blair RJ, Beierwaltes WH, Lieberman CM, Boyd RE, Counsell RE, et al. Radiolabeled Cholesterol as an Adrenal Scanning Agent. *J Nucl Med* (1971) 12:176.
16. Beierwaltes WH, Lieberman LM, Ansari AN, Nishiyama H. Visualization of Human Adrenal Glands In Vivo by Scintillation Scanning. *JAMA* (1971) 216:275–7. doi: 10.1001/jama.216.2.275
17. Lieberman LM, Beierwaltes WH, Conn JW, Ansari AN, Nishiyama H. Diagnosis of Adrenal Disease by Visualization of Human Adrenal Glands With 131 I-19-Iodocholesterol. *N Engl J Med* (1971) 285:1387–93. doi: 10.1056/NEJM197112162852501
18. Beierwaltes WH, Sturman MF, Ryo U, Ice RD. Imaging Functional Nodules of the Adrenal Glands With 131-I-19-Iodocholesterol. *J Nucl Med* (1974) 15:246–51.
19. Hogan MJ, McRae J, Schambelan M, Biglieri EG. Location of Aldosterone-Producing Adenomas With 131I-19-Iodocholesterol. *N Engl J Med* (1976) 294:410–4. doi: 10.1056/NEJM197602192940802
20. Sarkar SD, Beierwaltes H, Ice RD, Basmadjian GP, Hetzel KR, Kennedy WP, et al. A New and Superior Adrenal Scanning Agent, NP-59. *J Nucl Med* (1975) 16:1038–42.
21. Nomura K, Kusakabe K, Maki M, Ito Y, Aiba M, Demura H. Iodomethylnor cholesterol Uptake in an Aldosteronoma Shown by Dexamethasone-Suppression Scintigraphy: Relationship to Adenoma Size and Functional Activity. *J Clin Endocrinol Metab* (1990) 71:825–30. doi: 10.1210/jcem-71-4-825
22. Funder JW, Carey RM, Mantero F, Murad MH, Reincke M, Shibata H, et al. The Management of Primary Aldosteronism: Case Detection, Diagnosis, and Treatment: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab* (2016) 101:1889–916. doi: 10.1210/jc.2015-4061
23. Ishimura J, Kawanaka M, Fukuchi M. Clinical Application of SPECT in Adrenal Imaging With iodine-131 6 Beta-Iodomethyl-19-Norcholesterol. *Clin Nucl Med* (1989) 14:278–81. doi: 10.1097/00003072-198904000-00009
24. Hwang I, Balingit AG, Georgitis WJ, Sisson JC, Shapiro B. Adrenocortical SPECT Using iodine-131 Np-59. *J Nucl Med* (1998) 39:1460–3.
25. La Cava G, Imperiale A, Olianti C, Gheri GR, Ladu C, Mannelli M, et al. SPECT Semiquantitative Analysis of Adrenocortical (131)I-6 Beta Iodomethyl-Norcholesterol Uptake to Discriminate Subclinical and Preclinical Functioning Adrenal Incidentaloma. *J Nucl Med* (2003) 44:1057–64.
26. Chang CC, Chen YY, Lai TS, Zeng YH, Chen CK, Tu KH, et al. Taiwan Mini-Frontier of Primary Aldosteronism: Updating Detection and Diagnosis. *J Formos Med Assoc* (2021) 120:121–9. doi: 10.1016/j.jfma.2020.08.001
27. Kitamoto T, Omura M, Suematsu S, Saito J, Nishikawa T. KCNJ5 Mutation as a Predictor for Resolution of Hypertension After Surgical Treatment of Aldosterone-Producing Adenoma. *J Hypertens* (2018) 36:619–27. doi: 10.1097/HJH.0000000000001578
28. Vilela LAP, Rassi-Cruz M, Guimaraes AG, Moises CCS, Freitas TC, Alencar NP, et al. Kcnj5 Somatic Mutation Is a Predictor of Hypertension Remission After Adrenalectomy for Unilateral Primary Aldosteronism. *J Clin Endocrinol Metab* (2019) 104:4695–702. doi: 10.1210/jc.2019-00531
29. Chang YY, Tsai CH, Peng SY, Chen CC, Chang BC, Lee CW, et al. Kcnj5 Somatic Mutations in Aldosterone-Producing Adenoma Are Associated With a Worse Baseline Status and Better Recovery of Left Ventricular Remodeling and Diastolic Function. *Hypertension* (2020) Hypertensionaha12015679. doi: 10.1161/HYPERTENSIONAHA.120.15679
30. Lv Z, Fan J, Xu J, Wu F, Huang Q, Guo M, et al. Value of (18)F-FDG PET/CT for Predicting EGFR Mutations and Positive ALK Expression in Patients With non-Small Cell Lung Cancer: A Retrospective Analysis of 849 Chinese Patients. *Eur J Nucl Med Mol Imaging* (2018) 45:735–50. doi: 10.1007/s00259-017-3885-z
31. Yip SS, Kim J, Coroller TP, Parmar C, Velazquez ER, Huynh E, et al. Associations Between Somatic Mutations and Metabolic Imaging Phenotypes in Non-Small Cell Lung Cancer. *J Nucl Med* (2017) 58:569–76. doi: 10.2967/jnumed.116.181826
32. Kawada K, Toda K, Nakamoto Y, Iwamoto M, Hatano E, Chen F, et al. Relationship Between 18f-Fdg PET/CT Scans and KRAS Mutations in Metastatic Colorectal Cancer. *J Nucl Med* (2015) 56:1322–7. doi: 10.2967/jnumed.115.160614
33. Nagarajah J, Ho AL, Tuttle RM, Weber WA, Grewal RK. Correlation of BRAFV600E Mutation and Glucose Metabolism in Thyroid Cancer Patients: An (1)(8)F-Fdg PET Study. *J Nucl Med* (2015) 56:662–7. doi: 10.2967/jnumed.114.150607
34. Chen R, Zhou X, Liu J, Huang G. Relationship Between 18f-Fdg PET/CT Findings and HER2 Expression in Gastric Cancer. *J Nucl Med* (2016) 57:1040–4. doi: 10.2967/jnumed.115.171165
35. Seccia TM, Mantero F, Letizia C, Kuppusamy M, Caroccia B, Barisa M, et al. Somatic Mutations in the KCNJ5 Gene Raise the Lateralization Index: Implications for the Diagnosis of Primary Aldosteronism by Adrenal Vein Sampling. *J Clin Endocrinol Metab* (2012) 97:E2307–13. doi: 10.1210/jc.2012-2342
36. Zheng FF, Zhu LM, Nie AF, Li XY, Lin JR, Zhang K, et al. Clinical Characteristics of Somatic Mutations in Chinese Patients With Aldosterone-Producing Adenoma. *Hypertension* (2015) 65:622–8. doi: 10.1161/HYPERTENSIONAHA.114.03346
37. Osswald A, Fischer E, Degenhart C, Quinkler M, Bidlingmaier M, Pallauf A, et al. Lack of Influence of Somatic Mutations on Steroid Gradients During Adrenal Vein Sampling in Aldosterone-Producing Adenoma Patients. *Eur J Endocrinol* (2013) 169:657–63. doi: 10.1530/EJE-13-0551
38. Lechner B, Lechner K, Heinrich D, Adolf C, Holler F, Schneider H, et al. Therapy OF Endocrine DISEASE: Medical Treatment of Primary Aldosteronism. *Eur J Endocrinol* (2019) 181:R147–53. doi: 10.1530/EJE-19-0215
39. Dekkers T, Prejbisz A, Kool LJS, Groenewoud H, Velema M, Spiering W, et al. Adrenal Vein Sampling Versus CT Scan to Determine Treatment in Primary Aldosteronism: An Outcome-Based Randomised Diagnostic Trial. *Lancet Diabetes Endocrinol* (2016) 4:739–46. doi: 10.1016/S2213-8587(16)30100-0
40. Parthasarathy HK, Menard J, White WB, Young WF Jr, Williams GH, Williams B, et al. A Double-Blind, Randomized Study Comparing the Antihypertensive Effect of Eplerenone and Spironolactone in Patients With Hypertension and Evidence of Primary Aldosteronism. *J Hypertens* (2011) 29:980–90. doi: 10.1097/HJH.0b013e3283455ca5
41. de Gasparo M, Joss U, Ramjoue HP, Whitebread SE, Haenni H, Schenkel L, et al. Three New Epoxy-Spirolactone Derivatives: Characterization In Vivo and In Vitro. *J Pharmacol Exp Ther* (1987) 240:650–6. doi: 10.1530/EJE-17-0990
42. Williams TA, Reincke M. Management OF Endocrine DISEASE: Diagnosis and Management of Primary Aldosteronism: The Endocrine Society Guideline 2016 Revisited. *Eur J Endocrinol* (2018) 179:R19–29. doi: 10.1530/EJE-17-0990
43. Eide IK, Torjesen PA, Drolsum A, Babovic A, Lilledahl NP. Low-Renin Status in Therapy-Resistant Hypertension: A Clue to Efficient Treatment. *J Hypertens* (2004) 22:2217–26. doi: 10.1097/00004872-200411000-00026
44. Lim PO, Young WF, MacDonald TM. A Review of the Medical Treatment of Primary Aldosteronism. *J Hypertens* (2001) 19:353–61. doi: 10.1097/00004872-200103000-00001

45. Dietz JD, Du S, Bolten CW, Payne MA, Xia C, Blinn JR, et al. A Number of Marketed Dihydropyridine Calcium Channel Blockers Have Mineralocorticoid Receptor Antagonist Activity. *Hypertension* (2008) 51:742–8. doi: 10.1161/HYPERTENSIONAHA.107.103580
46. Deinum J, Riksen NP, Lenders JW. Pharmacological Treatment of Aldosterone Excess. *Pharmacol Ther* (2015) 154:120–33. doi: 10.1016/j.pharmthera.2015.07.006
47. Tauber P, Penton D, Stindl J, Humberg E, Tegtmeier I, Sterner C, et al. Pharmacology and Pathophysiology of Mutated KCNJ5 Found in Adrenal Aldosterone-Producing Adenomas. *Endocrinology* (2014) 155:1353–62. doi: 10.1210/en.2013-1944
48. Scholl UI, Abriola L, Zhang C, Reimer EN, Plummer M, Kazmierczak BI, et al. Macrolides Selectively Inhibit Mutant KCNJ5 Potassium Channels That Cause Aldosterone-Producing Adenoma. *J Clin Invest* (2017) 127:2739–50. doi: 10.1172/JCI91733
49. Caroccia B, Prisco S, Seccia TM, Piazza M, Maiolino G, Rossi GP. Macrolides Blunt Aldosterone Biosynthesis: A Proof-of-Concept Study in KCNJ5 Mutated Adenoma Cells Ex Vivo. *Hypertension* (2017) 70:1238–42. doi: 10.1161/HYPERTENSIONAHA.117.10226
50. Williams TA, Monticone S, Mulatero P. KCNJ5 Mutations are the Most Frequent Genetic Alteration in Primary Aldosteronism. *Hypertension* (2015) 65:507–9. doi: 10.1161/HYPERTENSIONAHA.114.04636
51. Monticone S, Buffolo F, Tetti M, Veglio F, Pasini B, Mulatero P. Genetics IN Endocrinology: The Expanding Genetic Horizon of Primary Aldosteronism. *Eur J Endocrinol* (2018) 178:R101–11. doi: 10.1530/EJE-17-0946

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

ORIGINAL RESEARCH
published: 14 May 2021
doi: 10.3389/fendo.2021.672557

CMR-Verified Myocardial Fibrosis Is Associated With Subclinical Diastolic Dysfunction in Primary Aldosteronism Patients

*Fangli Zhou[1], Tao Wu[2], Wei Wang[1], Wei Cheng[2], Shuang Wan[1], Haoming Tian[1], Tao Chen[1], Jiayu Sun[2] and Yan Ren[1]**

[1] Adrenal Center, Department of Endocrinology and Metabolism, West China Hospital, Sichuan University, Chengdu, China, [2] Departments of Radiology, West China Hospital, Sichuan University, Chengdu, China

OPEN ACCESS

Edited by:
Vin-Cent Wu,
National Taiwan University, Taiwan

Reviewed by:
Zheng-Wei Chen,
National Taiwan University, Taiwan
Michael Nelson,
University of Texas at Arlington, United States
Luigi Petramala,
Sapienza University of Rome, Italy

***Correspondence:**
Yan Ren
renyan@scu.edu.cn

Specialty section:
This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Endocrinology

Received: *01 March 2021*
Accepted: *28 April 2021*
Published: *14 May 2021*

Citation:
Zhou F, Wu T, Wang W, Cheng W, Wan S, Tian H, Chen T, Sun J and Ren Y (2021) CMR-Verified Myocardial Fibrosis Is Associated With Subclinical Diastolic Dysfunction in Primary Aldosteronism Patients. Front. Endocrinol. 12:672557. doi: 10.3389/fendo.2021.672557

Objectives: The main cardiac features of primary aldosteronism (PA) are impaired left ventricular (LV) diastolic function, and some articles also reported more cardiac fibrosis in PA patients. However, the correlation between LV dysfunction and diffuse myocardial fibrosis in PA remains unknown.

Methods: We enrolled 84 PA patients and 28 essential hypertension (EH) patients in West China Hospital. Cardiac magnetic resonance imaging (CMR) contrast enhancement was arranged for all subjects. Postcontrast T1 time and left ventricular myocardial strains and strain rates were measured.

Results: 76 PA patients and 27 essential hypertension (EH) patients were included in the final analysis. Blood pressure, LV mass indexes, and LV ejection fractions were comparable in both groups, while the global circumferential peak diastolic strain rate (PDSR) was lower (0.9 ± 0.3 vs. 1.1 ± 0.4, $p < 0.01$) and the postcontrast T1 time was shorter (520 ± 38 vs. 538 ± 27, $p = 0.01$) in PA patients than those in EH patients. Postcontrast T1 time ($p = 0.01$) was independently related to global circumferential PDSR after adjusting for age and duration of hypertension in PA patients. Furthermore, plasma aldosterone concentration was negatively associated with postcontrast T1 time ($R = –0.253$, $p = 0.028$) in PA patients.

Conclusions: The global circumferential PDSR derived by CMR is decreased, and the diffuse myocardial fibrosis is increased in PA patients compared to those in blood pressure matched EH patients. The severity of cardiac diastolic dysfunction independently relates to the degree of diffuse myocardial fibrosis in PA patients, and the diffuse myocardial fibrosis may be caused by high PAC level.

Clinical Trial Registration: http://www.chictr.org.cn/listbycreater.asp, identifier ChiCTR2000031792.

Keywords: primary aldosteronism, essential hypertension, left ventricular function, the global circumferential PDSR, myocardial fibrosis, CMR

INTRODUCTION

Primary aldosteronism (PA), characterized by the overproduction of aldosterone that seems autonomous from renin (1), is the most common endocrine cause of hypertension and contributes more than 10% to the etiology of hypertension (2) and 29.1% to the etiology of resistant hypertension (3). In addition to high blood pressure, increased aldosterone, which has proinflammatory (4) and profibrotic effects (5) on myocardial tissues, is another risk factor for cardiovascular diseases. Previous clinical studies have shown that PA patients are prone to cardiovascular complications (6–9). PA patients revealed a significantly higher prevalence of coronary artery disease (adjusted OR, 1.9), nonfatal myocardial infarction (adjusted OR, 2.6), heart failure (adjusted OR, 2.9), and atrial fibrillation (adjusted OR, 5.0) than essential hypertension (EH) patients (6). Moreover, Reincke et al. (8) reported that cardiovascular mortality was the leading cause of death in PA (50%) and occurred less frequently in EH controls (34%).

It is well known that PA patients' major cardiac damage is impaired left ventricular function (10, 11), leading to poor prognosis in PA patients. The pathophysiological mechanism of left ventricular dysfunction in PA patients remains unclear. Studies have found that cardiac dysfunction results from fibrosis of the myocardium in many diseases, such as heart failure (12), hypertrophic cardiomyopathy (13), and diabetic patients (14). As to if myocardial fibrosis increases in PA patients, the answer is still controversial (15–19). Cardiac biopsy samples from four male PA patients exhibited 1.5-fold more fibrosis than those from EH patients (14% vs. 6%) (18). A late gadolinium enhancement study using cardiac magnetic imaging (CMR) proved that patients with PA exhibit more frequent diffuse myocardial fibrosis than healthy volunteers (16) and EH patients (17). Moreover, adrenalectomy was proven to reverse myocardial fibrosis in PA patients (19). However, Gretaas et al. (15) reported that increased myocardial fibrosis was not found and may not represent a common clinical problem in PA.

CMR offers a non-invasive, highly accurate assessment of cardiac function and geometry, and contrast-enhanced T1 mapping can provide evidence of diffuse myocardial fibrosis (14, 20). Post-contrast myocardial T1 time is inversely correlated with histologically defined interstitial fibrosis, so shorter postcontrast T1 time represents more interstitial fibrosis (21, 22). However, the correlation of left ventricle (LV) dysfunction and myocardial fibrosis in PA patients remains unknown. Thus, the aims of the prospective observational study were as follows: (1) to compare global postcontrast T1 time between PA patients and age-, sex-, body mass index-, blood pressure-, and hypertension duration-controlled EH subjects; and (2) if the PA patients own shorter postcontrast T1 time, we will try to explore the relationship between cardiac left ventricular function and myocardial fibrosis in PA patients.

MATERIALS AND METHODS

Study Population

From April 2018 to May 2019, 84 PA patients were recruited from the inpatient department of Endocrinology and Metabolism of West China Hospital. 28 EH patients were recruited from the medical examination center of West China Hospital. All the subjects were of Han ethnicity and between 22 and 78 years old. All the patients completed the initial screening test of the plasma aldosterone/renin ratio (ARR) for PA, plasma renin activity (PRA) <0.2 ng/ml/h was set as 0.2 for the calculation of the ARR to avoid inflation due to a very low denominator. According to the guidelines, before the initial screening, all the subjects had been on stable antihypertensive treatment with an α1-adrenergic receptor antagonist alone or verapamil sustained-release agent or hydralazine, and with normal serum potassium levels. The duration of stable antihypertensive treatment depended on the antihypertensive drugs the patients were taking. MR antagonists (e.g., spironolactone, eplerenone), potassium-sparing diuretics (e.g., amiloride, triamterene), and potassium-wasting diuretics (e.g., hydrochlorothiazide, furosemide) should be withdrawn for at least four weeks before ARR testing. Angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, renin inhibitors, dihydropyridine calcium channel antagonists, β-Adrenergic blockers, and central α-2 agonists (e.g., clonidine, α-methyldopa) should be withdrawn for at least two weeks before ARR testing. The PA patients were further diagnosed by confirmatory tests of saline infusion and/or captopril challenge according to current guideline (23). The exclusion criteria included the following: ① subjects with a history of congestive heart failure, chronic steroid therapy or chronic kidney disease (estimated glomerular filtration rate <60 ml/min); ② subjects with clinical indications of other secondary causes of hypertension except for PA, such as renal artery stenosis, pheochromocytoma, Cushing's syndrome, and hyperthyroidism.

This study was revised and confirmed by the Ethics Committee of West China Hospital. Before the study, written informed consent was obtained from each individual. Our study was registered in the Chinese clinical trial registry (ChiCTR2000031792).

Demographic Characteristics and Laboratory Determinations

Two trained staff members recorded the clinical characteristics of subjects with a standard questionnaire. Physical examination was performed on all the subjects, including body height, weight, waist circumference, and blood pressure. Body mass index (BMI) was computed as body weight in kilograms (kg) divided by height in meters squared (m2). The body surface areas were calculated as $0.0057 \times$ height (cm) $+ 0.0121 \times$ weight (kg) $+ 0.0882$ for males and $0.0073 \times$ height (cm) $+ 0.0127 \times$ weight (kg) $- 0.2106$ for females. All patients underwent 24-hour ambulatory blood pressure monitoring three days before the CMR scan.

After overnight fasting (≥8 h), venous blood samples were collected to measure plasma aldosterone concentration (PAC), PRA, and other biochemical parameters. Radioimmunoassay was used to measure PAC and PRA (Beijing North Institute of Biotechnology Co., St. Panjia Miao, Beijing). Serum potassium, serum sodium, total cholesterol, triglycerides, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, pro-brain natriuretic peptide (pro-BNP), creatine kinase MB,

troponin T, and myoglobin concentrations were measured for all patients using automated, standardized equipment by the Clinical Laboratory at West China Hospital.

Cardiovascular Magnetic Resonance Protocol

CMR was performed with a 3.0 T MRI imager (Trio Tim; Siemens Medical Solution, Erlangen, Germany) using an eight-channel phased-array surface coil and prospective electrocardiographic triggering. Patients assumed a supine position, and ECG gating and respiratory gating were used throughout the scan. The cine images of the short-axis covering the LV and the long-axis (two-, three- and four-chamber views) were obtained with a segmented balanced steady-state-free-precession sequence. The scanning parameters were TR/TE 3.4/1.3 ms, flip angle 50°, a field of view 320–340 mm, matrix size 256 × 144, and section thickness, 8 mm with no gap.

Postcontrast T1 maps were acquired using modified look-locker inversion recovery sequence (total acquisition is 17 heartbeats, TR/TE 2.9/1.12 ms, 8 mm thickness, in-plane spatial resolution 2.4 mm × 1.8 mm, matrix size 192 × 144, flip angle 35°, bandwidth 930 Hz/pixel, TI of first experiment 100 ms, TI increment 80 ms, parallel imaging 2), which was recommended by the Society for Cardiovascular Magnetic Resonance (24). Postcontrast T1 mapping was performed 15 min after the intravenous bolus injection administration of gadobenate dimeglumine (MultiHance; 0.5 mmol/ml; Bracco, Milan, Italy) at a dose of 0.15 mmol/kg body weight. About the gadolinium-based contrast doses, 0.1–0.2 mmol/Kg is recommended for T1 mapping in the European Association for Cardiovascular Imaging recommendation (24). Our research center used 0.15 mmol/kg to detect T1 mapping for many years. Some researches have been reported (25–27). **Figure 1** shows the contrast-enhanced T1 maps of two PA patients and two EH patients.

CMR Data Analysis

All imaging data of PA patients and EH subjects were uploaded to Argus software (Siemens Healthcare, Erlangen, Germany). Two experienced radiologists blinded to clinical data defined the end-diastole, end-systole, and delineated LV endocardial and epicardial borders (**Figure 2**). LV end-diastolic volume, LV end-systolic volume, LV mass, and LV ejection fraction were then calculated, and volumes and mass were indexed to body surface area. The software also automatically calculated the global myocardial strain parameters, including radial, circumferential, and longitudinal peak strain (PS), peak systolic strain rate (PSSR), and peak diastolic strain rate (PDSR), and the intra- and inter-observer variability were calculated. To evaluate diffuse myocardial fibrosis, post-processing software (Qmass 7.6; Medis, The Netherlands) was used to measure the value of myocardial native T1 time and postcontrast T1 time at the mid-layer myocardium of left ventricular basal, middle and apical segments. To attain blood T1, the regions of interest in the blood pool of the LV cavity in pre- and post-contrast T1-mapping images were drawn. ECV was calculated as: ECV= (1 − hematocrit) × (ΔR1myocardium − ΔR1blood), in which R1 = 1/T1.

Statistical Analysis

Normally distributed data were expressed as the mean (± SD) for continuous variables, and categorical variables were expressed as percentages. Skewed variables were logarithmically transformed before analysis and expressed as medians (interquartile ranges). The quantitative variables were compared using a t-test, while the qualitative variables were compared using the $\chi 2$ test.

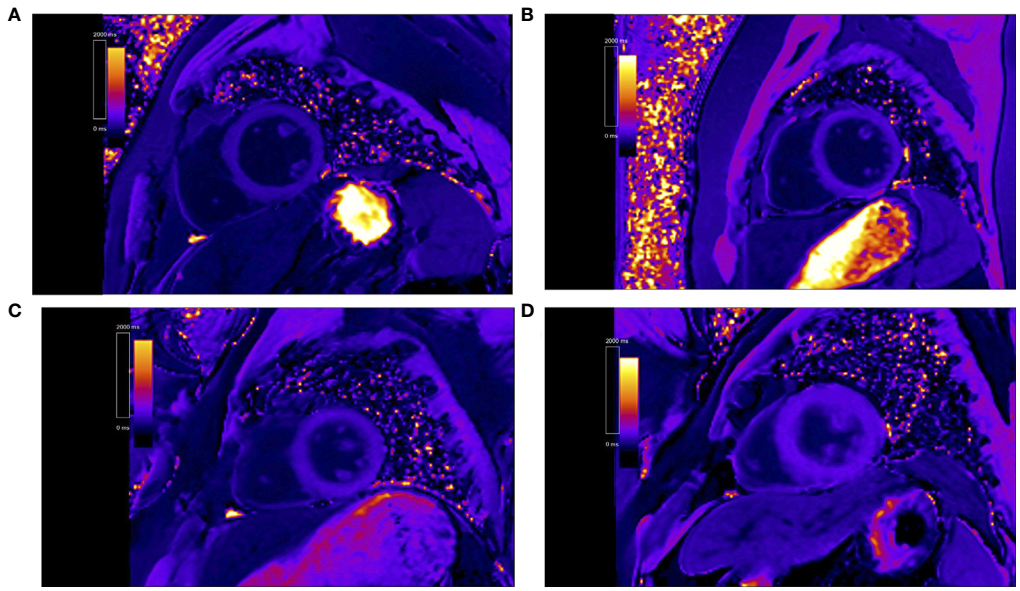

FIGURE 1 | Representative contrast-enhanced T1 maps of primary aldosteronism patients [**(A)** patient 1, post T1 of 451.1 ms. **(B)** patient 2, post T1 of 426.7 ms] and essential hypertension patients [**(C)** patient 3, post T1 of 568.4 ms. **(D)** patient 4, post T1 of 589.5 ms].

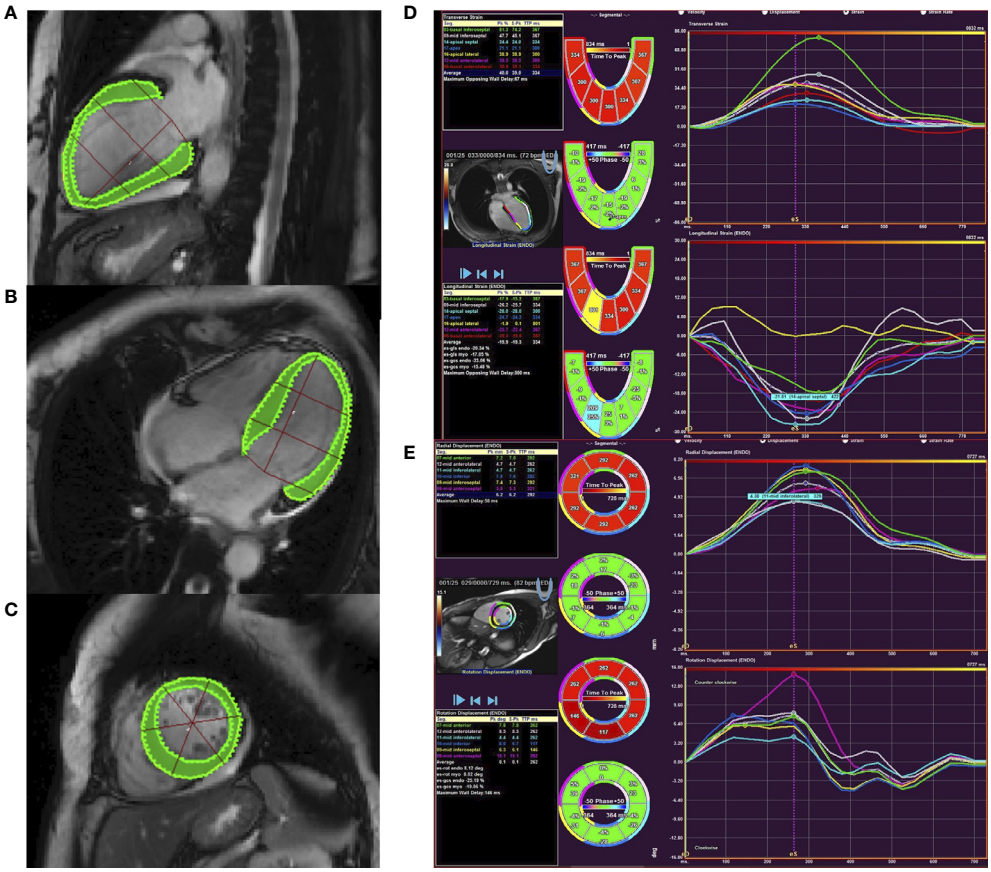

FIGURE 2 | Cardiac magnetic resonance feature tracking of the left ventricle in PA patient. Panels **(A–C)** showed countering for LV longitudinal **(A)**, circumferential **(B)**, and radial **(C)** strain and strain rate. Panel **(D)** showed tracking in the short-axis image with circumferential and radial strain curves. Panel **(E)** showed tracking from the four-chamber image to derive longitudinal strain.

Univariable analysis was performed to discover the correlation of the global circumferential PDSR with postcontrast T1 time and other risk factors. The association of the global circumferential PDSR with postcontrast T1 time in PA patients was analyzed with the stepwise multivariate analysis. Variables included in the regression model were those parameters p ≤0.1 in univariable analysis. P ≤0.05 was considered statistically significant. Pearson's analysis was used to test the relationship between PAC and postcontrast T1 time in PA patients. Analyses were performed with SPSS 17.0 (Chicago, IL) for Windows.

RESULTS

Characteristics and Metabolic Parameters of PA and EH Patients

All patients successfully underwent CMR except for nine patients (eight PA patients and one EH patient) who had severe arrhythmia, causing ECG synchronization failure during the study session. Therefore, 76 PA patients and 27 EH patients were included in the analysis. **Table 1** shows the demographic characteristics and laboratory data of the subjects. Age, duration of hypertension, waist circumference, BMI, systolic blood pressure, and diastolic blood pressure did not significantly differ between the EH and PA groups. PA patients had higher PAC levels, ARR, high-density lipoprotein cholesterol, serum sodium, and pro-BNP, and lower levels of PRA and serum potassium than those in EH patients.

CMR Data in PA and EH Patients

CMR results for LV mass, volumes, function, and contrast-enhanced T1 mapping are summarized in **Table 2**. No significant differences were found in LV mass index, LV end-diastolic volume index, LV end-systolic volume index, and LV ejection fraction between PA patients and EH patients. As to T1 mapping, PA patients possessed significantly shorter post-T1 time (520 ± 38 vs. 538 ± 27, p = 0.01), higher native T1 (1,228 ± 40 vs. 1,196 ± 44, p <0.01) and ECV (27 ± 4 vs. 25 ± 3, p = 0.02) than EH patients. Data on LV function are also presented in **Table 2**. The global circumferential PDSR (0.9 ± 0.3 vs. 1.1 ± 0.4, p <0.01) was decreased in PA patients than EH patients. Other LV function parameters, including the global radial, circumferential, and longitudinal PS, PSSR, and radial, longitudinal PDSR, did not show significant differences between the two groups.

TABLE 1 | Demographic characteristics and laboratory data of patients.

Variable	EH (n = 27)	PA (n = 76)	*P* value
Age, year	46 ± 15	48 ± 11	0.62
Women, number (% in total number)	15 (54)	54 (72)	0.16
Duration of hypertension, month	72 (96)	36 (114)	0.42
Waist, cm	89 ± 7	88 ± 10	0.80
BMI, kg/m^2	25 ± 3	25 ± 4	0.50
SBP, mmHg	146 ± 11	145 ± 17	0.86
DBP, mmHg	94 ± 12	93 ± 11	0.69
TG, mmol/L	1.5 (0.9)	1.3 (0.9)	0.09
TC, mmol/L	4.3 ± 1.0	4.5 ± 0.9	0.42
LDL-C, mmol/L	2.5 ± 0.8	2.7 ± 0.7	0.29
HDL-C, mmol/L	1.2 ± 0.4	1.4 ± 0.4	0.04
PAC, ng/dl	23 ± 9	32 ± 12	<0.01
PRA, ng/ml/h	3.0 (2.7)	0.2 (0.5)	<0.01
ARR, IU/L	9 (6)	102 (104)	<0.01
K, mmol/L	4.0 ± 0.4	3.5 ± 0.6	<0.01
Na, mmol/L	141.7 ± 1.6	142.8 ± 2.2	0.02
Pro-BNP, pg/ml	45 (38)	76 (93)	<0.01
CKMB, ng/ml	1.0 (0.5)	1.1 (0.7)	0.32
TPN-T, ng/L	6 (4)	7 (6)	0.64
Myo, ng/ml	28 (15)	29 (11)	0.54
Number of antihypertensive medications	2.2 ± 0.9	2.0 ± 1.1	0.35
Number (% in total number)			
CCB	24 (89)	59 (78)	0.20
ACEI	2 (7)	4 (5)	0.68
ARB	10 (37)	34 (45)	0.49
Diuretics	2 (7)	9 (12)	0.52
α-blocker	7 (26)	15 (20)	0.50
β-blpcker	11 (41)	19 (25)	0.12
MR antagonists	2 (7)	8 (11)	0.64

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PAC, plasma aldosterone concentration; PRA, plasma renin activity; ARR, aldosterone-to-renin ratio; Pro-BNP, pro-brain natriuretic peptide; CKMB, creatine kinase MB; TPN-T, troponin T; Myo, myoglobin; PTH, parathyroid hormone; CCB, calcium channel blockers; ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin II receptor blockers.

Factors Affecting Global Circumferential PDSR in PA Patients

To further identify the parameters affecting global circumferential PDSR in PA patients, we used univariate analysis to demonstrate the relationship between global circumferential PDSR and potentially related factors. Global circumferential PDSR statistically related to age (R = 0.39, p <0.01), duration of hypertension (R = −0.21, p = 0.08) and postcontrast T1 time (R = 0.30, p = 0.01).

Independent Determinants of LV Global PDSR in PA Patients

Parameters that are p ≤0.1 (**Table 3**) in univariable analysis were included in the multiple linear regression model. Specifically, age, hypertension duration, and postcontrast T1 time were brought into the multiple linear regression of global circumferential PDSR. **Table 4** showed the multiple linear regression results, which demonstrated that postcontrast T1 time was independently associated with the global circumferential PDSR (β = 0.257, p = 0.012, model R = 0.593) after adjusting for age and duration of hypertension.

TABLE 2 | Comparison of CMR results between EH and PA patients.

Variable	EH (n = 27)	PA (n = 76)	*P* value
LV massi, gm/m^2	58 ± 11	59 ± 16	0.87
LVEDVi, ml/m^2	79 ± 14	81 ± 15	0.44
LVESVi, ml/m^2	32 ± 9	34 ± 11	0.49
LVEF, %	62 ± 11	59 ± 8	0.26
Native T1 time, ms	1,196 ± 44	1,228 ± 40	<0.01
Postcontrast T1 time, ms	538 ± 27	520 ± 38	0.01
ECV, %	25 ± 3	27 ± 4	0.02
PS, %			
Radial	44 ± 7	43 ± 9	0.64
Circumferential	−15 ± 2	−15 ± 3	0.84
Longitudinal	−14 ± 2	−14 ± 3	0.80
PSSR, 1/s			
Radial	3.7 ± 0.8	3.4 ± 0.9	0.26
Circumferential	−1.5 ± 0.3	−1.5 ± 0.4	0.47
Longitudinal	−1.3 ± 0.3	−1.2 ± 0.3	0.19
PDSR, 1/s			
Radial	−2.7 ± 1.3	−2.2 ± 1.0	0.07
Circumferential	1.1 ± 0.4	0.9 ± 0.3	<0.01
Longitudinal	1.0 ± 0.3	1.0 ± 0.3	0.97

LVEDVi, Left ventricular end-diastolic volume index; LVESVi, left ventricular end-systolic volume index; LV massi, left ventricular mass indexed to body surface area; LVEF, left ventricular ejection fraction; PS, peak strain; PSSR, peak systolic strain rate; PDSR, peak diastolic strain rate.

TABLE 3 | Univariable linear regression analysis for global circumferential PDSR in PA patients.

Variable	Correlation Coefficient	*P* value
Age	0.39	<0.01
Gender	−0.14	0.22
Ln duration of hypertension	−0.21	0.08
BMI	0.11	0.37
SBP	0.05	0.68
DBP	−0.08	0.48
HDL-C	0.15	0.19
K	−0.17	0.15
Na	−0.05	0.68
PAC	0.07	0.58
Ln PRA	0.15	0.21
Ln ARR	−0.14	0.25
Ln Pro-BNP	−0.02	0.89
Native T1 time	−0.08	0.51
Postcontrast T1 time	0.30	0.01
ECV	0.04	0.71

*Abbreviations as in **Tables 1** and **2**.*

Postcontrast Myocardial T1 Time Negatively Related to PAC in PA Patients

As showed above, the global circumferential PDSR was lower in PA patients than EH patients but not statistically related to PAC. However, Pearson's analysis (**Figure 3**) showed that PAC was negatively related to postcontrast myocardial T1 time (R = −0.253, p = 0.028).

Postcontrast Myocardial T1 Time Negatively Related to PAC in PA Patients

Inter-observer variabilities of tissue tracking between two experienced radiologists were minimum, which were shown in **Table 5**. Correlation coefficient r were 0.749–0.957, p <0.001 for

TABLE 4 | Independent determinants of LV global PDSR in PA patients.

Variable	Unstardardized β	95% CI	*P* value
Age	0.994	0.619-1.369	<0.001
Ln duration of hypertension	–3.829	–6.371–1.287	0.004
Postcontrast T1 time	0.138	0.031–0.245	0.012

*Abbreviations as in **Table 2**.*

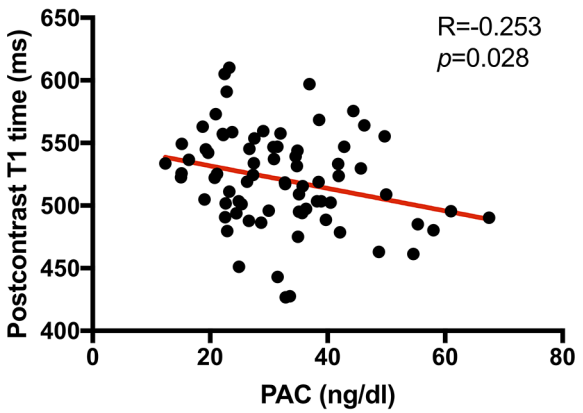

FIGURE 3 | Scatterplot showing the correlation between myocardial postcontrast T1 time (ms) and plasm aldosterone concentration (PAC, ng/dl) in PA patients. Thus, higher PAC was associated with a higher burden of interstitial myocardial collagen deposition (represented by a shorter postcontrast T1 time) in PA patients.

all. Intra-observer variabilities for tissue tracking were r = 0.816–0.955, p <0.001 for all. Myocardial tissue tracking was reproducible and reliable.

DISCUSSION

To the best of our knowledge, this is the first study to demonstrate the relationship between CMR-verified cardiac diastolic dysfunction and diffuse myocardial fibrosis assessed by postcontrast T1 time in PA patients. Although there was no significant difference was found in systolic cardiac functions between PA and EH patients, the global circumferential PDSR was lower, the native-T1 and ECV were higher, and the post-T1 time was shorter in PA patients than in blood pressure matched EH patients. In PA patients, postcontrast T1 time independently related to global circumferential PDSR after adjusting for confounding factors in the multivariate regression analysis. Additionally, the postcontrast T1 time is reversely related to PAC. Our study implies that diffuse myocardial fibrosis, which may be caused by elevated PAC level, affects left ventricular diastolic function in PA patients.

In the present study, we also found that the global circumferential PDSR is a more sensitive indicator for identifying the left ventricular dysfunction in PA patients than ejection fraction, PS, and PSSR. Catena found that PA patients had lower left ventricular diastolic function than EH patients but no systolic function differences with echocardiographic measurements (10), which was similar to our finding. However, Catena (10) and Muiesan (28) also reported greater left ventricular mass in PA patients than in EH patients, but we did not see a significant difference in left ventricular mass in this study. This may be due to our PA patients' short disease course, which was not long enough to cause a marked increase in left ventricular mass. The median of PA patients' hypertension duration was 3 years in this study. The PA patients' hypertension duration was 9.2 ± 8 years in Freel's study which also showed no significant difference in LV massi between PA and EH patients (17). In comparison, PA patients' mean hypertension duration was 15 years in Grytaas' study which reported higher LV massi in PA patients than healthy subjects (15). And the mechanisms of aldosterone-induced LV hypertrophy and aldosterone-induced LV fibrosis were different. The aldosterone promotes LV hypertrophy by activating mineralocorticoid receptors (MRs), extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and protein kinase C-α (PKC-α) (29). Basic and clinical studies found that aldosterone-induced cardiac fibrosis is associated with the activation of MRs and glucocorticoid receptors (GRs) through genomic and non-genomic pathways (30). Previous

TABLE 5 | Inter-and intra-observer variability of tissue tracking.

	Inter-observer r (n = 20)	95%CI	Intra-observer r (n = 20)	95%CI
PS (%)				
Radial	0.909	0.757–0.977	0.955	0.7896–0.980
Circumferential	0.874	0.781–0.946	0.823	0.617–0.933
Longitudinal	0.802	0.561–0.965	0.932	0.832–0.978
PSSR (1/s)				
Radial	0.941	0.873–0.983	0.940	0.843–0.982
Circumferential	0.939	0.784–0.995	0.887	0.756–0.954
Longitudinal	0.750	0.544–0.935	0.849	0.645–0.989
PDSR (1/s)				
Radial	0.889	0.779–0.961	0.937	0.858–0.977
Circumferential	0.957	0.835–0.990	0.816	0.639–0.918
Longitudinal	0.749	0.501–0.945	0.878	0.595–0.996

*Abbreviations as in **Table 2**.*

echocardiography studies showed a decreased global longitudinal strain in PA patients compared with EH (31, 32). It seems diverse to our finding. However, they found the lower longitudinal strain in PA patients, which showed not significantly different in our study, which may be related to our participant characteristics with a short duration of hypertension. On the other hand, the strain rate would be affected by the heart rate. Our study showed global circumferential PDSR was lower in the PA patients. Still, the difference of PDSR between PA and EH patients was not described in Chen's and Boulestreau's study, and additional research is needed to determine the change of PDSR in PA patients. Furthermore, we found a strong correlation ($\beta = 0.963$, $p < 0.001$) between the global longitudinal PDSR and averaged across all five layers and the inter-observer and intra-observer variabilities of global longitudinal PDSR were 0.749 and 0.878. The value of global longitudinal PDSR in our study was reliable.

Diastolic dysfunction plays a causative role in the process of cardiac failure (33). In one study that included 6,067 heart failure patients over 15 years, 47% of patients had a preserved ejection fraction, and the morbidity/mortality of preserved ejection fraction heart failure was comparable to that of reduced ejection fraction heart failure (34). In our study, the median duration of hypertension was only three years, but the global circumferential PDSR was decreased in PA patients, indicating that even patients with a short PA course have already developed subclinical cardiomyopathy. Therefore, it is essential to assess cardiac function, especially diastolic function, but not only to ejection fraction in PA patients with a course of more than three years to identify subclinical cardiomyopathy.

Several studies have demonstrated that PA patients have increased fibrosis than healthy volunteers (16, 35) and EH patients (17, 18, 35). However, Gretaas et al. (15) reported a reverse result in his research. In the present study, we found that diffuse myocardial fibrosis represented by postcontrast T1 time was increased in PA patients compared to blood pressure controlled EH patients. Abundant elementary experimental studies supported that aldosterone could promote myocardial fibrosis directly and indirectly. Aldosterone directly increases rat cardiac myofibroblast proliferation by activating Ki-RasA, the MAPK1/2 cascade (36), and insulin-like growth factor-I receptor (37). Aldosterone also has an indirectly profibrogenic function by upregulating inflammation in cardiomyocytes (38) and inhibiting antifibrotic factors, including BNP and ANP (39). Our study showed that the PAC was negatively related to postcontrast myocardial T1 time, which indicates that the diffuse myocardial fibrosis may be caused by high PAC level.

We speculate that the different salt consumption of included patients may explain the different myocardial fibrosis results in PA patients in different research. From a rat model of hyperaldosteronism, the myocardial fibrosis only developed in rats with high salt intake (40), and Ang II type 1 receptor might have some implications in this model (41). The habitual dietary sodium intake of the participants was not recorded in Gretaas' study (15). However, in Freel's (17) and our study, patients were recruited in the local population with consuming a salt-rich diet, and myocardial fibrosis was increased. Besides, the serum sodium of PA patients was higher than EH patients in our study.

CMR-verified diffuse myocardial fibrosis was proved to associate with diastolic dysfunction in heart failure (12), hypertrophic cardiomyopathy (13), and diabetic patients (14), but it remains unknown in PA. Su (16) reported increased myocardial fibrosis and left ventricular mass in PA patients compared with healthy controls, but they did not perform a correlation analysis between these two parameters. Our study proved an independent relationship between the global circumferential PDSR and CMR-verified diffuse myocardial fibrosis, suggesting that hyperaldosteronism in PA may contribute to cardiac dysfunction by promoting myocardial fibrosis.

Our study had some limitations. The myocardial biopsy is the gold standard for the diagnosis of cardiomyopathy. Considering its invasiveness and financial cost, we did not have histological evidence of myocardial fibrosis to validate the results of T1 mapping. Furthermore, because this was a single-center study, there is a need for multicenter, large-scale trials to confirm our findings.

CONCLUSIONS

The global circumferential PDSR derived by CMR is decreased, and the diffuse myocardial fibrosis is increased in PA patients compared to those in blood pressure matched EH patients. The severity of cardiac diastolic dysfunction independently relates to the degree of diffuse myocardial fibrosis in PA patients, and the diffuse myocardial fibrosis may be caused by high PAC level.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of West China Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

FZ designed research, acquisition, and analysis of data, writing the manuscript. TW, WW, and SW collection of data. WC analysis of the data. HT, TC, and JS designed research. YR designed the study, revising the manuscript, and final approval of the manuscript submitted. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by West China Hospital, Sichuan University (Grant Nos. ZYGD18022 and 2018HXFH009), Department of Science and Technology of Sichuan Province (Grant Nos. 2019YJ0040, 2020YFS0123 and 2021YFS0198) and Health Department of Sichuan Province (Grant No. 20PJ047).

ACKNOWLEDGMENTS

The authors thank the other investigators, staff, and participants of the ACWCH for their valuable contributions. We would also like to thank for Dr. Xiaoyue Zhou from Siemens Healthineers Ltd., Shanghai, China for the advices from technical perspective.

REFERENCES

1. Rossi GP, Bisogni V, Bacca AV, Belfiore A, Cesari M, Concistre A, et al. The 2020 Italian Society of Arterial Hypertension (SIIA) Practical Guidelines for the Management of Primary Aldosteronism. *Int J Cardiol Hypertens* (2020) 5:100029. doi: 10.1016/j.ijchy.2020.100029
2. Young WF, Calhoun DA, Lenders JWM, Stowasser M, Textor SC. Screening for Endocrine Hypertension: An Endocrine Society Scientific Statement. *Endocr Rev* (2017) 38(2):103–22. doi: 10.1210/er.2017-00054
3. Parasiliti-Caprino M, Lopez C, Prencipe N, Lucatello B, Settanni F, Giraudo G, et al. Prevalence of Primary Aldosteronism and Association With Cardiovascular Complications in Patients With Resistant and Refractory Hypertension. *J Hypertens* (2020) 38(9):1841–48. doi: 10.1097/HJH.0000000000002441
4. Buonafine M, Martinez-Martinez E, Amador C, Gravez B, Ibarrola J, Fernandez-Celis A, et al. Neutrophil Gelatinase-Associated Lipocalin From Immune Cells is Mandatory for Aldosterone-Induced Cardiac Remodeling and Inflammation. *J Mol Cell Cardiol* (2018) 115:32–8. doi: 10.1016/j.yjmcc.2017.12.011
5. Lijnen P, Petrov V. Induction of Cardiac Fibrosis by Aldosterone. *J Mol Cell Cardiol* (2000) 32(6):865–79. doi: 10.1006/jmcc.2000.1129
6. Savard S, Amar L, Plouin P-F, Steichen O. Cardiovascular Complications Associated With Primary Aldosteronism. *Hypertension* (2013) 62(2):331–36. doi: 10.1161/hypertensionaha.113.01060
7. Milliez P, Girerd X, Plouin P-F, Blacher J, Safar ME, Mourad J-J. Evidence for an Increased Rate of Cardiovascular Events in Patients With Primary Aldosteronism. *J Am Coll Cardiol* (2005) 45(8):1243–48. doi: 10.1016/j.jacc.2005.01.015
8. Reincke M, Fischer E, Gerum S, Merkle K, Schulz S, Pallauf A, et al. Observational Study Mortality in Treated Primary Aldosteronism. *Hypertension* (2012) 60(3):618–24. doi: 10.1161/hypertensionaha.112.197111
9. Seccia TM, Letizia C, Muiesan ML, Lerco S, Cesari M, Bisogni V, et al. Atrial Fibrillation as Presenting Sign of Primary Aldosteronism: Results of the Prospective Appraisal on the Prevalence of Primary Aldosteronism in Hypertensive (PAPPHY) Study. *J Hypertens* (2020) 38(2):332–39. doi: 10.1097/HJH.0000000000002250
10. Catena C, Colussi G, Lapenna R, Nadalini E, Chiuch A, Gianfagna P, et al. Long-Term Cardiac Effects of Adrenalectomy or Mineralocorticoid Antagonists in Patients With Primary Aldosteronism. *Hypertension* (2007) 50(5):911–18. doi: 10.1161/hypertensionaha.107.095448
11. Cesari M, Letizia C, Angeli P, Sciomer S, Rosi S, Rossi GP. Cardiac Remodeling in Patients With Primary and Secondary Aldosteronism. *Circulation: Cardiovasc Imaging* (2016) 9(6). doi: 10.1161/circimaging.116.004815
12. Su M-YM, Lin L-Y, Tseng Y-HE, Chang C-C, Wu C-K, Lin J-L, et al. CMR-Verified Diffuse Myocardial Fibrosis Is Associated With Diastolic Dysfunction In Hfpef. *JACC: Cardiovasc Imaging* (2014) 7(10):991–97. doi: 10.1016/j.jcmg.2014.04.022
13. Ellims AH, Iles LM, Ling LH, Hare JL, Kaye DM, Taylor AJ. Diffuse Myocardial Fibrosis in Hypertrophic Cardiomyopathy can be Identified by Cardiovascular Magnetic Resonance, and is Associated With Left Ventricular Diastolic Dysfunction. *J Cardiovasc Magn Reson* (2012) 14(1):76. doi: 10.1186/1532-429X-14-76
14. Ng ACT, Auger D, Delgado V, van Elderen SGC, Bertini M, Siebelink H-M, et al. Association Between Diffuse Myocardial Fibrosis by Cardiac Magnetic Resonance Contrast-Enhanced T1 Mapping and Subclinical Myocardial Dysfunction in Diabetic Patients. *Circulation: Cardiovasc Imaging* (2012) 5(1):51–9. doi: 10.1161/circimaging.111.965608
15. Grytaas MA, Sellevag K, Thordarson HB, Husebye ES, Lovas K, Larsen TH. Cardiac Magnetic Resonance Imaging of Myocardial Mass and Fibrosis in Primary Aldosteronism. *Endocr Conn* (2018) 7(3):413–24. doi: 10.1530/EC-18-0039
16. Su M-YM, Wu V-C, Yu H-Y, Lin Y-H, Kuo C-C, Liu K-L, et al. Contrast-Enhanced MRI Index of Diffuse Myocardial Fibrosis is Increased in Primary Aldosteronism. *J Magn Reson Imaging* (2012) 35(6):1349–55. doi: 10.1002/jmri.23592
17. Freel EM, Mark PB, Weir RAP, McQuarrie EP, Allan K, Dargie HJ, et al. Demonstration of Blood Pressure-Independent Noninfarct Myocardial Fibrosis in Primary Aldosteronism. *Circulation: Cardiovasc Imaging* (2012) 5(6):740–47. doi: 10.1161/circimaging.112.974576
18. Frustaci A, Letizia C, Verardo R, Grande C, Francone M, Sansone L, et al. Primary Aldosteronism-Associated Cardiomyopathy: Clinical-Pathologic Impact of Aldosterone Normalization. *Int J Cardiol* (2019) 292:141–47. doi: 10.1016/j.ijcard.2019.06.055
19. Lin YH, Wu XM, Lee HH, Lee JK, Liu YC, Chang HW, et al. Adrenalectomy Reverses Myocardial Fibrosis in Patients With Primary Aldosteronism. *J Hypertens* (2012) 30(8):1606–13. doi: 10.1097/HJH.0b013e3283550f93
20. Mascherbauer J, Marzluf BA, Tufaro C, Pfaffenberger S, Graf A, Wexberg P, et al. Cardiac Magnetic Resonance Postcontrast T1 Time is Associated With Outcome in Patients With Heart Failure and Preserved Ejection Fraction. *Circ Cardiovasc Imaging* (2013) 6(6):1056–65. doi: 10.1161/CIRCIMAGING.113.000633
21. Iles L, Pfluger H, Phrommintikul A, Cherayath J, Aksit P, Gupta SN, et al. Evaluation of Diffuse Myocardial Fibrosis in Heart Failure With Cardiac Magnetic Resonance Contrast-Enhanced T1 Mapping. *J Am Coll Cardiol* (2008) 52(19):1574–80. doi: 10.1016/j.jacc.2008.06.049
22. Sibley CT, Noureldin RA, Gai N, Nacif MS, Liu S, Turkbey EB, et al. T1 Mapping in Cardiomyopathy at Cardiac MR: Comparison With Endomyocardial Biopsy. *Radiology* (2012) 265(3):724–32. doi: 10.1148/radiol.12112721
23. Funder JW, Carey RM, Mantero F, Murad MH, Reincke M, Shibata H, et al. The Management of Primary Aldosteronism: Case Detection, Diagnosis, and Treatment: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab* (2016) 101(5):1889–916. doi: 10.1210/jc.2015-4061
24. Messroghli DR, Moon JC, Ferreira VM, Grosse-Wortmann L, He T, Kellman P, et al. Clinical Recommendations for Cardiovascular Magnetic Resonance Mapping of T1, T2, T2* and Extracellular Volume: A Consensus Statement by the Society for Cardiovascular Magnetic Resonance (SCMR) Endorsed by the European Association for Cardiovascular Imaging (EACVI). *J Cardiovasc Magn Reson* (2017) 19(1). doi: 10.1186/s12968-017-0389-8
25. Yu L, Sun J, Sun J, Li J, Dong Y, Zhou X, et al. Early Detection of Myocardial Involvement by T1 Mapping of Cardiac MRI in Idiopathic Inflammatory Myopathy. *J Magn Reson Imaging* (2018) 48(2):415–22. doi: 10.1002/jmri.25945
26. Wan K, Li W, Sun J, Xu Y, Wang J, Liu H, et al. Regional Amyloid Distribution and Impact on Mortality in Light-Chain Amyloidosis: A T1 Mapping Cardiac Magnetic Resonance Study. *Amyloid* (2019) 26(1):45–51. doi: 10.1080/13506129.2019.1578742
27. Wang J, Yang F, Liu W, Sun J, Han Y, Li D, et al. Radiomic Analysis of Native T1 Mapping Images Discriminates Between MYH7 and MYBPC3-Related Hypertrophic Cardiomyopathy. *J Magn Reson Imaging* (2020) 52(6):1714–21. doi: 10.1002/jmri.27209
28. Muiesan ML, Salvetti M, Paini A, Agabiti-Rosei C, Monteduro C, Galbassini G, et al. Inappropriate Left Ventricular Mass in Patients With Primary Aldosteronism. *Hypertension* (2008) 52(3):529–34. doi: 10.1161/hypertensionaha.108.114140

29. Okoshi MP, Yan X, Okoshi K, Nakayama M, Schuldt AJ, O'Connell TD, et al. Aldosterone Directly Stimulates Cardiac Myocyte Hypertrophy. *J Card Fail* (2004) 10(6):511–8. doi: 10.1016/j.cardfail.2004.03.002
30. Tsai CH, Pan CT, Chang YY, Chen ZW, Wu VC, Hung CS, et al. Left Ventricular Remodeling and Dysfunction in Primary Aldosteronism. *J Hum Hypertens* (2021) 35(2):131–47. doi: 10.1038/s41371-020-00426-y
31. Boulestreau R, Cremer A, Delarche N, Gosse P. [Alteration of Left Ventricular Longitudinal Systolic Function in 2D-Strain in Primary Aldosteronism: A New Target Organ Damage Marker]. *Ann Cardiol Angeiol (Paris)* (2018) 67 (5):315–20. doi: 10.1016/j.ancard.2018.08.007
32. Chen ZW, Huang KC, Lee JK, Lin LC, Chen CW, Chang YY, et al. Aldosterone Induces Left Ventricular Subclinical Systolic Dysfunction: A Strain Imaging Study. *J Hypertens* (2018) 36(2):353–60. doi: 10.1097/HJH.0000000000001534
33. Obokata M, Reddy YNV, Borlaug BA. Diastolic Dysfunction and Heart Failure With Preserved Ejection Fraction: Understanding Mechanisms by Using Noninvasive Methods. *JACC Cardiovasc Imaging* (2020) 13(1 Pt 2):245–57. doi: 10.1016/j.jcmg.2018.12.034
34. Owan TE, Hodge DO, Herges RM, Jacobsen SJ, Roger VL, Redfield MM. Trends in Prevalence and Outcome of Heart Failure With Preserved Ejection Fraction. *N Engl J Med* (2006) 355(3):251–9. doi: 10.1056/NEJMoa052256
35. Redheuil A, Blanchard A, Pereira H, Raissouni Z, Lorthioir A, Soulat G, et al. Aldosterone-Related Myocardial Extracellular Matrix Expansion in Hypertension in Humans: A Proof-of-Concept Study by Cardiac Magnetic Resonance. *JACC Cardiovasc Imaging* (2020) 13(10):2149–59. doi: 10.1016/j.jcmg.2020.06.026
36. Stockand JD, Meszaros JG. Aldosterone Stimulates Proliferation of Cardiac Fibroblasts by Activating Ki-Rasa and MAPK1/2 Signaling. *Am J Physiol Heart Circ Physiol* (2003) 284(1):H176–84. doi: 10.1152/ajpheart.00421.2002
37. Bunda S, Wang Y, Mitts TF, Liu P, Arab S, Arabkhari M, et al. Aldosterone Stimulates Elastogenesis in Cardiac Fibroblasts Via Mineralocorticoid Receptor-Independent Action Involving the Consecutive Activation of Gα13, C-Src, the Insulin-Like Growth Factor-I Receptor, and Phosphatidylinositol 3-Kinase/Akt. *J Biol Chem* (2009) 284(24):16633–47. doi: 10.1074/jbc.M109.008748
38. Latouche C, Sainte-Marie Y, Steenman M, Castro Chaves P, Naray-Fejes-Toth A, Fejes-Toth G, et al. Molecular Signature of Mineralocorticoid Receptor Signaling in Cardiomyocytes: From Cultured Cells to Mouse Heart. *Endocrinology* (2010) 151(9):4467–76. doi: 10.1210/en.2010-0237
39. Azibani F, Fazal L, Chatziantoniou C, Samuel JL, Delcayre C. Aldosterone Mediates Cardiac Fibrosis in the Setting of Hypertension. *Curr Hypertens Rep* (2013) 15(4):395–400. doi: 10.1007/s11906-013-0354-3
40. Brilla CG, Weber KT. Reactive and Reparative Myocardial Fibrosis in Arterial Hypertension in the Rat. *Cardiovasc Res* (1992) 26(7):671–7. doi: 10.1093/cvr/26.7.671
41. Katayama IA, Pereira RC, Dopona EP, Shimizu MH, Furukawa LN, Oliveira IB, et al. High-Salt Intake Induces Cardiomyocyte Hypertrophy in Rats in Response to Local Angiotensin II Type 1 Receptor Activation. *J Nutr* (2014) 144(10):1571–8. doi: 10.3945/jn.114.192054

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

SYSTEMATIC REVIEW
published: 17 May 2021
doi: 10.3389/fendo.2021.644260

Composite Cardiovascular Outcomes in Patients With Primary Aldosteronism Undergoing Medical Versus Surgical Treatment: A Meta-Analysis

Wei-Chieh Huang[1,2], *Ying-Ying Chen*[3,4], *Yen-Hung Lin*[4,5] *and Jeff S. Chueh*[6*]

[1] Division of Cardiology, Department of Internal Medicine, Taipei Veterans General Hospital, Taipei, Taiwan, [2] School of Medicine, National Yang-Ming Chiao-Tung University, Taipei, Taiwan, [3] Division of Nephrology, Department of Internal Medicine, MacKay Memorial Hospital, Taipei, Taiwan, [4] Graduate Institute of Clinical Medicine, College of Medicine, National Taiwan University Hospital, Taipei, Taiwan, [5] Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan, [6] Glickman Urological and Kidney Institute, and Cleveland Clinic Lerner College of Medicine, Cleveland Clinic, Cleveland, OH, United States

OPEN ACCESS

Edited by:
Qiang Wei,
Sichuan University, China

Reviewed by:
Silvia Monticone,
University of Turin, Italy
Leonardo Sechi,
University of Udine, Italy
Yoshiyu Takeda,
Kanazawa University, Japan

***Correspondence:**
Jeff S. Chueh
jeffchueh@gmail.com

Specialty section:
This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Endocrinology

Received: *20 December 2020*
Accepted: *12 April 2021*
Published: *17 May 2021*

Citation:
Huang W-C, Chen Y-Y, Lin Y-H and Chueh JS (2021) Composite Cardiovascular Outcomes in Patients With Primary Aldosteronism Undergoing Medical Versus Surgical Treatment: A Meta-Analysis. Front. Endocrinol. 12:644260. doi: 10.3389/fendo.2021.644260

Background: Superior outcomes after surgical treatment over medical treatment for primary aldosteronism (PA) has been reported in small-scale clinical studies, but no solid conclusion has been drawn as results of large randomized trials are lacking.

Methods: We performed a search of PubMed, MEDLINE, Embase and Cochrane Library for randomized or observational studies that investigated cardiovascular outcomes in patients with PA undergoing medical *versus* surgical treatment. Meta-analyses of both composite and individual outcomes were conducted. Risks of bias of the included studies were assessed with Risk Of Bias In Non-randomized Studies of Interventions (ROBINS-I) checklist. Trial sequential analysis (TSA) was performed to control the risk of random errors and assess whether the results in our meta-analysis were conclusive.

Results: A total of 12 studies, including a total of 6148 PA patients, were included in the meta-analysis. The results of meta-analyses demonstrated lower incidence of composite cardiovascular outcomes among PA patients who underwent surgical treatment over medical treatment (odds ratio (OR): 0.49). Surgical treatment also led to less incidence of persistence of hypertension (OR of non-cure hypertension: 0.31). Fewer major cardiovascular events and mortality events were observed (OR: 0.60) after surgical treatment. TSA result showed that the required information size was 2151 and the cumulative Z curve crossed the futility boundary and reached the required information size.

Conclusion: Superior performance of surgical treatment over medical treatment is confirmed with meta-analyses in terms of lower incidences of composite cardiovascular outcomes and non-cure of hypertension. Hence, adrenalectomy could now be concluded as the treatment of choice for lateralized PA.

Keywords: primary aldosteronism, adrenalectomy, mineralocorticoid receptor antagonists, surgical treatment, medical treatment, ROBINS-I

INTRODUCTION

Primary aldosteronism (PA) was first described in 1955 as hypertension, hypokalemia and overproduction of aldosterone (1). Once thought to be a rare cause of hypertension, PA is now recognized as the most frequent form of secondary hypertension (2) and affects about 5% to 10% of patients with high blood pressure (3). Many studies have shown that PA cases account for around 5% of the general hypertensive population (3), and 11% of hypertension patients are referred to specialized centers (2). Far from being a benign form of hypertension, PA is associated with higher risks of cardiovascular, renal, and metabolic sequelae, including left ventricular hypertrophy, myocardial infarction, atrial fibrillation, stroke, microalbuminuria, osteoporosis, and metabolic syndrome (4–8).

The two most common subtypes of PA are lateralized PA (including aldosterone-producing adenoma (APA), (multiple) aldosterone-producing micronodule(s) (mAPM), or rarely unilateral hyperplasia) and idiopathic hyperaldosteronism (IHA) (9). Targeted treatment with either adrenalectomy (surgery) and mineralocorticoid receptor antagonist (MRA) are the two common comparative, well-documented treatments to improve the outcomes of lateralized PA patients (10, 11). Although adrenalectomy is currently considered as the standard treatment in lateralized PA patients (9, 12, 13), some patients might have residual hypertension and still need anti-hypertensive medications after surgery. The current practice guideline recommends adrenalectomy for lateralized aldosterone excess (9, 12), whereas bilateral disease is treated using MRA (9, 14). However, there are still some patients with lateralized disease who do not undergo surgical intervention. Additionally, the reported discordance rate between a lateralizing adrenal venous sampling (AVS) test and a localizing adrenal CT scan could be as high as 45% (15, 16), which may lead to uncertainty of the final diagnosis and hence management with medical treatment. For these reasons, the relative efficacy of medical *versus* surgical therapy for lateralized PA should be evaluated more clearly.

Previous studies showed similar cardiovascular (10), blood pressure, and hypokalemia (17) outcomes between surgical or medical treatment. However, other studies found adrenalectomy associated with better cure rate of hypertension and hypokalemia (18), improved quality of life (19), as well as lower incidence of cardiovascular adverse outcomes (20). Moreover, one recent study suggested that surgical intervention is superior to medical therapy for lateralized PA due to excess cortisol co-existing in PA patients (21). Therefore, we performed this meta-analysis to elucidate the efficacy between surgical *versus* medical therapy for patients with PA.

MATERIALS AND METHODS

Search Strategy and Selection Criteria

We searched PubMed, MEDLINE, Embase, and Cochrane Library to identify all eligible studies using terms associated with PA. Searches were limited to human studies and patients aged over 18 years old. In order to enhance the number of studies included for screening, broad searching strategies were applied with no language restriction and any relevant reports published after 1985 were included. The key terms used were "hyperaldosteronism," "hyperaldosertonaemia," "aldoseteronism," "aldosteronaemia," "adrenalectomy (-ies)," "surgery," "surgical," "resection," "mineralocorticoid receptor antagonists," and "medical treatment." Searches were done up to Feb 28, 2021.

The searched articles were first evaluated at the title or abstract level and consensus between two independent investigators (WC Huang, YY Chen) or comments from a third reviewer (J Chueh) were sought for any disagreements identified. If the searched articles were potentially relevant, full articles were further retrieved and evaluated as complete reports according to the selection criteria. Studies including prospective study and retrospective studies were selected if the setting of the studies was to evaluate the outcomes (either hypertension-related or clinical events) of PA patients comparing the performance of medical and surgical treatments. Exclusion criteria were non-human setting, duplicate reporting, studies without relevant outcome data, absence of a control group, which referred to the studies presenting the outcomes of either medical or surgical treatments only, or different settings on the intervention and control arms. For example, studies using patients with essential hypertension (EH) as the control group to demonstrate the superior performance of PA patients after either medical or surgical treatment were excluded.

Data Extraction and Quality Assessment

Two investigators (WC Huang and YY Chen) independently extracted data using a standardized data extraction spreadsheet, with discrepancies resolved by consensus or through a third reviewer (J Chueh). Extracted data included author, journal, year of publication, location of the study group, study design, details of the treatments received by PA patients in both groups, length of follow-up, numbers of participants enrolled in the studies and those who reached the desired end-points of the specific studies. For studies reported in more than one publication, data from the most complete publication was extracted.

The quality of the included studies was evaluated independently by two investigators (WC Huang and YY Chen) using the Risk Of Bias In Non-randomized Studies of Interventions (ROBINS-I) checklist (22), scoring each study for the following 7 domains: "Confounding", "Selection", "Classification of intervention", "Deviation from intervention", "Missing data", "Measurement of outcomes" and "Selection of repeated result." Domains were scored as "No information" (0), "Low" (1—low risk of bias), "Moderate" (2—moderate risk of bias), "Serious" (3—serious risk of bias) and "Critical" (4—critical risk of bias). A study was categorized as high quality if most of the domains were judged to be at low risk of bias.

The pre-defined review protocol was registered at the PROSPERO international prospective register of systematic reviews (http://www.crd.york.ac.uk/PROSPERO, registration number CRD42019119175). The protocol for this trial and

supporting CONSORT checklist are available as supporting information; see Checklist S1 and Protocol S1.

Outcomes of Interests

Efficacy outcomes of interest included: (1) Persistence of hypertension and (2) incidence of major adverse cardiovascular events (MACEs; the composite occurrence of myocardial infarction, stroke, coronary revascularization, or hospitalization because of heart failure) or all-cause mortality. The 'composite outcome' [defined as the existence of either (1) or (2)] was considered to overcome the challenges caused by diverse clinical parameters reported in different studies. We considered the presence of either type of outcomes as the incidence of unwanted outcomes after treatments, and the composite outcome was first analyzed to demonstrate the overall effect of the various treatments, and then the treatment efficacy on the individual components was also reported.

Statistical Analysis

Data were extracted with the use of a standardized data form and any discrepancies were resolved by consensus. In order to compare the differences on the incidence of events between the 2 treatment groups, pooled odds ratios (ORs) with corresponding 95% CI using the Mantel-Haenszel random-effects models was constructed with the aid of RevMan 5.3 (The Cochran Collaboration, The Nordic Cochrane Centre, Copenhagen, Denmark). The assigned weights in the random-effects model considered the variances of both within-studies and the between-studies.

The heterogeneity between the studies was evaluated using the Cochran Q test and the null hypothesis of statistical homogeneity was rejected if p values found were less than 0.10. The extent of heterogeneity was further categorized based on the value of I^2: mild (less than 30%), moderate (30–50%) and substantial (>50%). Graphical inspection on generated funnel plots was applied to detect publication bias.

Trial Sequential Analysis

Trial sequential analysis (TSA) was performed with the tool developed by the Copenhagen Trial Unit, Centre for Clinical Intervention Research, Denmark. In order to see the different temporal traceries or clinical outcome of current targeted treatments, e.g. adrenalectomy or MRA, we applied TSA in enrolled 12 studies to control the risk of random errors and simultaneously minimize the chance of having type I errors (5%) due to random error from the included studies with small sample size, potential bias and low methodological quality, together with the influence of the inclusion of studies with comparatively large sample sizes. We used a random effects model to construct the cumulative Z curve and used an anticipated relative risk reduction (RRR) of 15.0% with a power of 90% to calculate the required information size to detect or reject an intervention effect. The incidence in control arm was adjusted to 37.5% in this study. The conventional nonsuperiority boundaries were calculated assuming significance levels of .05 and .05, and a power of 90%. The a-spending boundaries were also calculated using significance levels of .01 and .05 and the O'Brien-Fleming multiple testing procedure (23). When the cumulative Z curve crossed the trial sequential monitoring boundary or entered the futility area, a sufficient level of evidence for accepting or rejecting the anticipated intervention effect may have been reached, and no further studies were needed. If the Z curve did not cross any of the boundaries, and the required information size had not been reached, evidence to reach a conclusion was insufficient, and more studies would be required (24). We used TSA version 0.9.5.5 (reviewed in November 2016) b software was used for these analyses the cumulative effect of randomized trials on mortality.

RESULTS

Included Studies

Our initial literature search yielded a total of 372 articles from the selected databases and hand-searching references through the applied searching strategy and they were further evaluated for eligibility at title or abstract level (**Figure 1**). After the removal of duplicated and irrelevant records, 59 articles with full-text articles were further reviewed. Among them, 32 did not show relevant data, while other 15 articles reported outcomes non-uniformly with regard to the target treatments. Finally, 12 studies (6, 10, 18, 20, 25–32) were included in this meta-analysis (**Figure 1**), resulting in a total of 6148 PA patients who received either surgical (35.98%) or medical treatment (64.02%). The descriptive summary for each included study was recapitulated in **Table 1**. Types of studies included prospective studies (n = 6) and retrospective studies (n = 6). Moreover, our previous study had a heavy weight in this meta-analysis as even the analysis of the outcomes to either surgical or medical treatment was carried out at the sub-group analysis level, its large sample size gave the advantage to maintain study quality, although a separate EH database was used as a control group which underwent standardized PA screening evaluation to exclude the possibility of subclinical PA (30).

Quality Assessment

The result of ROBINS-I tool analysis showed that the overall risk of bias of the majority of the included studies was from low (1) to moderate (2) (**Table 2**). Two of the included retrospective studies failed to report the handling methods of missing data (18, 30), which might introduce bias, hence their overall risks of the bias were relatively higher. Due to the restriction of small sample size of the included clinical studies, most of the included studies showed limitations on adjusting the outcome measures with potential confounding variables (domain 1), and in two of the included studies serious (25, 30) (3) to critical (4) bias in this domain was further pointed out. In addition, the risk of bias due to selection of reported results (domain 7) among the included studies reached moderate level.

Trial Sequential Analysis

In a TSA on the composite negative outcomes including both non-cure of hypertension and incidence of adverse cardiovascular events and all-cause mortality, random-effects

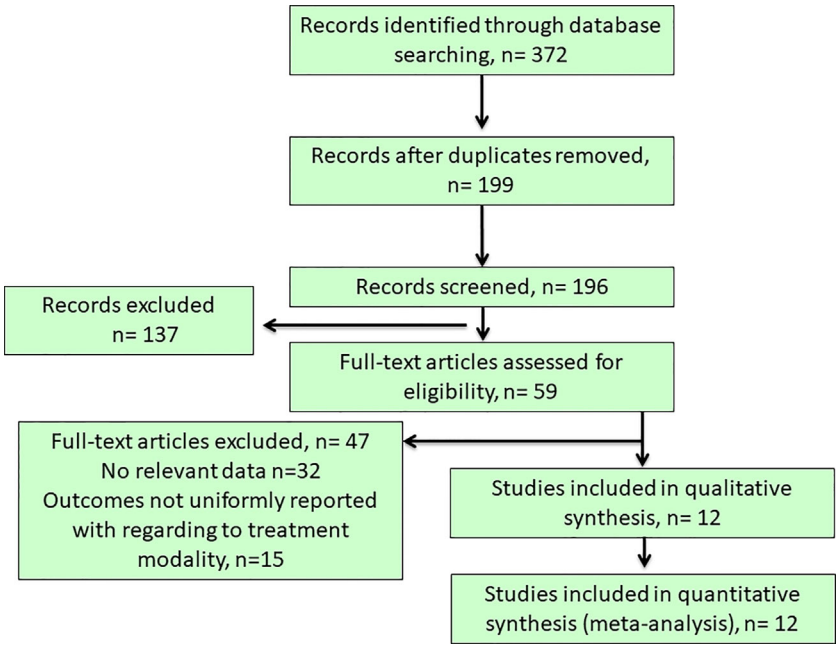

FIGURE 1 | Flow chart of literature search and study selection. Our initial literature search yielded a total of 372 articles from the selected databases and after the removal of duplicated and irrelevant records, 59 articles with full-text articles were further reviewed. Among them, 32 did not show relevant data, while other 15 articles reported outcomes non-uniformly with regard to the target treatments. Finally, 12 studies was included in this study.

TABLE 1 | Characteristics of the trials included in the meta-analysis.

Reference	Study nature	Relevant outcomes	Mean duration of follow-up (months)	Number of patients	
				Surgical	Medical
Bernini et al. (20)	Prospective clinical trial	Hypertension-related	Surgical: 31.5 Medical: 32.1	19	41
Catena et al. (10)	Prospective clinical trial	Hypertension-related	76.8	24	30
Catena et al. (27)	Prospective clinical trial	Composite cardiovascular end-points	60.0 (selected)	24	30
Giacchetti et al. (29)	Prospective clinical trial	Hypertension-related	34.4	25	36
Kline et al. (18)	Retrospective study	Hypertension-related	Surgical: 6.5 Medical 13.4	38	39
Miyake et al. (25)	Retrospective study	Hypertension-related	60.0 (max)	755	800
Mulatero et al. (6)	Retrospective study	Composite cardiovascular end-points	144.0 (median)	57	213
Park et al. (31)	Retrospective study	Hypertension-related	Surgical: 45.6 Medical: 55.2	206	63
Rossi et al. (28)	Prospective clinical trial	Hypertension-related	36.0 (median)	110	70
Wu et al. (30)	Retrospective study	Composite cardiovascular end-points or all-cause mortality	69.0	846	2516
Zacharieva et al. (26)	Prospective clinical trial	Hypertension-related	Surgical: 3.0 Medical: 3.0-6.0	22	30
Puar et al. (32)	Retrospective study	Composite cardiovascular end-points	68.4	86	68

model (DL) was applied in the TSA for all 12 included trials. TSA performed using a significance level of 0.05 and considering that homogeneity of results was stable showed that around 2151 patients would be needed to reach a stopping boundary of superiority. However, the Z-curve was parallel to superior boundary, but crossed the neutrality boundary including all trials. In our TSA, we included trials of patients, which yielded enough information size to conclude that the current evidence reached preliminary conclusion for supporting the superior outcome of surgical treatment over medical treatment (**Figure 2**).

Primary Outcome: Incidence of Outcomes

Among the 12 included studies, 8 of them reported the persistence of hypertension as primary outcome of the studies, while the other 4 studies reported the occurrence of MACEs or all-cause mortality as the primary outcome of the studies. Superior performance of surgical treatment over medical

TABLE 2 | Range of overall assessment by study and bias domains.

	Domain 1: confounding	Domain 2: selection	Domain 3: classification of intervention	Domain 4: deviation from interventions	Domain 5: missing data	Domain 6: measurement of outcomes	Domain 7: selection of reported result	ROBINS-I overall
Bernini et al. (20)	2-3	1	1	1-2	1	2-3	2	1-2 Low – Moderate
Catena et al. (10)	3	1	1	1-2	1	2-3	2	1-2 Low – Moderate
Catena et al. (27)	2	1	1	1-2	1	2-3	2-3	1-2 Low – Moderate
Giacchetti et al. (29)	3	1	1	1-2	1	2-3	2	1-2 Low – Moderate
Kline et al. (18)	3-4	1	1	1-2	2	2-3	2	2-3 Moderate – Serious
Miyake et al. (25)	2	1	1	1-2	3-4	2-3	2	2-3 Moderate – Serious
Mulatero et al. (6)	2-3	1	1	1-2	1	2-3	3	3 Serious
Park et al. (31)	2	1	1	1-2	1	2-3	2-3	1-2 Low – Moderate
Rossi et al. (28)	3-4	1	1	1-2	1	2-3	2	2 Moderate
Wu et al. (30)	2	1	1	1-2	3-4	2-3	2-3	3 Serious
Zacharieva et al. (26)	3-4	1	1	1-2	1	2-3	2	2 Moderate
Puar et al. (32)	2	1	1	1-2	1	2-3	2	1-2 Low – Moderate

Risk of bias assessment: 0 No information; 1 Low; 2 Moderate; 3 Serious; 4 Critical

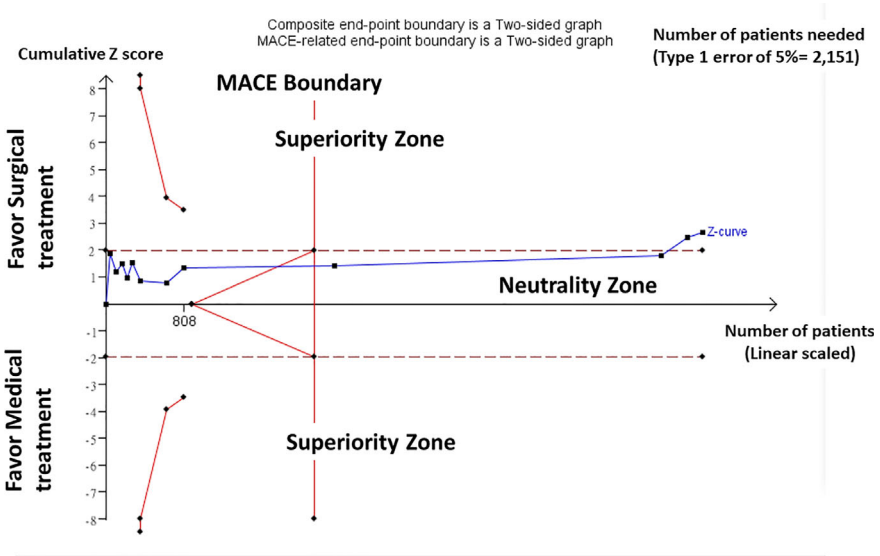

FIGURE 2 | Trial Sequential Analysis (TSA). Result of TSA showed homogeneity of results was stable and around 2151 patients would be needed to reach a stopping boundary of superiority. The Z-curve was parallel to superior boundary, but crossed the neutrality boundary including all trials. Trials of patients yielded enough information size to conclude that the current evidence reached preliminary conclusion for supporting the superior outcome of surgical treatment over medical treatment.

treatment was reported in 4 included studies, in regard to cure of hypertension and event-free of MACEs or all-cause mortality. Conversely, two included studies reported superior performance of medical treatment for PA patients over surgical treatment, in term of lower incidence of persistence of hypertension. The remaining 6 studies failed to give a definite conclusion on whether the medical or the surgical treatment was better for PA patients. Overall, when the composite outcomes were considered with the use of random effects model, the significance of better outcome in adrenalectomy (surgery) group was observed with the odds ratio [(OR) (OR: 0.49 (95% CI: 0.30-0.80, p = 0.005] (**Figure 3**). However, there was a significant heterogeneity among the enrolled studies [I (2) = 82%, p < 0.00001]. In the random effect models of meta-analysis on the composite outcomes, symmetry in the funnel plots was observed and this implied low publication bias among the included studies, but outliers also existed due to the diversity on the scales of the included studies (**Figure S1** and **Supplemental File**).

When the cure of hypertension after targeted treatment was considered, superior performance of the adrenalectomy was demonstrated in the random effects model (OR: 0.31, 95% CI: 0.11 – 0.85, p=0.020) (**Figure S2** and **Supplemental File**). For significant clinical events including both mortality or MACEs, surgical treatment led to lower incidence of outcomes when compared with that of medical treatment (OR: 0.60, 95% CI: 0.36 – 1.01, p = 0.05) (**Figure S3** and **Supplemental File**).

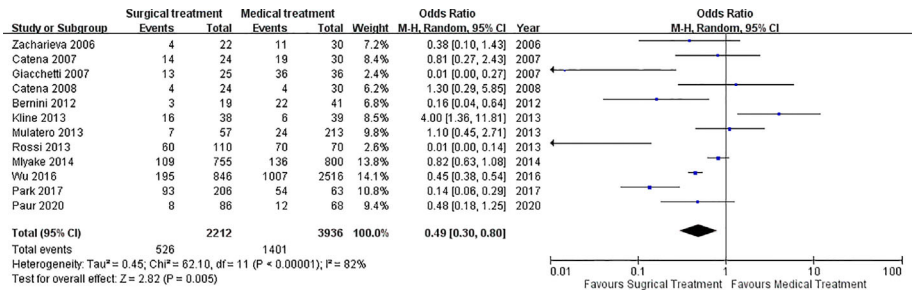

FIGURE 3 | Forest plot of the OR of primary outcome. Forest plot of the OR of primary outcome (composite outcomes) in patients with primary aldosteronism under medical treatment or surgical treatment. Central squares of each horizontal line represent the OR for each study. Horizontal lines indicate the range of the 95% CI and the vertical line indicates an OR of 1.0 (which indicates no differences in the odds ratio between medical treatment or surgical treatment). OR, odds ratio.

DISCUSSION

Main Findings

In this meta-analysis of 12 studies that included 6148 patients with PA, both composite and individual outcomes after surgical *versus* medical treatment have been reviewed systematically. Among 8 out of these 12 included studies were related to cure of hypertension of the enrolled PA patients at follow-up period after targeted treatments, and the integrated result suggested that adrenalectomy has a high possibility of achieving cure of hypertension. This result echoed with the findings of a retrospective study with large sample sizes (25). In addition, our finding suggested the significance of superior outcomes of the adrenalectomy in the meta-analysis of composite outcomes, including MACEs, all-cause mortality and persistence of hypertension among the PA patients and, hence, adrenalectomy might be considered in the treatment of choice for uPA.

In the Primary Aldosteronism Surgical Outcome (PASO) study (33), which included relevant data of 705 PA patients who underwent adrenalectomy, the prevalence rate of complete ("normal blood pressure without the aid of antihypertensive medication") and partial ("the same blood pressure with less antihypertensive medication or a reduction in blood pressure with either the same amount or less antihypertensive medication") clinical success was summed up as high as 84.2%, together with 93.9% biochemical success, which refers to "correction of hypokalemia and normalization of aldosterone to renin ratio (ARR)". In this meta-analysis, the percentage of PA patients with hypertension cured after surgical treatment was higher than that with medical treatment (73.98% vs 68.08%). This remarkable finding suggests that adrenalectomy is an effective treatment for lateralized PA patients. In addition, a systematic review on comparing the outcomes of PA patients after surgical *versus* medical treatment also reported reduced usage of antihypertensive medications, improved quality of life and potential lower all-cause mortality after surgical treatment (34). Additionally, a number of large case series have confirmed that surgical treatment (unilateral adrenalectomy) of lateralized PA is safe with low morbidity. No major differences in cure rate between various surgical techniques were seen, but some authors have identified predictive characteristics for cure (35–37). Reported complication rates of adrenalectomy ranged between 2 and 10%, with the highest rate occurred in an early series (38).

Long-term treatment with the MRA, including both spironolactone and/or eplerenone, was reported to have 5 – 8% remission rate of PA, this additional beneficial effect made the medical treatment an alternative of surgical treatment, especially for those patients with bilateral PA (12). However, based on our findings in this meta-analysis, the efficacy of the medical treatment for lateralized PA patients was not comparable to that of surgery. One of the potential causes is that the mineralocorticoid receptors, with high bonding affinity with both mineralocorticoids and glucocorticoids, were reported to be involved in the maturation of pre-adipocytes to adipocytes (34), which could be associated with the higher prevalence of hyperglycemia among PA patients over patients with EH (39). Since there is no clear evidence that MRA could cause better control on both glucose and lipid levels in PA patients, the efficacy of medical treatment becomes questionable in regard to its influence on metabolic syndrome. Meanwhile, many case reports and case series reported excess glucocorticoid secretion among PA patients (40, 41). MRA treatment may induce a further increase in aldosterone and subsequently trigger a vicious cycle that leads to an insufficient effect of prescribed MRA on blocking MRs which are activated by high plasma aldosterone levels. Arlt et al. further reported that excess glucocorticoid secretion could be associated with several risk factors which might cause adverse metabolic events among PA patients, and additional glucocorticoid-opposing treatment should be provided for PA patients in addition to MRA treatment, in order to reduce the potential risks of having cardiovascular diseases and other comorbidities (21). Although, there were many studies supporting that glycometabolic abnormalities when excess aldosterone is not removed with use of MRAs, Catena et al. (42) stated that in primary aldosteronism, normal sensitivity to insulin is rapidly restored after treatment with either adrenalectomy or aldosterone antagonists, whereas no further change of glucose metabolism parameters occurs during the long-term follow-up. In recent studies, adrenalectomy in patients with APA was found to decrease glucocorticoid secretion, reverse osteoporosis, attenuate adverse metabolic risks and improve the quality of life (13, 21, 43, 44); all of which could be attributed to decreased glucocorticoid levels after adrenalectomy, in addition to correction of mineralocorticoid excess. Such finding further adds insights on our results, and it could at least in part explain the relatively inferior performance of medical treatment over adrenalectomy, although the adoption of medical treatment itself is not the direct cause of higher incidence of cardiovascular comorbidities.

Patients treated medically need more anti-hypertensive drugs (34), and require a longer follow-up and more clinical visits at specialized referral center than those treated surgically. Similarly, the incidence of cardiovascular events during the follow-up period was greater in IHA patients than that in EH controls, although there were no differences in their BP levels (6). It implicates that aldosterone may play a detrimental role independent of its effect on blood pressure. MRA may also induce an increase in aldosterone and subsequently trigger a vicious cycle that leads to an insufficient effect of prescribed MRA on blocking MRs activated by high plasma aldosterone levels. There are also concerns about MRA prescription, such as medication compliance with inadequate dose, probably partially due to frequent occurrences of dose-dependent side effects, especially gynecomastia or dysmenorrhea, and a subsequent decrease in efficacy (30).

Strengths and Limitations

Recommendations on treatment are hampered by the lack of systematic reporting of clearly defined outcomes and randomized clinical trials (RCTs). The present data supported surgical resection of lateralized PA disease, which can be performed with low morbidity. Some researchers (34) have attempted to answer this question before, but only qualitative comparisons between these targeted treatments were made due to diverse and heterogeneous clinical outcomes reported from clinical studies and lack of RCTs on this research topic, which is considered as critical evidence for constructing such a meta-

analysis. In this meta-analysis, composite outcome approach expanded the scope of the included studies successfully and achieved a sufficiently large sample size to embrace the diversity on the settings of clinical studies and overcome the limitations of small-scale clinical studies. With sufficient sample size, a more concrete conclusion on the superior performance of surgical over medical treatment for lateralized PA patients could be drawn with good-quality quantitative evidence.

In addition, ROBINS-I was adopted in this meta-analysis to assess the risks of bias among the included studies. ROBINS-I was developed by Sterne et al. and it was designed to evaluate the potential risk of bias in the non-randomized studies of the effects of interventions (22). Through answering the signaling questions included in the checklist, the risks of bias for 7 domains of each included study were first reviewed and subsequently, overall risk of bias of the entire study was then concluded. The adoption of ROBINS-I in this study provided a comprehensive evaluation on the quality of the included studies and revealed that most of the included clinical trials were with low to moderate risks of bias while the retrospective studies possessed higher risks of bias.

We acknowledge several limitations of our study. First, there was no adjustment on the results of the meta-analyses according to other influential variables such as patient demographics. Furthermore, lack of the dosage of MRA, types of medication or targets in the medical treatment might further influence the outcomes of PA patients. Second, the drawback of composite outcome approach introduced large heterogeneity within the meta-analysis, especially since there was significant difference on sample sizes of the included studies. Furthermore, the pathology of APA and IHA differ, and these differences lead to diversity in clinical outcomes after the same treatment (45). Because surgery is not an option for patients with IHA (46), our findings are clinically relevant only to patients with APA/lateralized PA. Especially, it is important to accurately diagnosis aldosterone-producing microadenoma in which has better rate of cure of hypertension than macroadenoma and is only detected by AVS (47). Finally, due to so small enrolled patients and the different temporal traceries or clinical outcome of current targeted treatments, e.g. adrenalectomy or MRA, we performed trial sequential analysis (TSA) and in our TSA, was obvious that the included trials over the years (from 2006 to 2020) showed certain extent of consensus on demonstrating constant performance of adrenalectomy under the adjustment of TSA.

CONCLUSION

In conclusion, superior performance of surgical treatment over medical treatment for patients with lateralized PA has been confirmed with this meta-analysis approach on both composite and individual clinical cardiovascular outcomes. Adrenalectomy should be concluded as the treatment of choice for the management of lateralized PA patients, especially in regard to attenuating unwanted cardiovascular events.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

W-CH contributed to study design, data collection, statistical analysis, data interpretation, and drafting of the manuscript. W-CH and Y-YC contributed to study design, data collection, data interpretation, and critical review of the manuscript. Y-YC and Y-HL contributed to statistical analysis and critical review of the manuscript. JC contributed to data interpretation and critical review of the manuscript. JC is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

We thank Dr. Yvonne Chueh for English editing.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.644260/full#supplementary-material

REFERENCES

1. Conn JW. Presidential Address. I. Painting Background. II. Primary Aldosteronism, a New Clinical Syndrome. *J Lab Clin Med* (1955) 45(1):3–17. doi: 10.5555/uri:pii:0022214355900482
2. Rossi GP, Bernini G, Caliumi C, et al. A Prospective Study of the Prevalence of Primary Aldosteronism in 1,125 Hypertensive Patients. *J Am Coll Cardiol* (2006) 48(11):2293–300. doi: 10.1016/j.jacc.2006.07.059
3. Monticone S, Burrello J, Tizzani D, et al. Prevalence and Clinical Manifestations of Primary Aldosteronism Encountered in Primary Care Practice. *J Am Coll Cardiol* (2017) 69(14):1811–20. doi: 10.1016/j.jacc.2017.01.052
4. Young WF. Primary Aldosteronism: Renaissance of a Syndrome. *Clin Endocrinol (Oxf)* (2007) 66(5):607–18. doi: 10.1111/j.1365-2265.2007.02775.x
5. Pitkala KH, Strandberg TE, Tilvis RS. Inappropriate Drug Prescribing in Home-Dwelling, Elderly Patients: A Population-Based Survey. *Arch Intern Med* (2002) 162(15):1707–12. doi: 10.1001/archinte.162.15.1707
6. Mulatero P, Monticone S, Bertello C, et al. Long-Term Cardio- and Cerebrovascular Events in Patients With Primary Aldosteronism. *J Clin Endocrinol Metab* (2013) 98(12):4826–33. doi: 10.1210/jc.2013-2805
7. Savard S, Amar L, Plouin PF, Steichen O. Cardiovascular Complications Associated With Primary Aldosteronism: A Controlled Cross-Sectional Study. *Hypertension* (2013) 62(2):331–6. doi: 10.1161/HYPERTENSIONAHA.113.01060
8. Rossi GP, Sechi LA, Giacchetti G, Ronconi V, Strazzullo P, Funder JW. Primary Aldosteronism: Cardiovascular, Renal and Metabolic Implications. *Trends Endocrinol Metab* (2008) 19(3):88–90. doi: 10.1016/j.tem.2008.01.006

9. Lenders JWM, Eisenhofer G, Reincke M. Subtyping of Patients With Primary Aldosteronism: An Update. *Horm Metab Res* (2017) 49(12):922–8. doi: 10.1055/s-0043-122602
10. Catena C, Colussi G, Lapenna R, et al. Long-Term Cardiac Effects of Adrenalectomy or Mineralocorticoid Antagonists in Patients With Primary Aldosteronism. *Hypertension* (2007) 50(5):911–8. doi: 10.1161/HYPERTENSIONAHA.107.095448
11. Catena C, Colussi G, Di Fabio A, et al. Mineralocorticoid Antagonists Treatment Versus Surgery in Primary Aldosteronism. *Horm Metab Res* (2010) 42(6):440–5. doi: 10.1055/s-0029-1246185
12. Funder JW, Carey RM, Mantero F, et al. The Management of Primary Aldosteronism: Case Detection, Diagnosis, and Treatment: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab* (2016) 101 (5):1889–916. doi: 10.1210/jc.2015-4061
13. Wu VC, Hu YH, Er LK, et al. Case Detection and Diagnosis of Primary Aldosteronism - The Consensus of Taiwan Society of Aldosteronism. *J Formos Med Assoc* (2017) 116(12):993–1005. doi: 10.1016/j.jfma.2017.06.004
14. Huang KH, Yu CC, Hu YH, et al. Targeted Treatment of Primary Aldosteronism - The Consensus of Taiwan Society of Aldosteronism. *J Formos Med Assoc* (2019) 118(1 Pt 1):72–82. doi: 10.1016/j.jfma.2018.01.006
15. Mathur A, Kemp CD, Dutta U, et al. Consequences of Adrenal Venous Sampling in Primary Hyperaldosteronism and Predictors of Unilateral Adrenal Disease. *J Am Coll Surg* (2010) 211(3):384–90. doi: 10.1016/j.jamcollsurg.2010.05.006
16. Magill SB, Raff H, Shaker JL, et al. Comparison of Adrenal Vein Sampling and Computed Tomography in the Differentiation of Primary Aldosteronism. *J Clin Endocrinol Metab* (2001) 86(3):1066–71. doi: 10.1210/jc.86.3.1066
17. Reincke M, Rump LC, Quinkler M, et al. Risk Factors Associated With a Low Glomerular Filtration Rate in Primary Aldosteronism. *J Clin Endocrinol Metab* (2009) 94(3):869–75. doi: 10.1210/jc.2008-1851
18. Kline GA, Pasieka JL, Harvey A, So B, Dias VC. Medical or Surgical Therapy for Primary Aldosteronism: Post-Treatment Follow-Up as a Surrogate Measure of Comparative Outcomes. *Ann Surg Oncol* (2013) 20(7):2274–8. doi: 10.1245/s10434-013-2871-3
19. Velema M, Dekkers T, Hermus A, et al. Quality of Life in Primary Aldosteronism: A Comparative Effectiveness Study of Adrenalectomy and Medical Treatment. *J Clin Endocrinol Metab* (2018) 103(1):16–24. doi: 10.1210/jc.2017-01442
20. Bernini G, Bacca A, Carli V, et al. Cardiovascular Changes in Patients With Primary Aldosteronism After Surgical or Medical Treatment. *J Endocrinol Invest* (2012) 35(3):274–80.
21. Arlt W, Lang K, Sitch AJ, et al. Steroid Metabolome Analysis Reveals Prevalent Glucocorticoid Excess in Primary Aldosteronism. *JCI Insight* (2017) 2(8). doi: 10.1172/jci.insight.93136
22. Sterne JA, Hernan MA, Reeves BC, et al. Robins-I: A Tool for Assessing Risk of Bias in non-Randomised Studies of Interventions. *BMJ* (2016) 355:i4919. doi: 10.1136/bmj.i4919
23. O'Brien PC, Fleming TR. A Multiple Testing Procedure for Clinical Trials. *Biometrics* (1979) 35(3):549–56. doi: 10.2307/2530245
24. Wetterslev J, Jakobsen JC, Gluud C. Trial Sequential Analysis in Systematic Reviews With Meta-Analysis. *BMC Med Res Methodol* (2017) 17(1):39. doi: 10.1186/s12874-017-0315-7
25. Miyake Y, Tanaka K, Nishikawa T, et al. Prognosis of Primary Aldosteronism in Japan: Results From a Nationwide Epidemiological Study. *Endocr J* (2014) 61(1):35–40. doi: 10.1507/endocrj.EJ13-0353
26. Zacharieva S, Orbetzova M, Elenkova A, et al. Diurnal Blood Pressure Pattern in Patients With Primary Aldosteronism. *J Endocrinol Invest* (2006) 29(1):26–31. doi: 10.1007/BF03349173
27. Catena C, Colussi G, Nadalini E, et al. Cardiovascular Outcomes in Patients With Primary Aldosteronism After Treatment. *Arch Intern Med* (2008) 168 (1):80–5. doi: 10.1001/archinternmed.2007.33
28. Rossi GP, Cesari M, Cuspidi C, et al. Long-Term Control of Arterial Hypertension and Regression of Left Ventricular Hypertrophy With Treatment of Primary Aldosteronism. *Hypertension* (2013) 62(1):62–9. doi: 10.1161/HYPERTENSIONAHA.113.01316
29. Giacchetti G, Ronconi V, Turchi F, et al. Aldosterone as a Key Mediator of the Cardiometabolic Syndrome in Primary Aldosteronism: An Observational Study. *J Hypertens* (2007) 25(1):177–86. doi: 10.1097/HJH.0b013e3280108e6f
30. Wu VC, Wang SM, Chang CH, et al. Long Term Outcome of Aldosteronism After Target Treatments. *Sci Rep* (2016) 6:32103. doi: 10.1038/srep32103
31. Park KS, Kim JH, Yang YS, et al. Outcomes Analysis of Surgical and Medical Treatments for Patients With Primary Aldosteronism. *Endocr J* (2017) 64 (6):623–32. doi: 10.1507/endocrj.EJ16-0530
32. Puar TH, Loh LM, Loh WJ, et al. Outcomes in Unilateral Primary Aldosteronism After Surgical or Medical Therapy. *Clin Endocrinol (Oxf)* (2021) 94(2):158–67. doi: 10.1111/cen.14351
33. Williams TA, Lenders JWM, Mulatero P, et al. 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):689–99. doi: 10.1111/cen.14351
34. Muth A, Ragnarsson O, Johannsson G, Wangberg B. Systematic Review of Surgery and Outcomes in Patients With Primary Aldosteronism. *Br J Surg* (2015) 102(4):307–17. doi: 10.1002/bjs.9744
35. Walz MK, Gwosdz R, Levin SL, et al. Retroperitoneoscopic Adrenalectomy in Conn's Syndrome Caused by Adrenal Adenomas or Nodular Hyperplasia. *World J Surg* (2008) 32(5):847–53. doi: 10.1007/s00268-008-9513-0
36. Zarnegar R, Lee J, Brunaud L, et al. Good Blood Pressure Control on Antihypertensives, Not Only Response to Spironolactone, Predicts Improved Outcome After Adrenalectomy for Aldosteronoma. *Surgery* (2007) 142(6):921–9. discussion -9. doi: 10.1016/j.surg.2007.09.001
37. Zhang X, Zhu Z, Xu T, Shen Z. Factors Affecting Complete Hypertension Cure After Adrenalectomy for Aldosterone-Producing Adenoma: Outcomes in a Large Series. *Urol Int* (2013) 90(4):430–4. doi: 10.1159/000347028
38. Meria P, Kempf BF, Hermieu JF, Plouin PF, Duclos JM. Laparoscopic Management of Primary Hyperaldosteronism: Clinical Experience With 212 Cases. *J Urol* (2003) 169(1):32–5. doi: 10.1016/S0022-5347(05)64028-1
39. Fallo F, Veglio F, Bertello C, et al. Prevalence and Characteristics of the Metabolic Syndrome in Primary Aldosteronism. *J Clin Endocrinol Metab* (2006) 91(2):454–9. doi: 10.1210/jc.2005-1733
40. Yoon V, Heyliger A, Maekawa T, et al. Benign Adrenal Adenomas Secreting Excess Mineralocorticoids and Glucocorticoids. *Endocrinol Diabetes Metab Case Rep* (2013) 2013:130042. doi: 10.1530/EDM-13-0042
41. Fallo F, Bertello C, Tizzani D, et al. Concurrent Primary Aldosteronism and Subclinical Cortisol Hypersecretion: A Prospective Study. *J Hypertens* (2011) 29(9):1773–7. doi: 10.1097/HJH.0b013e32834937f3
42. Catena C, Lapenna R, Baroselli S, et al. Insulin Sensitivity in Patients With Primary Aldosteronism: A Follow-Up Study. *J Clin Endocrinol Metab* (2006) 91(9):3457–63. doi: 10.1210/jc.2006-0736
43. Wu VC, Chueh SJ, Chen L, et al. Risk of New-Onset Diabetes Mellitus in Primary Aldosteronism: A Population Study Over 5 Years. *J Hypertens* (2017) 35(8):1698–708. doi: 10.1097/HJH.0000000000001361
44. Sukor N, Kogovsek C, Gordon RD, Robson D, Stowasser M. Improved Quality of Life, Blood Pressure, and Biochemical Status Following Laparoscopic Adrenalectomy for Unilateral Primary Aldosteronism. *J Clin Endocrinol Metab* (2010) 95(3):1360–4. doi: 10.1210/jc.2009-1763
45. Young WFJr. Primary Aldosteronism: Management Issues. *Ann N Y Acad Sci* (2002) 970:61–76. doi: 10.1111/j.1749-6632.2002.tb04413.x
46. Chao CT, Wu VC, Kuo CC, et al. Diagnosis and Management of Primary Aldosteronism: An Updated Review. *Ann Med* (2013) 45(4):375–83. doi: 10.3109/07853890.2013.785234
47. Omura M, Sasano H, Saito J, Yamaguchi K, Kakuta Y, Nishikawa T. Clinical Characteristics of Aldosterone-Producing Microadenoma, Macroadenoma, and Idiopathic Hyperaldosteronism in 93 Patients With Primary Aldosteronism. *Hypertens Res* (2006) 29(11):883–9. doi: 10.1291/hypres.29.883

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

SYSTEMATIC REVIEW
published: 24 May 2021
doi: 10.3389/fendo.2021.646933

New-Onset Atrial Fibrillation in Patients With Primary Aldosteronism Receiving Different Treatment Strategies: Systematic Review and Pooled Analysis of Three Studies

*Cheng-Hsuan Tsai[1,2], Ya-Li Chen[2], Chien-Ting Pan[3], Yen-Tin Lin[4], Po-Chin Lee[5], Yu-Wei Chiu[6,7], Che-Wei Liao[8], Zheng-Wei Chen[3], Chin-Chen Chang[5], Yi-Yao Chang[6], Chi-Sheng Hung[2] and Yen-Hung Lin[2]**

[1] Department of Internal Medicine, National Taiwan University Hospital Jin-Shan Branch, New Taipei City, Taiwan, [2] Department of Internal Medicine, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan, [3] Department of Internal Medicine, National Taiwan University Hospital Yun-Lin Branch, Yun-Lin, Taiwan, [4] Department of Internal Medicine, Taoyuan General Hospital, Taoyuan, Taiwan, [5] Department of Medical Imaging, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan, [6] Cardiology Division of Cardiovascular Medical Center, Far Eastern Memorial Hospital, New Taipei City, Taiwan, [7] Department of Computer Science and Engineering, Yuan Ze University, Taoyuan City, Taiwan, [8] Department of Medicine, National Taiwan University Cancer Center, Taipei, Taiwan

OPEN ACCESS

Edited by:
Qiang Wei,
Sichuan University, China

Reviewed by:
Heng-Chih Pan,
Keelung Chang Gung Memorial Hospital, Taiwan
Jiguang Wang,
Shanghai Jiao Tong University, China

***Correspondence:**
Yen-Hung Lin
austinr34@gmail.com

Specialty section:
This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Endocrinology

Received: 28 December 2020
Accepted: 03 May 2021
Published: 24 May 2021

Citation:
Tsai C-H, Chen Y-L, Pan C-T, Lin Y-T, Lee P-C, Chiu Y-W, Liao C-W, Chen Z-W, Chang C-C, Chang Y-Y, Hung C-S and Lin Y-H (2021) New-Onset Atrial Fibrillation in Patients With Primary Aldosteronism Receiving Different Treatment Strategies: Systematic Review and Pooled Analysis of Three Studies. Front. Endocrinol. 12:646933. doi: 10.3389/fendo.2021.646933

Background: Primary aldosteronism (PA) is a common cause of secondary hypertension and associated with higher incidence of new-onset atrial fibrillation (NOAF). However, the effects of surgical or medical therapies on preventing NOAF in PA patents remain unclear. The aim of this meta-analysis study was to assess the risk of NOAF among PA patients receiving mineralocorticoid receptor antagonist (MRA) treatment, PA patients receiving adrenalectomy, and patients with essential hypertension.

Methods: We performed the meta-analysis of the randomized or observational studies that investigated the incidence rate of NOAF in PA patients receiving MRA treatment versus PA patients receiving adrenalectomy from database inception until December 01, 2020 which were identified from PubMed, Embase, and Cochrane Library.

Results: A total of 172 related studies were reviewed, of which three fulfilled the inclusion criteria, including a total of 2,705 PA patients. The results of meta-analysis demonstrated a higher incidence of NOAF among the PA patients receiving MRA treatment compared to the PA patients receiving adrenalectomy (pooled odds ratio [OR]: 2.83, 95% confidence interval [CI]: 1.76–4.57 in the random effects model, I^2 = 0%). The pooled OR for the PA patients receiving MRA treatment compared to the patients with essential hypertension was 1.91 (95% CI: 1.11–3.28). The pooled OR for the PA patients receiving adrenalectomy compared to the patients with essential hypertension was 0.70 (95% CI: 0.28–1.79).

Conclusion: Compared to the essential hypertension patients and the PA patients receiving adrenalectomy, the patients with PA receiving MRA treatment had a higher risk of NOAF.

Systematic Review Registration: https://www.crd.york.ac.uk/prospero/, identifier CRD42021222022.

Keywords: hyperaldosteronism, primary aldosteronism, adrenalectomy, mineralocorticoid receptor antagonist, atrial fibrillation

INTRODUCTION

Primary aldosteronism (PA) is a state of autonomous aldosterone secretion which is unresponsive to renin regulation, resulting in hypertension and electrolyte imbalance (1). The prevalence of PA has been reported to be 4.3 to 9.5% in all patients with hypertension and 17 to 23% in PA patients with resistant hypertension (2, 3). Compared to essential hypertension, PA is associated with higher risks of cardiovascular, renal, and metabolic complications (4–8). The excess aldosterone in PA will cause atrial structural and electrical remodeling which induce atrial fibrillation genesis. A correlation between PA and atrial fibrillation has been identified in previous studies, although the complicated interplay has yet to be completely elucidated (9). Milliez et al. reported that PA patients had a 12.1-fold higher risk of atrial fibrillation compared to essential hypertension patients (10), and a recent meta-analysis reported the risk of atrial fibrillation was 3.5-fold higher in PA patients compared to essential hypertension patients (11).

Atrial fibrillation is the most prevalent arrhythmia among adults that is associated with cerebro-cardiovascular complications (12, 13). The prevalence of atrial fibrillation has been reported to be 1.1% in adults aged above 35 years in Taiwan (14). The prevalence of atrial fibrillation was even higher in the elderly and in patients with chronic illnesses (15). Since that, the detection of new-onset atrial fibrillation (NOAF) is important to allow for timely risk stratification and interventions to prevent stroke or embolic events in PA patients.

Current guideline suggests that PA can be classified as lateralized PA, including aldosterone-producing adenoma and less commonly unilateral hyperplasia, and idiopathic hyperaldosteronism (16). Adrenalectomy is currently the standard treatment for lateralized PA (2, 16–19). However, there are still some PA patients with lateralized disease do not receive adrenalectomy in real world practice due to unwilling to receive surgery or limited equipment or capacity to receive adrenal vein sampling to confirmed the diagnosis (20). For these patients, mineralocorticoid receptor antagonist (MRA) therapy is the alternative treatment strategy for the lateralized PA and MRA is also the suggested treatment strategy for idiopathic hyperaldosteronism (19, 21). Recently, Pan et al. demonstrated that PA patients receiving adrenalectomy have a lower incidence of NOAF than essential hypertension patients and this finding has not been found in PA patients receiving MRA treatment (22).

The findings of these studies indicate that the over-secretion of aldosterone in PA patients may be correlated with the atrial fibrillation genesis, and that the active treatment of PA can reduce the risk of NOAF. Although there is abundant evidence of the strong correlation between PA and atrial fibrillation, few studies have investigated the effects of different treatment strategies on the prevention of NOAF. Therefore, we designed this study to compare the effects of adrenalectomy and MRA treatment on the development of NOAF in PA patients.

MATERIALS AND METHODS

Search Strategy and Selection Criteria

This meta-analysis was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (23). We searched PubMed, Embase, and Cochrane Library for randomized or observational studies using Mesh terms related to PA (e.g. 'primary aldosteronism', 'hyperaldosteronism', 'primary aldosteronism/hyperaldosteronism') and the following terms: 'adrenalectomy', 'atrial fibrillation, arrhythmia' from the database inception up to December 01, 2020. The studies were examined independently by the same authors as full-text reports according to the following criteria: (i) inclusion of patients with aldosterone-producing adenoma, idiopathic hyperaldosteronism, and essential hypertension; (ii) studies with both adrenalectomy and MRA treatment of patients with PA; (iii) NOAF included as an outcome treatment variable; (iv) exclusion of patients with a history of atrial fibrillation; and (v) limited to human studies. If there was more than one report from the same study group, we selected the report with the largest sample of patients. Review articles or meta-analyses were not included for analysis, but their citations and references were searched for additional relevant studies. The details of the search algorithm were provided in the supplemental materials.

The identified articles were first evaluated at the title or abstract level after consensus between two independent investigators (CHT and YLC). If the articles were potentially relevant, full articles were further retrieved and evaluated as complete reports according to the selection criteria. Studies including prospective clinical trials and retrospective studies were selected if the setting of the studies was to evaluate the outcomes of PA patients comparing the performance of MRA treatment and adrenalectomy. The exclusion criteria were non-

human studies, duplicate reports, studies without relevant outcome data, absence of a control group, studies presenting the outcomes of either MRA treatment or adrenalectomy only, or different settings in the intervention and control arms.

Data Extraction and Quality Assessment

Two independent reviewers (CHT and YLC) extracted the following data: author, journal, year of publication, location of the study group, design of the study, baseline features of the included patients, length of follow-up, numbers of participants enrolled and data of outcome in the studies and those who reached the desired endpoints of the specific studies. For studies reported in more than one publication, data from the most completed publication were extracted.

The quality of the included studies was evaluated using the Risk Of Bias In Non-randomized Studies of Interventions (ROBINS-I) checklist (24), scoring each study for the following seven domains: "Confounding", "Selection", "Classification of intervention", "Deviation from intervention", "Missing data", "Measurement of outcomes" and "Selection of repeated results". The domains were scored as "No information" (0), "Low" (1—low risk of bias), "Moderate" (2—moderate risk of bias), "Serious" (3—Serious risk of bias), and "Critical" (4—Critical risk of bias). A study was categorized as being of high quality if most of the domains were judged to be at low risk of bias.

Outcomes of Interest

The primary outcome of interest was the risk of NOAF in PA patients receiving MRA therapy versus adrenalectomy. We also analyzed two secondary outcomes: (1) PA patients receiving MRA therapy versus essential hypertension patients; and (2) PA patients receiving adrenalectomy versus essential hypertension patients.

Statistical Analysis

Data were extracted with the use of a standardized data form. In order to compare differences on the incidence of events between the two treatment groups, we extracted the number of events (NOAF) and total patients in each treatment arm. The pooled odds ratios (OR) with corresponding 95% confidence intervals (CIs) were calculated using inverse variance (IV) fixed and Laird and Ware random effects models with RevMan 5.3 (The Cochran Collaboration, The Nordic Cochrane Centre, Copenhagen, Denmark). The weights in the fixed effects model were assigned according to inverse within-study variance based on the assumption that all studies were sampled from a population with the same effect size. In contrast, the assigned weights in the random effects model considered both within-study and between-study variance.

Heterogeneity across studies was evaluated using the I^2 index, which was considered to be low if I^2 was ≤50%, moderate if >50% to <75%, and high if ≥75% (25). We assessed publication bias by visually inspecting funnel plots and Egger's regression asymmetry test, using Comprehensive Meta-Analysis Version 3.3.070 (Biostat Inc., Englewood, NJ, USA, 2014).

RESULTS

Included Studies

The electronic search yielded a total of 172 studies. The references of these studies were examined using the search strategy, and further evaluated for eligibility at the title or abstract level (**Figure 1**). Of the 172 studies assessed, seven were excluded because they were duplicate reports, and 157

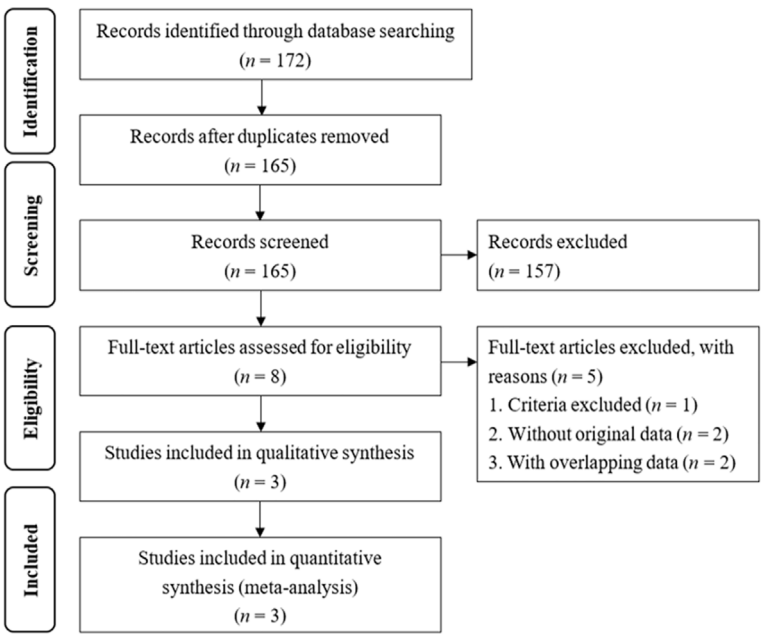

FIGURE 1 | Flow chart of the literature search. The 175 identified studies were from PubMed (11), Embase (152) and Cochrane (9).

studies were excluded after screening the title and abstract. Of the remaining eight full-text articles, five were excluded. In these five excluded studies, one is the editorial comments which not matching the searching criteria and two studies did not have the treatment results of adrenalectomy and MRA treatment. The two studies with overlapping data with current enrolled studies. Compared with current enrolled studies, both excluded studies were the conference abstracts including one with shorter follow-up and another with the same result. One study from our study group did not include details of the number of NOAF events in the study (22). We enrolled the raw data form our study to perform the meta-analysis. Finally, three studies were included in the final meta-analysis (22, 26, 27). In total, these studies included 2,705 PA patients, of who 776 received adrenalectomy and 1,929 received MRA treatment, and 49,794 essential hypertension patients.

Descriptive summaries of each included study are shown in **Table 1**. The types of study included one prospective study (26) and two retrospective studies (22, 27). In these three studies, the median duration of follow-up was 10 years (IQR 7.2–10.9). The dosages of spironolactone used in the MRA treatment groups were listed in **Table 1**.

Risk of Bias Assessment

The results of ROBINS-I tool analysis showed that the overall risk of bias of all three included studies were moderate (2) (**Supplement Table**).

Outcomes

Associations among PA patients receiving MRA treatment, PA patients receiving adrenalectomy, and essential hypertension patients with regards to NOAF events were analyzed.

Primary Outcome: Risk of NOAF in the PA Patients Receiving MRA Treatment Compared to the PA Patients Receiving Adrenalectomy

MRA treatment was significantly associated with a higher incidence of NOAF compared to adrenalectomy in both the fixed effects model (OR: 2.83, 95% CI: 1.76–4.57) (**Figure 2A**) and random effects model (OR: 2.83, 95% CI: 1.76–4.57) (**Figure 2B**). The heterogeneity of the included studies was low (I^2 = 0). The funnel plot (**Supplement Figure**) was generally symmetry and the Egger's regression asymmetry test (p = 0.91) did not reveal a statistical significance.

TABLE 1 | Characteristics of the studies meeting the inclusion criteria.

Reference	Study nature	Country	Number of patients			Duration of follow-up* (years)	Initial MRA Dosage	Exclusion criterion
			PA		EH			
			MRA	adrenalectomy				
Hundemer, (27)	Retrospective	USA	195	201	40,092	10	43 to 50 mg†	AF, CHF, MI, Stroke
Pan, (12)	Retrospective	Taiwan	1,668	534	8,808	4.4	50 mg	AF, MVD, Hyperthyroidism
Rossi, (26)	Prospective	Italy	66	41	894	11.8	/	AF, secondary hypertension

AF, atrial fibrillation; CHF, congestive heart failure; EH, essential hypertension; MI, myocardial infarction; MRA, mineralocorticoid receptor antagonist; MVD, mitral valve disease; PA, primary aldosteronism.

**For retrospective studies, the duration of follow-up is indicated as mean value; Prospective study is indicated as medium value.*

†The initial prescription dose of spironolactone was 43 mg in PRA >1 group and 50 mg in PRA <1 group.

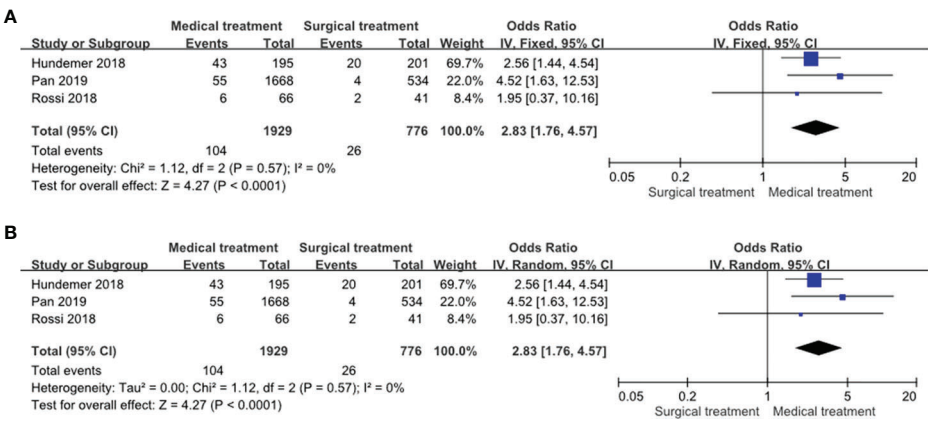

FIGURE 2 | Forest plots of NOAF in PA patients receiving MRA treatment vs adrenalectomy. Forest plots for the fixed effects model **(A)** and random effects model **(B)**. CI, confidence interval; OR, odds ratio; NOAF, new-onset atrial fibrillation; PA, primary aldosteronism; MRA, mineralocorticoid receptor antagonist.

Risk of NOAF in the PA Patients Receiving MRA Treatment Compared to the Essential Hypertension Patients

The PA patients receiving MRA treatment had a significantly higher incidence of NOAF events compared to the essential hypertension patients in the fixed effects model (OR: 1.61, 95% CI: 1.30–2.00) (**Figure 3A**) and random effects model (OR: 1.91, 95% CI: 1.11–3.28) (**Figure 3B**). Of note, the heterogeneity of this comparison was high (I^2 = 78%).

Risk of NOAF in the PA Patients Receiving Adrenalectomy Compared to the Essential Hypertension Patients

The PA patients receiving adrenalectomy had similar risk of NOAF compared to the essential hypertension patients in the fixed effects model (OR: 0.71, 95% CI: 0.48–1.07) (**Figure 4A**) and in the random effects model (OR: 0.70, 95% CI: 0.28–1.79) (**Figure 4B**). The heterogeneity of this comparison was moderate (I^2 = 67%).

DISCUSSION

This is the first meta-analysis to compare the long-term risk of NOAF among PA patients receiving MRA treatment, PA patients receiving adrenalectomy, and essential hypertension patients. The pooled results suggested that the PA patients receiving MRA treatment had a higher risk of NOAF compared to the PA patients receiving adrenalectomy and the patients with essential hypertension. In addition, there was no significant difference in the risk of NOAF between the PA patients receiving adrenalectomy and the essential hypertension patients. These results provide strong evidence of the higher long-term risk of NOAF in PA patients receiving MRA treatment compared to PA patients receiving adrenalectomy and essential hypertension patients.

Atrial fibrillation is associated with increased risks of stroke, heart failure and mortality (28). Excessive aldosterone is a major contributing factor to atrial fibrillation genesis (9, 12), as shown in the German Conn's Registry which reported a prevalence rate

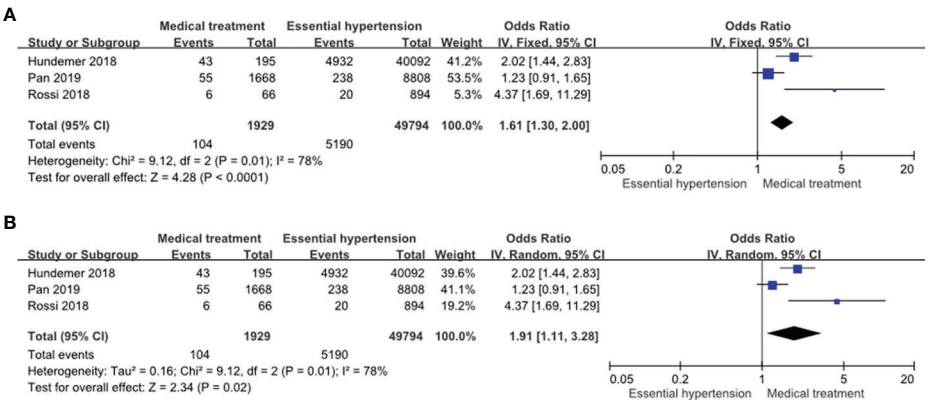

FIGURE 3 | Forest plot of NOAF in PA patients receiving MRA treatment vs EH patients. Forest plots for the fixed effects model **(A)** and random effects model **(B)**. CI, confidence interval; OR, odds ratio; NOAF, new-onset atrial fibrillation; PA, primary aldosteronism; MRA, mineralocorticoid receptor antagonist; EH, essential hypertension.

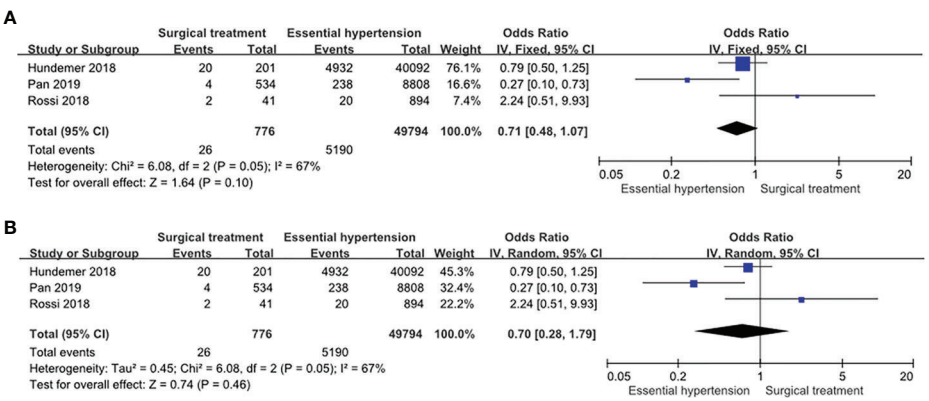

FIGURE 4 | Forest plot of NOAF in PA patients receiving adrenalectomy vs EH patients. Forest plots for the fixed effects model **(A)** and random effects model **(B)**. CI, confidence interval; OR, odds ratio; NOAF, new-onset atrial fibrillation; PA, primary aldosteronism; EH, essential hypertension.

of atrial fibrillation of 7.1% among 553 PA patients (29). Seccia et al. also demonstrated that the PA was highly prevalent in hypertensive patients with unexplained atrial fibrillation in prospective appraisal on the prevalence of primary aldosteronism in hypertensive (PAPPHY) study (30). The MRA also significantly reduced new-onset atrial fibrillation and recurrent atrial fibrillation in general patient population in one recent meta-analysis (31). However, the interactions among hypertension, hyperaldosteronism, and atrial fibrillation genesis in PA patients are complex (9, 12).

In bench studies, excessive aldosterone has been shown to stimulate the development of atrial fibrillation through atrial fibrosis and conduction disturbances (32–34). Atrial fibrosis is arrhythmogenic and the atrial fibrillation also promotes atrial fibrosis (35). The aldosterone increases inflammatory cytokines and increased oxidative stress which result in atrial fibrosis (12). In addition, left ventricular remodeling, which is also strongly correlated to atrial fibrillation. Some clinical studies have shown that aldosterone induces left ventricular hypertrophy and fibrosis which is associated with left ventricular diastolic dysfunction (8, 36–40). The left ventricular diastolic dysfunction has a prominent influence on atrial structure and function which also contributed to the atrial fibrillation genesis (8, 12, 41).

Both lateralized adrenalectomy and medical MRAs treatment can reverse left ventricular remodeling and improve outcomes in PA patients. However, lateralized adrenalectomy can potentially achieve a completely biochemical cure by removing the lesion responsible for hyperaldosteronism (42). Furthermore, the adrenalectomy can achieve the completed therapeutic effects more rapidly compared with medical therapy (43). In PA patients received MRA treatment, it takes longer time to observe a greater reduction in left ventricular wall thickness compare to PA patient receiving adrenalectomy (17). In addition, Rossi et al. compared the long-term outcomes of PA patients after adrenalectomy versus PA patients receiving MRA treatment, and reported a potentially lower left ventricular mass index after adrenalectomy (44). Since that, lateralized adrenalectomy is the standard treatment for PA patients who are suitable to receiving surgery (2, 21). In addition, PA patients received lateralized adrenalectomy had lower risk of new-onset diabetes mellitus (45), better quality of life (46) and less osteoporosis (47) compared with those received medical MRAs treatment. Another benefit of adrenalectomy over MRA treatment is that it can decrease the incidence of atrial fibrillation (26). However, the studies which compared the treatment effects from lateralized adrenalectomy or MRAs treatment to lower NOAF occurrence were still limited.

In the current meta-analysis, we enrolled three large cohort studies which showed consistent results that lateralized adrenalectomy had significantly lower NOAF events compared with medical therapy with low heterogeneity in the analysis. In addition, compared with patients with essential hypertension, only lateralized adrenalectomy could neutralize the risk of NOAC due to excess aldosterone but not MRA treatment. However, the heterogeneity of the results was moderate to high in secondary analysis of essential hypertension and PA patients possibly due to the diversity of patient characteristics and the different dosages of MRA use in these studies. The risk of NOAF in PA patients with MRA compared others receiving adrenalectomy or essential hypertension patients may be contributed form the insufficient MRA dosage and treatment effects (27). Due to limited studies, the subgroup analysis was not applicable. In contrast, the result of primary analysis compared MRA treatment to adrenalectomy revealed a very low heterogeneity.

The optimal dosage of spironolactone has yet to be established, and the current recommendation dosage in guidelines is from a daily dose of 12.5 mg with slow titration to a maximum daily dose of 100 mg (2). Catena et al. evaluated the effect of high-dose spironolactone, and found no significant difference in the occurrence of the combined cardiovascular endpoint of myocardial infarction, stroke, revascularization procedures, and sustained arrhythmias between patients with PA who received adrenalectomy and those who received high-dose MRA treatment (daily spironolactone dose: 121 mg) (HR, 1.26; 95% CI, 0.36–4.44; P = 0.71) (43). However, higher incidences of drug adverse effects were found in the high-dose spironolactone treatment group such as gynecomastia. In 2018, Hundemer et al. demonstrated that the level of plasma renin activity (PRA) after spironolactone treatment (<1 or ≥1 ng/ml/h) may be associated with NOAF and worse cardiovascular outcomes, and that the level of PRA after MRA treatment may be a better predictor than MRA dosage to predict clinical outcomes (48). To identify the ideal spironolactone dose in each patient, titrating the dose according to the PRA may be a reasonable approach in those PA patients received medical therapy. The dosages of MRA in the two retrospective studies enrolled in this meta-analysis were relatively low. Pan et al. showed the first prescript spironolactone dosage was only 50 and 75 mg as maximum dosage. Hundemer et al. showed that the initial spironolactone dosages were about 43 to 50 mg and were titrated up to 71 to 84 mg during follow-up. The relatively low spironolactone dosages may contribute to the higher risk of NOAF in PA patients receiving medical MRA treatment.

This study has some limitations. First, the choice of treatment is largely depended on lateralization. When diagnosed as aldosterone-producing adenoma, most patients are treated by adrenalectomy, while those diagnosed as idiopathic hyperaldosteronism are usually treated with MRAs. The different nature between two subtypes may influence the incidence of NOAF. However, data of aldosterone-producing adenoma patients receiving only MRA treatment were limited, and the information of different treatments in aldosterone-producing adenoma were not available in cohorts collected in this study. Further randomized study with different treatment strategies (surgery versus MRA therapy) in aldosterone-producing adenoma patients is needed to solve this issue. Second, many important baseline characteristics such as anti-hypertension medication use, the dosage of MRA, body mass index, waistline, and baseline cardiac function were not available in the enrolled studies which could be the confounding factors and the origin of the heterogeneity. In addition, the follow-up durations were varied between three enrolled studies which

might interfere with the results. However, the benefit of adrenalectomy was consistent in this pooled analysis. Third, only limited studies have been conducted to investigate the NOAF in PA in different treatment strategies and no randomized control trial has been conducted to investigate this issue. Fourth, the heterogeneity was moderate to high in secondary analysis of essential hypertension and PA patients possibly due to the diversity of patient characteristics and the different dosages of MRA use in these studies. However, due to limited studies, the subgroup analysis or meta-regression could not be done to explore the source of heterogeneity.

CONCLUSIONS

The PA patients receiving MRA treatment had a higher risk of NOAF compared to the PA patients receiving adrenalectomy and the patients with essential hypertension.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

Y-HL conceived and designed the experiments. C-HT, Y-LC, C-TP, Y-TL P-CL, C-WL, Z-WC, C-CC, and Y-YC analyzed the data. C-HT, Y-LC, and Y-HL wrote the paper. C-SH and Y-WC made scientific comments on the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This research was supported by grants from the Ministry of Science and Technology (MOST 105-2314-B-002-122-MY3, MOST 106-2314-B-002-169-MY3 and MOST 107-2314-B-002 -264 -MY3), National Taiwan University Hospital (NTUH 107-A141, 108-A141, 109-A141, 108-N01, 108-S4382, UN108-37), and the Excellent Translational Medicine Research Projects of National Taiwan University College of Medicine, and National Taiwan University Hospital (109C 101-43).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.646933/full#supplementary-material

REFERENCES

1. Conn JW. Presidential Address: Part I. Painting Background Part Ii. Primary Aldosteronism, a New Clinical Syndrome. *J Lab Clin Med* (1955) 45:3–17. doi: https://doi.org/10.5555/uri:pii:0022214355900482
2. Funder JW, Carey RM, Mantero F, Murad MH, Reincke M, Shibata H, et al. The Management of Primary Aldosteronism: Case Detection, Diagnosis, and Treatment: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab* (2016) 101:1889–916. doi: 10.1210/jc.2015-4061
3. 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) 44:157–62. doi: 10.1055/s-0031-1295438
4. Savard S, Amar L, Plouin P-F, Steichen O. Cardiovascular Complications Associated With Primary Aldosteronism: A Controlled Cross-Sectional Study. *Hypertension* (2013) 62:331–6. doi: 10.1161/HYPERTENSIONAHA.113.01060
5. Mulatero P, Monticone S, Bertello C, Viola A, Tizzani D, Iannaccone A, et al. Long-Term Cardio- and Cerebrovascular Events in Patients With Primary Aldosteronism. *J Clin Endocrinol Metab* (2013) 98:4826–33. doi: 10.1210/jc.2013-2805
6. Rossi G-P, Sechi LA, Giacchetti G, Ronconi V, Strazzullo P, Funder JW. Primary Aldosteronism: Cardiovascular, Renal and Metabolic Implications. *Trends Endocrinol Metab* (2008) 19:88–90. doi: 10.1016/j.tem.2008.01.006
7. Young WF. Primary Aldosteronism: Renaissance of a Syndrome. *Clin Endocrinol (Oxf)* (2007) 66:607–18. doi: 10.1111/j.1365-2265.2007.02775.x
8. Tsai CH, Pan CT, Chang YY, Chen ZW, Wu VC, Hung CS, et al. Left Ventricular Remodeling and Dysfunction in Primary Aldosteronism. *J Hum Hypertens* (2021) 35(2):131–47. doi: 10.1038/s41371-020-00426-y
9. Seccia TM, Caroccia B, Adler GK, Maiolino G, Cesari M, Rossi GP, et al. Atrial Fibrillation, and Hyperaldosteronism: The Triple Trouble. *Hypertension* (2017) 69:545–50. doi: 10.1161/HYPERTENSIONAHA.116.08956
10. 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) 45:1243–8. doi: 10.1016/j.jacc.2005.01.015
11. Monticone S, D'Ascenzo F, Moretti C, Williams TA, Veglio F, Gaita F, et al. Cardiovascular Events and Target Organ Damage in Primary Aldosteronism Compared With Essential Hypertension: A Systematic Review and Meta-Analysis. *Lancet Diabetes Endocrinol* (2018) 6:41–50. doi: 10.1016/S2213-8587(17)30319-4
12. Pan CT, Tsai CH, Chen ZW, Chang YY, Wu VC, Hung CS, et al. Atrial Fibrillation in Primary Aldosteronism. *Horm Metab Res* (2020) 52:357–65. doi: 10.1055/a-1141-5989
13. Manolis A, Doumas M. Atrial Fibrillation, Arterial Hypertension, and Primary Aldosteronism: A Dangerous and Unexpected Trio. *J Hypertens* (2020) 38:208–10. doi: 10.1097/HJH.0000000000002273
14. Chiang CE, Wu TJ, Ueng KC, Chao TF, Chang KC, Wang CC, et al. 2016 Guidelines of the Taiwan Heart Rhythm Society and the Taiwan Society of Cardiology for the Management of Atrial Fibrillation. *J Formos Med Assoc* (2016) 115:893–952. doi: 10.1016/j.jfma.2016.10.005
15. Kirchhof P, Benussi S, Kotecha D, Ahlsson A, Atar D, Casadei B, et al. 2016 ESC Guidelines for the Management of Atrial Fibrillation Developed in Collaboration With EACTS. *Eur Heart J* (2016) 37:2893–962. doi: 10.5603/KP.2016.0172
16. Wu VC, Hu YH, Er LK, Yen RF, Chang CH, Chang YL, et al. Case Detection and Diagnosis of Primary Aldosteronism - The Consensus of Taiwan Society of Aldosteronism. *J Formos Med Assoc* (2017) 116:993–1005. doi: 10.1016/j.jfma.2017.06.004
17. Catena C, Colussi G, Lapenna R, Nadalini E, Chiuch A, Gianfagna P, et al. Long-Term Cardiac Effects of Adrenalectomy or Mineralocorticoid Antagonists in Patients With Primary Aldosteronism. *Hypertension* (2007) 50:911–8. doi: 10.1161/HYPERTENSIONAHA.107.095448
18. Catena C, Colussi G, Di Fabio A, Valeri M, Marzano L, Uzzau A, et al. Mineralocorticoid Antagonists Treatment Versus Surgery in Primary Aldosteronism. *Horm Metab Res* (2010) 42:440–5. doi: 10.1055/s-0029-1246185
19. Lenders JWM, Eisenhofer G, Reincke M. Subtyping of Patients With Primary Aldosteronism: An Update. *Horm Metab Res* (2017) 49:922–8. doi: 10.1055/s-0043-122602

20. Pillai PR, Griffith M, Schwarcz MD, Weiss IA. Primary Aldosteronism: Cardiovascular Risk, Diagnosis, and Management. *Cardiol Rev* (2020) 28:84–91. doi: 10.1097/CRD.0000000000000281
21. Huang KH, Yu CC, Hu YH, Chang CC, Chan CK, Liao SC, et al. Targeted Treatment of Primary Aldosteronism - The Consensus of Taiwan Society of Aldosteronism. *J Formos Med Assoc* (2019) 118:72–82. doi: 10.1016/j.jfma.2018.01.006
22. Pan CT, Liao CW, Tsai CH, Chen ZW, Chen L, Hung CS, et al. Influence of Different Treatment Strategies on New-Onset Atrial Fibrillation Among Patients With Primary Aldosteronism: A Nationwide Longitudinal Cohort-Based Study. *J Am Heart Assoc* (2020) 9:e013699. doi: 10.1161/JAHA.119.013699
23. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JPA, et al. The PRISMA Statement for Reporting Systematic Reviews and Meta-Analyses of Studies That Evaluate Healthcare Interventions: Explanation and Elaboration. *BMJ* (2009) 339:b2700. doi: 10.1136/bmj.b2700
24. Sterne JAC, Hernán MA, Reeves BC, Savović J, Berkman ND, Viswanathan M, et al. Robins-I: A Tool for Assessing Risk of Bias in Non-Randomised Studies of Interventions. *BMJ* (2016) 355:i4919. doi: 10.1136/bmj.i4919
25. Higgins JPT, Thompson SG, Deeks JJ, Altman DG. Measuring Inconsistency in Meta-Analyses. *BMJ* (2003) 327:557. doi: 10.1136/bmj.327.7414.557
26. Rossi GP, Maiolino G, Flego A, Belfiore A, Bernini G, Fabris B, et al. Adrenalectomy Lowers Incident Atrial Fibrillation in Primary Aldosteronism Patients At Long Term. *Hypertension* (2018) 71:585–91. doi: 10.1161/HYPERTENSIONAHA.117.10596
27. Hundemer GL, Curhan GC, Yozamp N, Wang M, Vaidya A. Incidence of Atrial Fibrillation and Mineralocorticoid Receptor Activity in Patients With Medically and Surgically Treated Primary Aldosteronism. *JAMA Cardiol* (2018) 3:768–74. doi: 10.1001/jamacardio.2018.2003
28. Hindricks G, Potpara T, Dagres N, Arbelo E, Bax JJ, Blomstrom-Lundqvist C, et al. 2020 ESC Guidelines for the Diagnosis and Management of Atrial Fibrillation Developed in Collaboration With the European Association for Cardio-Thoracic Surgery (Eacts). *Eur Heart J* (2021) 42:373–498. doi: 10.1093/eurheartj/ehaa612
29. Born-Frontsberg E, Reincke M, Rump LC, Hahner S, Diederich S, Lorenz R, et al. Cardiovascular and Cerebrovascular Comorbidities of Hypokalemic and Normokalemic Primary Aldosteronism: Results of the German Conn's Registry. *J Clin Endocrinol Metab* (2009) 94:1125–30. doi: 10.1210/jc.2008-2116
30. Seccia TM, Letizia C, Muiesan ML, Lerco S, Cesari M, Bisogni V, et al. Atrial Fibrillation as Presenting Sign of Primary Aldosteronism: Results of the Prospective Appraisal on the Prevalence of Primary Aldosteronism in Hypertensive (Papphy) Study. *J Hypertens* (2020) 38:332–9. doi: 10.1097/HJH.0000000000002250
31. Neefs J, van den Berg NW, Limpens J, Berger WR, Boekholdt SM, Sanders P, et al. Aldosterone Pathway Blockade to Prevent Atrial Fibrillation: A Systematic Review and Meta-Analysis. *Int J Cardiol* (2017) 231:155–61. doi: 10.1016/j.ijcard.2016.12.029
32. Lavall D, Selzer C, Schuster P, Lenski M, Adam O, Schäfers HJ, et al. The Mineralocorticoid Receptor Promotes Fibrotic Remodeling in Atrial Fibrillation. *J Biol Chem* (2014) 289:6656–68. doi: 10.1074/jbc.M113.519256
33. Tsai CF, Yang SF, Chu HJ, Ueng KC. Cross-Talk Between Mineralocorticoid Receptor/Angiotensin II Type 1 Receptor and Mitogen-Activated Protein Kinase Pathways Underlies Aldosterone-Induced Atrial Fibrotic Responses in HL-1 Cardiomyocytes. *Int J Cardiol* (2013) 169:17–28. doi: 10.1016/j.ijcard.2013.06.046
34. Reil JC, Hohl M, Selejan S, Lipp P, Drautz F, Kazakow A, et al. Aldosterone Promotes Atrial Fibrillation. *Eur Heart J* (2012) 33:2098–108. doi: 10.1093/eurheartj/ehr266
35. Dzeshka MS, Lip GY, Snezhitskiy V, Shantsila E. Cardiac Fibrosis in Patients With Atrial Fibrillation: Mechanisms and Clinical Implications. *J Am Coll Cardiol* (2015) 66:943–59. doi: 10.1016/j.jacc.2015.06.1313
36. Lin YH, Wu XM, Lee HH, Lee JK, Liu YC, Chang HW, et al. Adrenalectomy Reverses Myocardial Fibrosis in Patients With Primary Aldosteronism. *J Hypertens* (2012) 30:1606–13. doi: 10.1097/HJH.0b013e3283550f93
37. Hung CS, Chou CH, Liao CW, Lin YT, Wu XM, Chang YY, et al. Aldosterone Induces Tissue Inhibitor of Metalloproteinases-1 Expression and Further Contributes to Collagen Accumulation: From Clinical to Bench Studies. *Hypertension* (2016) 67:1309–20. doi: 10.1161/HYPERTENSIONAHA.115.06768
38. Chou CH, Hung CS, Liao CW, Wei LH, Chen CW, Shun CT, et al. IL-6 Trans-Signalling Contributes to Aldosterone-Induced Cardiac Fibrosis. *Cardiovasc Res* (2018) 114:690–702. doi: 10.1093/cvr/cvy013
39. Hung CS, Chou CH, Wu XM, Chang YY, Wu VC, Chen YH, et al. Circulating Tissue Inhibitor of Matrix Metalloproteinase-1 Is Associated With Aldosterone-Induced Diastolic Dysfunction. *J Hypertens* (2015) 33:1922–30; discussion 1930. doi: 10.1097/HJH.0000000000000619
40. Chang YY, Lee HH, Hung CS, Wu XM, Lee JK, Wang SM, et al. Association Between Urine Aldosterone and Diastolic Function in Patients With Primary Aldosteronism and Essential Hypertension. *Clin Biochem* (2014) 47:1329–32. doi: 10.1016/j.clinbiochem.2014.05.062
41. Rosenberg MA, Manning WJ. Diastolic Dysfunction and Risk of Atrial Fibrillation: A Mechanistic Appraisal. *Circulation* (2012) 126:2353–62. doi: 10.1161/CIRCULATIONAHA.112.113233
42. Katabami T, Fukuda H, Tsukiyama H, Tanaka Y, Takeda Y, Kurihara I, et al. Clinical and Biochemical Outcomes After Adrenalectomy and Medical Treatment in Patients With Unilateral Primary Aldosteronism. *J Hyperten* (2019) 37:1513–20. doi: 10.1097/HJH.0000000000002070
43. Catena C, Colussi G, Nadalini E, Chiuch A, Baroselli S, Lapenna R, et al. Cardiovascular Outcomes in Patients With Primary Aldosteronism After Treatment. *Arch Internal Med* (2008) 168:80–5. doi: 10.1001/archinternmed.2007.33
44. Rossi GP, Cesari M, Cuspidi C, Maiolino G, Cicala MV, Bisogni V, et al. Long-Term Control of Arterial Hypertension and Regression of Left Ventricular Hypertrophy With Treatment of Primary Aldosteronism. *Hypertension* (2013) 62:62–9. doi: 10.1161/HYPERTENSIONAHA.113.01316
45. Wu VC, Chueh SCJ, Chen L, Chang CH, Hu YH, Lin YH, et al. Risk of New-Onset Diabetes Mellitus in Primary Aldosteronism: A Population Study Over 5 Years. *J Hyperten* (2017) 35:1698–708. doi: 10.1097/HJH.0000000000001361
46. Velema M, Dekkers T, Hermus A, Timmers H, Lenders J, Groenewoud H, et al. Quality of Life in Primary Aldosteronism: A Comparative Effectiveness Study of Adrenalectomy and Medical Treatment. *J Clin Endocrinol Metab* (2018) 103:16–24. doi: 10.1210/jc.2017-01442
47. Wu VC, Chang CH, Wang CY, Lin YH, Kao TW, Lin PC, et al. Risk of Fracture in Primary Aldosteronism: A Population-Based Cohort Study. *J Bone Miner Res* (2017) 32:743–52. doi: 10.1002/jbmr.3033
48. Hundemer GL, Curhan GC, Yozamp N, Wang M, Vaidya A. Renal Outcomes in Medically and Surgically Treated Primary Aldosteronism. *Hyperten (Dallas Tex 1979)* (2018) 72:658–66. doi: 10.1161/HYPERTENSIONAHA.118.11568

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

REVIEW
published: 29 June 2021
doi: 10.3389/fendo.2021.683588

Approaches to Gene Mutation Analysis Using Formalin-Fixed Paraffin-Embedded Adrenal Tumor Tissue From Patients With Primary Aldosteronism

Kazutaka Nanba[1,2]*, *William E. Rainey*[1,3] *and Aaron M. Udager*[4,5,6]

[1] Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI, United States, [2] Department of Endocrinology and Metabolism, National Hospital Organization Kyoto Medical Center, Kyoto, Japan, [3] Division of Metabolism, Endocrinology, and Diabetes, Department of Internal Medicine, University of Michigan, Ann Arbor, MI, United States, [4] Department of Pathology, University of Michigan, Ann Arbor, MI, United States, [5] Rogel Cancer Center, University of Michigan, Ann Arbor, MI, United States, [6] Michigan Center for Translational Pathology, University of Michigan, Ann Arbor, MI, United States

OPEN ACCESS

Edited by:
Vin-Cent Wu,
National Taiwan University, Taiwan

Reviewed by:
Michael Stowasser,
The University of Queensland, Australia
John Funder,
Hudson Institute of Medical Research, Australia
Brasilina Caroccia,
University of Padua, Italy

***Correspondence:**
Kazutaka Nanba
knamba@umich.edu

Specialty section:
This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Endocrinology

Received: *21 March 2021*
Accepted: *07 June 2021*
Published: *29 June 2021*

Citation:
Nanba K, Rainey WE and Udager AM (2021) Approaches to Gene Mutation Analysis Using Formalin-Fixed Paraffin-Embedded Adrenal Tumor Tissue From Patients With Primary Aldosteronism. Front. Endocrinol. 12:683588. doi: 10.3389/fendo.2021.683588

Aldosterone production is physiologically under the control of circulating potassium and angiotensin II as well as adrenocorticotropic hormone and other secretagogues such as serotonin. The adrenal's capacity to produce aldosterone relies heavily on the expression of a single enzyme, aldosterone synthase (CYP11B2). This enzyme carries out the final reactions in the synthesis of aldosterone and is expressed almost solely in the adrenal zona glomerulosa. From a disease standpoint, primary aldosteronism (PA) is the most common of all adrenal disorders. PA results from renin-independent adrenal expression of CYP11B2 and production of aldosterone. The major causes of PA are adrenal aldosterone-producing adenomas (APA) and adrenal idiopathic hyperaldosteronism. Our understanding of the genetic causes of APA has significantly improved through comprehensive genetic profiling with next-generation sequencing. Whole-exome sequencing has led to the discovery of mutations in six genes that cause renin-independent aldosterone production and thus PA. To facilitate broad-based prospective and retrospective studies of APA, recent technologic advancements have allowed the determination of tumor mutation status using formalin-fixed paraffin-embedded (FFPE) tissue sections. This approach has the advantages of providing ready access to archival samples and allowing CYP11B2 immunohistochemistry-guided capture of the exact tissue responsible for inappropriate aldosterone synthesis. Herein we review the methods and approaches that facilitate the use of adrenal FFPE material for DNA capture, sequencing, and mutation determination.

Keywords: primary aldosteronism, CYP11B2, somatic mutation, immunohistochemistry, next-generation sequencing

INTRODUCTION

Technologic advances in genetic analysis have provided us a better understanding of the molecular pathogenesis of endocrine-related tumors. Aldosterone-producing adenoma (APA) is one of the major subtypes of primary aldosteronism (PA), the most common cause of endocrine-related hypertension. The application of next-generation sequencing (NGS) has resulted in the identification of disease-causing mutations in APA and familial PA. Aldosterone-driver mutations can occur in genes encoding membrane ion channels or pumps (1–9). Thus far, APA have been found with mutations in *KCNJ5* (1), *ATP1A1* (2, 4), *ATP2B3* (2), *CACNA1D* (3, 4), *CACNA1H* (9), and *CLCN2* (8) (aldosterone-driver mutations). These mutations cause excess aldosterone production by raising intracellular calcium levels which leads to enhanced CYP11B2 (aldosterone synthase) expression and renin-independent aldosterone production (10). Like other adrenocortical tumors, activating mutations in *CTNNB1* gene (encoding β-catenin) have also been documented in a subset of APA (11–14) but the mechanism of *CTNNB1* mutation activation of aldosterone production remains to be clearly defined. So far more than 90 APA somatic mutations have been reported (**Table 1**). Of note, only part of the previously reported somatic mutations has been functionally tested so far. To assess the pathologic role of these mutations, it would be ideal to perform cell-based studies for each mutation. In addition to tumor somatic mutations, PA aldosterone production may be regulated by hormones that include adrenocorticotropic hormone, serotonin, or luteinizing hormone (37–44).

Since the development of specific antibodies against human CYP11B2, which is required for aldosterone biosynthesis, CYP11B2 immunohistochemistry (IHC) has played an important role in defining the histopathologic characteristics of adrenals from patients with PA (45, 46). CYP11B2 IHC has revealed diversities in the histopathology of adrenals from patients with PA, including APA (CYP11B2-expressing adrenocortical adenoma) and adrenals with small CYP11B2-expressing cell nests, called aldosterone-producing cell clusters (APCCs) (45) or aldosterone-producing micronodules (APMs) (47). Advanced sequencing methods combined with CYP11B2 IHC have significantly improved the detection rate of somatic mutations in APA (14, 48, 49). CYP11B2 IHC-guided targeted NGS has also allowed the detection of aldosterone-driver mutations in APCCs (APMs) using small amounts of DNA (50–52). Herein, we provide an overview of recent advances in the genetic analysis of APA and introduce a streamlined sequencing approach using formalin-fixed paraffin-embedded (FFPE) tumor tissue material.

IMPORTANCE OF CYP11B2 IHC AND TARGETED DNA CAPTURE

Development of specific antibodies against human CYP11B2 has allowed detection of the source of pathologic aldosterone production in the resected adrenal tissue (45, 46). Unique characteristics of adrenals from patient with PA have been documented by CYP11B2 IHC. Importantly, adrenal tumors detected by cross-sectional imaging study are not always the cause of aldosterone excess even when adrenal vein sampling lateralizes autonomous aldosterone production to the tumor side (53). In such cases, APA can be below the detection limit of imaging studies and/or imaging-detected tumors can be non-functioning adrenocortical adenomas (CYP11B2-negative tumors by IHC). Cases with multiple APAs within one adrenal have also been documented (14, 48, 49, 54).

Traditionally, DNA and RNA have been isolated from snap frozen tumor pieces obtained during pathologic gross dissection at the time of adrenalectomy. Mutational analysis has subsequently been performed without consideration of CYP11B2 expression prior to sequencing. In the largest mutation prevalence study using this conventional approach, aldosterone-driver somatic mutations were detected in 54% of 474 adrenal tumors from PA patients (55). Considering the aforementioned diversities in the histology of PA, this approach could negatively affect the accuracy of mutational analysis. As such, we recently developed an advanced molecular profiling method using selective DNA isolation from FFPE sections based on CYP11B2 IHC, followed by NGS (14, 56). The step-by-step sequencing method using the CYP11B2 IHC-guided approach is shown in **Figure 1**. Many laboratories, including ours, use a mouse monoclonal antibody specific for human CYP11B2 that was produced and characterized by Dr. Celso Gomez-Sanchez (46). This antibody is commercially available from Millipore Sigma (MABS1251, RRID: AB_2783793), making it useful for both research and pathologic diagnosis purposes. As is needed for most antibodies, laboratory testing for individual in-house protocols should be done to optimize specificity and sensitivity for CYP11B2 detection. Initial protocol testing is particularly important due to the variable CYP11B2 expression seen between APAs. The scanned slide images of adrenal tumor tissue from a PA patient are shown in **Figure 2**. The adrenal contains two distinct adrenocortical tumors (an APA and a CYP11B2-negative tumor) which exist close to each other. This example highlights the importance of targeted DNA capture method for accurate mutation analysis. Importantly, past studies demonstrated that no aldosterone-driver mutation was detected in CYP11B2-negative adrenocortical tumors from PA patients (49, 57).

Using this CYP11B2 IHC-guided approach, aldosterone-driver mutations have been identified in 88-96% of APAs (14, 48, 49). A recent study demonstrated a better mutation detection rate using CYP11B2-IHC guided sequencing (94%) as compared to the authors' previous use of conventional tumor tissue approaches (71%) (58).

For the laboratories using traditional material, i.e., DNA/RNA from macro-dissected snap frozen tumor pieces, confirmation of *CYP11B2* mRNA expression by quantitative reverse transcription polymerase chain reaction (RT-qPCR) prior to sequencing could also improve the mutation detection

TABLE 1 | Previously reported somatic mutations in aldosterone-producing adenomas.

Gene	Somatic Mutations	
KCNJ5	c.343C>T (p.R115W) (15)	c.445_446insTGG (p.T149delinsMA) (49)
	c.376T>C (p.W126R) (16)	c.447_448insATT (p.T149delinsTI) (14)
	c.414_425dupGCTTTCCTGTTC (p.A139_F142dup) (17)	c.450_451insATG (p.I150_G151insM) (13)
	c.420C>G (p.F140L) (14)[a]	c.451G>A (p.G151R) (1)
	c.433_434insCCATTG (p.I144_E145insAI) (13)	c.451G>C (p.G151R) (24)
	c.433G>C (p.E145Q) (18)	(p.G151_Y152del)* (25)
	c.433G>A (p.E145K) (4)	c.457_492dupG_G (p.G153_G164dup) (20)
	c.432_439delTGAGACCGinsCA (p.E145_E147delinsK) (19)	c.461T>G (p.F154C) (13)
	c.439G>C and c.448_449insCAACAACCA (p.E147Q_T149_I150insTTT) (20)	c.467_469delTCA (p.I157del) (26)
	c.443C>T (p.T148I) (21)[b]	c.470_471delinsAA (p.I157K) (13)
	c.445_446insGAA (p.T148_T149insR) (22)	(p.I157_E159del)* (25)
	c.446insAAC (p.T149_I150insT) (23)	c.472A>G (p.T158A) (27)
	c.445A>T (p.T149S) (21)	c.503T>G (p.L168R) (1)
		(p.G184E)* (25)
		c.737A>G (p.E246G) (15)
ATP1A1	c.295G>A (p.G99R) (16)	c.2874_2882delCTTTGAAGA (p.F959_E961del) (29)
	c.299_313delTCTCAATGTTACTGT (p.F100_L104del) (2)	c.2877_2882delTGAAGA (p.F959_E961delinsL) (28)
	c.304_309delATGTTA (p.M102_L103del) (28)	c.2878_2895delGAAGAGACAGCCCTGGCTinsGCCCTGGTT (p.E960_A965delinsALV) (48)
	c.306_317delGTTACTGTGGAT (p.M102_I106delinsW) (28)	c.2877_2888delTGAAGAGACAGC (p.E960_A963del) (29)
	c.308_313delTACTGT (p.L103_L104del) (28)	c.2878_2887delGAAGAGACAGinsT (p.E960_A963delinsS) (4)
	c.311T>G (p.L104R) (2)	c.2879_2890delAAGAGACAGCCC (p.E960_L964delinsV) (28)
	c.995T>G (p.V332G) (2)	c.2878_2892delGAAGAGACAGCCCTGinsGCCGTG (p.E960_L964delinsAV) (14)
	c.2864_2878delTATTTGGCCTCTTTG (p.I955_E960delinsK) (49)	
	c. 2867_2882delTTGGCCTCTTTGAAGAinsG (p.F956_E961delinsW) (28)	
ATP2B3	c.367G>C (p.G123R) (30)	c.1273_1278delCTGGTC (p.L425_V426del) (2)
	c.1228T>G (p.Y410D) (31)	c.1277_1282delTCGTGG (p.V426_V427del) (2)
	c.1264_1278delGTCACTGTGCTGGTCinsAGCACACTC (p.V422_V426delinsSTL) (22)	c.1276_1287delGTCGTGGCTGTC (p.V426_V429del) (28)
	c.1264_1275delGTCACTGTGCTGinsATCACT (p.V422_L425delinsIT) (14)	c.1276_1298insGACA_delTCGTGGCTGTCCCAGAGGGCCT (p.V426G_V427Q_A428_L433del) (13)
	c.1269_1274delTGTGCT (p.V424_L425del) (32)	c.1279_1284delGTGGCT (p.V427_A428del) (33)
	c.1270_1275delGTGCTG (p.V424_L425del) (55)	c.1281_1286delGGCTGT (p.A428_V429del) (34)
	c.1272_1277delGCTGGT (p.L425_V426del) (2)	
CACNA1D	c.776T>A (p.V259D) (4)	c.2906C>T (p.S969L) (48)
	c.776T>G (p.V259G) (14)	c.2936T>A (p.V979D) (55)
	c.926T>C (p.V309A) (49)	c.2943G>C (p.V981N) (55)
	c.1201C>G (p.V401L) (28)	c.2968C>G (p.R990G) (49)
	c.1207G>C [p.G403R (exon8A)] (3, 4)	c.2969G>A (p.R990H) (4)
	c.1207G>C [p.G403R (exon8B)] (3)**	c.2978G>C (p.R993T) (49)
	c.1229C>T (p.S410L) (30)	c.2978G>T (p.R993M) (29)
	c.1856G>C (p.R619P) (49)	c.2992_2993GC>AT (p.A998I) (55)
	c.1955C>T (p.S652L) (55)	c.2993C>T (p.A998V) (55)
	c.1964T>C (p.L655P) (55)	c.3019T>C (p.C1007R) (49)
	c.2182G>A (p.V728I) (20)	c.3044T>G (p.I1015S) (49)
	c.2222A>G (p.Y741C) (55)	c.3044T>C (p.I1015T) (58)
	c.2239T>G (p.F747V) (3)	c.3451G>T (p.V1151F) (55)
	c.2239T>C (p.F747L) (4)	c.3452T>C (p.V1151A) (29)
	c.2241C>G (p.F747L) (4)	c.3455T>A (p.I1152N) (55)
	c.2240T>G (p.F747C) (56)	c.3458T>G (p.V1153G) (35)
	c.2240T>C (p.F747S) (29)[c]	c.4007C>G (p.P1336R) (4)
	c.2250C>G (p.I750M) (3, 4)	c.4012G>A (p.V1338M) (3)
	c.2248A>T (p.I750F) (55)	c.4062G>A (p.M1354I) (4)
	c.2261A>G (p.N754S) (29)	
CACNA1H	c.4289T>C (p.I1430T) (9)	
CLCN2	c.71G>A (p.G24D) (8)	
	c.64-2_74del (36)	

*[a-c]Associated with another somatic mutation ([a]KCNJ5 p.G151R; [b]KCNJ5 p.T149S; [c]CACNA1D p.N754S). * Base change information was not provided in the original article. For the CACNA1D mutations, amino acid substitutions are described based on the reference sequence NM_001128839 otherwise noted (**NM_000720 for the mutation in exon 8B). Mutations that are covered by the primer sets in* ***Table 2*** *are highlighted in blue.*

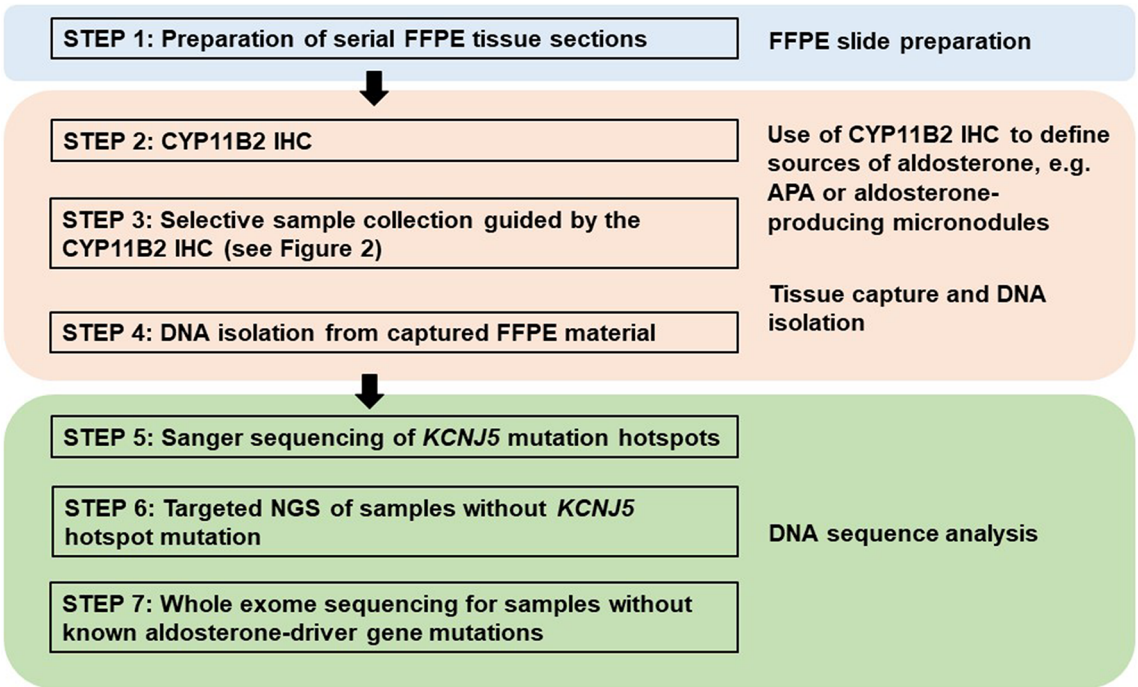

FIGURE 1 | Proposed method for DNA mutation analysis using excised adrenal tissue sections from patients with primary aldosteronism. This approach uses CYP11B2 immunohistochemistry (IHC) to define the source of aldosterone for DNA capture in FFPE tissue sections. Captured DNA is then used for Sanger or gene-targeted deep sequencing to detect known and/or novel drivers of aldosterone production.

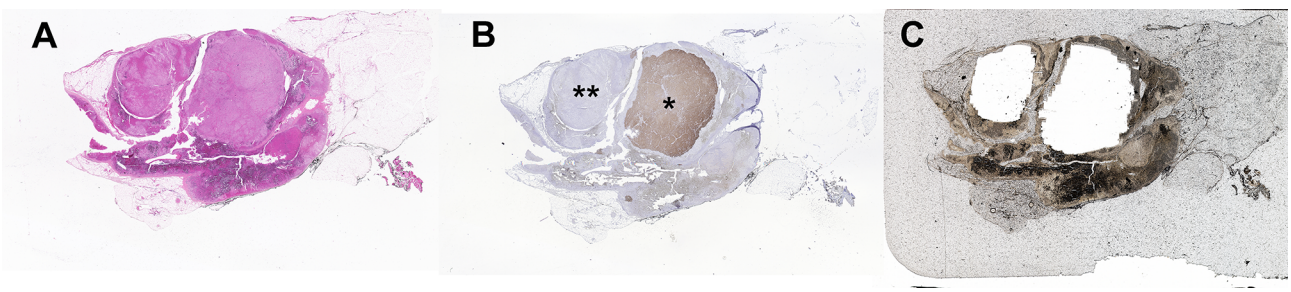

FIGURE 2 | Example of a multinodular adrenal sample from a patient with primary aldosteronism that illustrates the benefit of CYP11B2 IHC-guided DNA capture. **(A)** Hematoxylin and eosin staining, **(B)** CYP11B2 immunohistochemistry; *, aldosterone-producing adenoma (APA); **, CYP11B2-negative tumor, **(C)** Post-captured unstained FFPE adrenal tissue section. For DNA isolation, an APA and a CYP11B2-negative tumor were selectively scraped based on the results of CYP11B2 immunohistochemistry.

rate. A proposed method for mutational analysis using banked snap frozen material is shown in **Figure 3**.

DEFINING SOMATIC MUTATIONS IN ALDOSTERONE-PRODUCING LESIONS

Sanger Sequencing

Traditional direct Sanger sequencing has been widely used for the mutational analysis of APA. As new APA-related genes have continuously been identified, it is challenging to perform Sanger sequencing for the screening of multiple genes – particularly for genes like *CACNA1D*, which have a large coding region with dispersed mutation hotspot areas. Targeted NGS is rapidly becoming the preferred method due to its high sensitivity and ability to utilize small amounts of DNA; however, Sanger sequencing is still an attractive method considering the high per sample cost of NGS. As the prevalence of *KCNJ5* hotspot mutations in APA is relatively high, one option to decrease sample throughput is screening for *KCNJ5* mutation hotspots

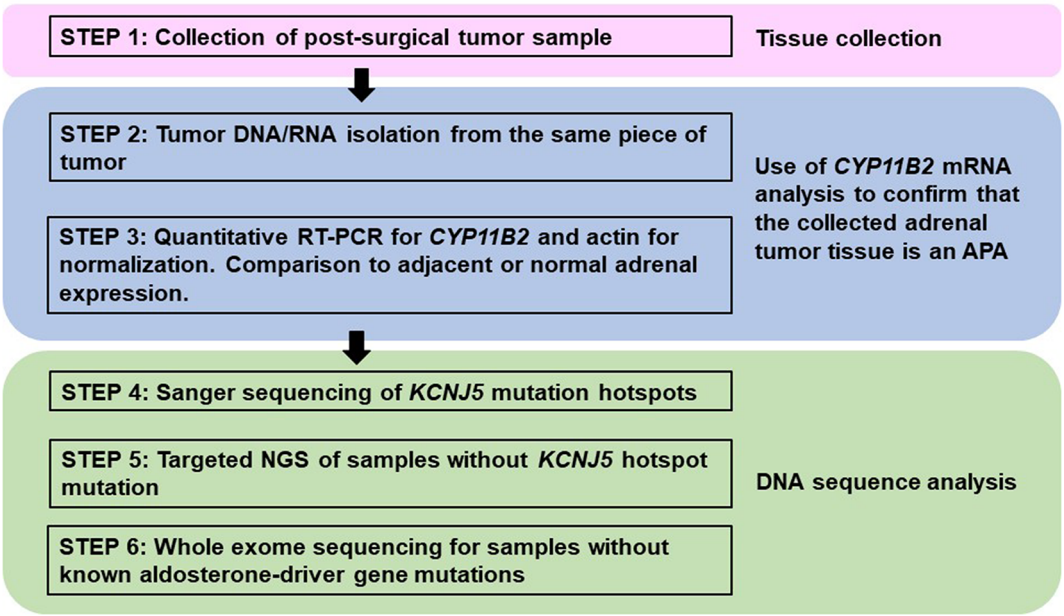

FIGURE 3 | Proposed method for processing fresh or frozen adrenal tumor tissue from patients with primary aldosteronism for mutational analysis. This approach varies from FFPE processing (Figure 1) by the method of tissue collection and the need to use *CYP11B2* mRNA detection for confirmation of APA status. However, mutational analysis is similar using Sanger and/or next-generation sequencing to detect known and/or novel drivers of aldosterone production.

using Sanger sequencing, followed by targeted NGS of *KCNJ5* mutation-negative samples (29, 48) (**Figure 1**). This approach significantly reduces cost and can also be applied to material isolated from traditional snap frozen tissue (**Figure 3**). For researchers without available NGS to screen entire coding regions, targeted Sanger sequencing that covers the majority of known aldosterone-driver mutations can be done in a systematic manner. Based on the APA mutation prevalence from our previous study (14), the use of five primer pairs (one for the *KCNJ5*, one for *ATP1A1*, one for *ATP2B3*, and two for *CACNA1D*, **Table 2**) appear to be able to identify over 70% of mutations by direct Sanger sequencing in a Caucasian American cohort. Special consideration is required for primer design when using genomic DNA (gDNA) from FFPE as a template, since FFPE-extracted DNA can be heavily degraded and fragmented. The authors recommend designing primer sets that target the amplicon size below 250 base pairs (bp) if possible.

Next-Generation Sequencing

NGS has rapidly become the standard approach for comprehensive molecular profiling of human tumors due to its ability to generate sequence-level genetic data simultaneously for tens, hundreds, or even thousands of genes. Although a variety of NGS methods and platforms exist, there are two broad approaches: amplicon-based and hybridization capture-based (**Table 3**). Amplicon-based approaches utilize multiplex PCR reactions to amplify genomic regions of interest, while hybridization capture-based methods utilize biotinylated oligonucleotide baits to pull down target regions from pools of sheared gDNA. In general, amplicon-based methods are

TABLE 2 | PCR primer sets for aldosterone-driver mutation hotspots.

Gene	Exon		Primer Sequences	Amplicon Size (bp)	Reference
KCNJ5	2	Forward	GGACCATGTTGGCGACCAAGAGTG	211	(21)
		Reverse	GACAAACATGCACCCCACCATGAAG		
ATP1A1	4	Forward	ATTAACATCTGCTCGTGCAGCTGAG	227	
		Reverse	CCATATGCTGAATTACAGAACTCAC		
ATP2B3	8	Forward	TGTCTGCCATCACCGTCATCATC	255	(14)
		Reverse	CCCAGTTTCCGAGTCTGTAAACAG		
CACNA1D	8A	Forward	CCCACTCCTATGAGACCATC	190	
		Reverse	TCTTGGCAACTGTCCTCAGG		
	16	Forward	GGTGTGTGGCGTTGCCATTG	253	(29)
		Reverse	AACTGTTGCAGGGCTCCCA		

TABLE 3 | Comparison of NGS approaches for molecular profiling of aldosterone-producing adrenal cortical lesions.

	Amplicon-based	Hybridization Capture-based
Enrichment method	Multiplex PCR	Biotinylated oligonucleotide baits
Input DNA	Less	More
# of genomic targets	Fewer	More
Experimental time	Less	More
Cost per sample	Lower*	Higher*
Application(s)	Targeted sequencing	Targeted sequencing or WES

**Depends on depth of sequencing and # of genomic targets.*
WES, whole-exome sequencing.

preferred for targeted sequencing of small numbers of genomic regions or when available input DNA for NGS library preparation is very low – particularly for FFPE samples – while hybridization capture-based approaches are favored for analyzing a large number of genomic regions [e.g., whole-exome sequencing (WES)] when ample input DNA is available. These and other differences between the NGS approaches inform how they may be best utilized for molecular profiling of aldosterone-producing lesions using FFPE tissue (**Figure 1**).

Given the relatively limited number of established aldosterone-driver mutations – coupled with the fact that most of these mutations occur at specific hotspot regions within the affected genes – targeted amplicon-based NGS is ideal for characterizing FFPE APA samples. As mentioned earlier, recent studies utilizing this approach have identified somatic aldosterone-driver mutations in the vast majority of APA. In addition to the ability to interrogate multiple genomic regions simultaneously, one of the important advantages of NGS over Sanger sequencing is improved sensitivity for detecting genetic variants. This is particularly important for detecting somatic mutations in microscopic lesions (i.e., APCC/APM), for which the expected allelic variant fraction may be less than 20% (depending on the purity of the isolated tissue for sequencing). Application of targeted amplicon-based NGS to APCC in normal adrenal glands and from patients with adrenal idiopathic hyperaldosteronism has identified somatic aldosterone-driver mutations in 34-58% of these lesions (50–52). For aldosterone-producing lesions that are mutation-negative by targeted amplicon-based NGS, hybridization capture-based WES of CYP11B2 IHC-guided FFPE tissue may identify novel aldosterone-driver mutations (9, 36). Finally, despite several clear advantages of NGS-based molecular profiling, application of these approaches to FFPE tissue is potentially limited by FFPE-associated DNA degradation (e.g., increased genomic fragmentation, artifactual nucleotide deamination) and technical issues (e.g., PCR amplification bias, sequencing error). Emerging NGS methods, including the use of unique molecular identifiers (UMI; as known as "molecular barcodes"), and novel NGS technologies may begin to address some of these limitations and will continue to revolutionize genomic characterization of human tumors, including aldosterone-producing lesions.

CONCLUSIONS

Recent advances in sequencing technology have significantly accelerated PA research to elucidate its molecular pathogenesis. Unique histologic characteristics of adrenals from patients with PA require special attention to tumor CYP11B2 expression for accurate somatic mutation identification. The streamlined approach using CYP11B2 IHC-guided DNA capture combined with NGS appears to be a preferred method for mutational analysis of adrenals from patients with PA. The use of this CYP11B2 IHC-guided sequencing approach in a large prospective cohort will allow us to accurately determine APA mutation prevalence as well as genotype-phenotype correlations.

AUTHOR CONTRIBUTIONS

KN and WR conceived the idea of this review article. KN and AU drafted the manuscript. WR reviewed the manuscript and made edits on the contents. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by grants from National Institutes of Diabetes and Digestive and Kidney Disease (DK106618 and DK043140) to WR. KN is supported by the Japan Heart Foundation Research Grant.

ACKNOWLEDGMENTS

We would like to thank Amy R. Blinder for her editorial assistance.

REFERENCES

1. Choi M, Scholl UI, Yue P, Bjorklund P, Zhao B, Nelson-Williams C, et al. K+ Channel Mutations in Adrenal Aldosterone-Producing Adenomas and Hereditary Hypertension. *Science* (2011) 331:768–72. doi: 10.1126/science.1198785
2. Beuschlein F, Boulkroun S, Osswald A, Wieland T, Nielsen HN, Lichtenauer UD, et al. Somatic Mutations in ATP1A1 and ATP2B3 Lead to Aldosterone-Producing Adenomas and Secondary Hypertension. *Nat Genet* (2013) 45:440–4, 444e1-2. doi: 10.1038/ng.2550
3. Scholl UI, Goh G, Stolting G, de Oliveira RC, Choi M, Overton JD, et al. Somatic and Germline CACNA1D Calcium Channel Mutations in Aldosterone-Producing Adenomas and Primary Aldosteronism. *Nat Genet* (2013) 45:1050–4. doi: 10.1038/ng.2695
4. Azizan EA, Poulsen H, Tuluc P, Zhou J, Clausen MV, Lieb A, et al. Somatic Mutations in ATP1A1 and CACNA1D Underlie a Common Subtype of Adrenal Hypertension. *Nat Genet* (2013) 45:1055–60. doi: 10.1038/ng.2716
5. Scholl UI, Stolting G, Nelson-Williams C, Vichot AA, Choi M, Loring E, et al. Recurrent Gain of Function Mutation in Calcium Channel CACNA1H

Causes Early-Onset Hypertension With Primary Aldosteronism. *Elife* (2015) 4:e06315. doi: 10.7554/eLife.06315
6. Scholl UI, Stolting G, Schewe J, Thiel A, Tan H, Nelson-Williams C, et al. CLCN2 Chloride Channel Mutations in Familial Hyperaldosteronism Type II. *Nat Genet* (2018) 50:349–54. doi: 10.1038/s41588-018-0048-5
7. Fernandes-Rosa FL, Daniil G, Orozco IJ, Goppner C, El Zein R, Jain V, et al. A Gain-of-Function Mutation in the CLCN2 Chloride Channel Gene Causes Primary Aldosteronism. *Nat Genet* (2018) 50:355–61. doi: 10.1038/s41588-018-0053-8
8. Dutta RK, Arnesen T, Heie A, Walz M, Alesina P, Soderkvist P, et al. A Somatic Mutation in CLCN2 Identified in a Sporadic Aldosterone-Producing Adenoma. *Eur J Endocrinol* (2019) 181:K37–41. doi: 10.1530/EJE-19-0377
9. Nanba K, Blinder AR, Rege J, Hattangady NG, Else T, Liu CJ, et al. Somatic CACNA1H Mutation As a Cause of Aldosterone-Producing Adenoma. *Hypertension* (2020) 75:645–9. doi: 10.1161/HYPERTENSIONAHA.119.14349
10. Fernandes-Rosa FL, Boulkroun S, Zennaro MC. Genetic and Genomic Mechanisms of Primary Aldosteronism. *Trends Mol Med* (2020) 26:819–32. doi: 10.1016/j.molmed.2020.05.005
11. Akerstrom T, Maharjan R, Sven Willenberg H, Cupisti K, Ip J, Moser A, et al. Activating Mutations in CTNNB1 in Aldosterone Producing Adenomas. *Sci Rep* (2016) 6:19546. doi: 10.1038/srep19546
12. Wu VC, Wang SM, Chueh SJ, Yang SY, Huang KH, Lin YH, et al. The Prevalence of CTNNB1 Mutations in Primary Aldosteronism and Consequences for Clinical Outcomes. *Sci Rep* (2017) 7:39121. doi: 10.1038/srep39121
13. Scholl UI, Healy JM, Thiel A, Fonseca AL, Brown TC, Kunstman JW, et al. Novel Somatic Mutations in Primary Hyperaldosteronism Are Related to the Clinical, Radiological and Pathological Phenotype. *Clin Endocrinol (Oxf)* (2015) 83:779–89. doi: 10.1111/cen.12873
14. Nanba K, Omata K, Else T, Beck PCC, Nanba AT, Turcu AF, et al. Targeted Molecular Characterization of Aldosterone-Producing Adenomas in White Americans. *J Clin Endocrinol Metab* (2018) 103:3869–76. doi: 10.1210/jc.2018-01004
15. Cheng CJ, Sung CC, Wu ST, Lin YC, Sytwu HK, Huang CL, et al. Novel KCNJ5 Mutations in Sporadic Aldosterone-Producing Adenoma Reduce Kir3.4 Membrane Abundance. *J Clin Endocrinol Metab* (2015) 100(1):E155–63.
16. Williams TA, Monticone S, Schack VR, Stindl J, Burrello J, Buffolo F, et al. Somatic ATP1A1, ATP2B3, and KCNJ5 Mutations in Aldosterone-Producing Adenomas. *Hypertension* (2014) 63(1):188–95.
17. Hardege I, Xu S, Gordon RD, Thompson AJ, Figg N, Stowasser M, et al. Novel Insertion Mutation in KCNJ5 Channel Produces Constitutive Aldosterone Release From H295R Cell. *Mol Endocrinol* (2015) 29(10):1522–30.
18. Akerstrom T, Crona J, Delgado Verdugo A, Starker LF, Cupisti K, Willenberg HS, et al. Comprehensive Re-Sequencing of Adrenal Aldosterone Producing Lesions Reveal Three Somatic Mutations Near the KCNJ5 Potassium Channel Selectivity Filter. *PloS One* (2012) 7(7):e41926.
19. Zheng FF, Zhu LM, Zhou WL, Zhang Y, Li MY, Zhu YC, et al. A Novel Somatic Mutation 145-147deleteinsk in KCNJ5 Increases Aldosterone Production. *J Hum Hypertens* (2017) 31(11):756–9.
20. Wang B, Li X, Zhang X, Ma X, Chen L, Zhang Y, et al. Prevalence and Characterization of Somatic Mutations in Chinese Aldosterone-Producing Adenoma Patients. *Med (Baltimore)* (2015) 94(16):e708.
21. Nanba K, Omata K, Tomlins SA, Giordano TJ, Hammer GD, Rainey WE, et al. Double Adrenocortical Adenomas Harboring Independent KCNJ5 and PRKACA Somatic Mutations. *Eur J Endocrinol* (2016) 175(2):K1–6.
22. Zheng FF, Zhu LM, Nie AF, Li XY, Lin JR, Zhang K, et al. Clinical Characteristics of Somatic Mutations in Chinese Patients With Aldosterone-Producing Adenoma. *Hypertension* (2015) 65(3):622–8.
23. Kuppusamy M, Caroccia B, Stindl J, Bandulik S, Lenzini L, Gioco F, et al. A Novel KCNJ5-Inst149 Somatic Mutation Close to, But Outside, the Selectivity Filter Causes Resistant Hypertension by Loss of Selectivity for Potassium. *J Clin Endocrinol Metab* (2014) 99(9):E1765–73.
24. Taguchi R, Yamada M, Nakajima Y, Satoh T, Hashimoto K, Shibusawa N, et al. Expression and Mutations of KCNJ5 mRNA in Japanese Patients With Aldosterone-Producing Adenomas. *J Clin Endocrinol Metab* (2012) 97(4):1311–9.
25. Kitamoto T, Omura M, Suematsu S, Saito J, Nishikawa T. KCNJ5 Mutation as a Predictor for Resolution of Hypertension After Surgical Treatment of Aldosterone-Producing Adenoma. *J Hypertens* (2018) 36(3):619–27.
26. Azizan EA, Lam BY, Newhouse SJ, Zhou J, Kuc RE, Clarke J, et al. Microarray, qPCR, and KCNJ5 Sequencing of Aldosterone-Producing Adenomas Reveal Differences in Genotype and Phenotype Between Zona Glomerulosa- and Zona Fasciculata-Like Tumors. *J Clin Endocrinol Metab* (2012) 97(5):E819–29.
27. Mulatero P, Tauber P, Zennaro MC, Monticone S, Lang K, Beuschlein F, et al. KCNJ5 Mutations in European Families With Nonglucocorticoid Remediable Familial Hyperaldosteronism. *Hypertension* (2012) 59(2):235–40.
28. Akerstrom T, Willenberg HS, Cupisti K, Ip J, Backman S, Moser A, et al. Novel Somatic Mutations and Distinct Molecular Signature in Aldosterone-Producing Adenomas. *Endocr Relat Cancer* (2015) 22(5):735–44.
29. Guo Z, Nanba K, Udager A, McWhinney BC, Ungerer JPJ, Wolley M, et al. Biochemical, Histopathological, and Genetic Characterization of Posture-Responsive and Unresponsive APA. *J Clin Endocrinol Metab* (2020) 105(9):e3224–35.
30. Backman S, Akerstrom T, Maharjan R, Cupisti K, Willenberg HS, Hellman P, et al. RNA Sequencing Provides Novel Insights Into the Transcriptome of Aldosterone Producing Adenoma. *Sci Rep* (2019) 9(1):6269.
31. Wu VC, Huang KH, Peng KY, Tsai YC, Wu CH, Wang SM, et al. Prevalence and Clinical Correlates of Somatic Mutation in Aldosterone Producing Adenoma-Taiwanese Population. *Sci Rep* (2015) 5:11396.
32. Murakami M, Yoshimoto T, Minami I, Bouchi R, Tsuchiya K, Hashimoto K, et al. A Novel Somatic Deletion Mutation of ATP2B3 in Aldosterone-Producing Adenom. *Endocr Pathol* (2015) 26(4):328–33.
33. Kitamoto T, Suematsu S, Yamazaki Y, Nakamura Y, Sasano H, Matsuzawa Y, et al. Clinical and Steroidogenic Characteristics of Aldosterone-Producing Adenomas With ATPase or CACNA1D Gene Mutation. *J Clin Endocrinol Metab* (2016) 101(2):494–503.
34. Dutta RK, Welander J, Brauckhoff M, Walz M, Alesina P, Arnesen T, et al. Complementary Somatic Mutations of KCNJ5, ATP1A1, and ATP2B3 in Sporadic Aldosterone Producing Adrenal Adenomas. *Endocr Relat Cancer* (2014) 21(1):L1–4.
35. Tan GC, Negro G, Pinggera A, Tizen Laim NMS, Mohamed Rose I, Ceral J, et al. Aldosterone-Producing Adenomas: Histopathology-Genotype Correlation and Identification of a Novel CACNA1D Mutatio. *Hypertension* (2017) 70(1):129–36.
36. Rege J, Nanba K, Blinder AR, Plaska S, Udager AM, Vats P, et al. Identification of Somatic Mutations in CLCN2 in Aldosterone-Producing Adenomas. *J Endocr Soc* (2020) 4:bvaa123. doi: 10.1210/jendso/bvaa123
37. Kem DC, Weinberger MH, Higgins JR, Kramer NJ, Gomez-Sanchez C, Holland OB. Plasma Aldosterone Response to ACTH in Primary Aldosteronism and in Patients With Low Renin Hypertension. *J Clin Endocrinol Metab* (1978) 46:552–60. doi: 10.1210/jcem-46-4-552
38. Lefebvre H, Cartier D, Duparc C, Lihrmann I, Contesse V, Delarue C, et al. Characterization of Serotonin(4) Receptors in Adrenocortical Aldosterone-Producing Adenomas: *In Vivo* and *In Vitro* Studies. *J Clin Endocrinol Metab* (2002) 87:1211–6. doi: 10.1210/jcem.87.3.8327
39. Zwermann O, Suttmann Y, Bidlingmaier M, Beuschlein F, Reincke M. Screening for Membrane Hormone Receptor Expression in Primary Aldosteronism. *Eur J Endocrinol* (2009) 160:443–51. doi: 10.1530/EJE-08-0711
40. Albiger NM, Sartorato P, Mariniello B, Iacobone M, Finco I, Fassina A, et al. A Case of Primary Aldosteronism in Pregnancy: Do LH and GNRH Receptors Have a Potential Role in Regulating Aldosterone Secretion? *Eur J Endocrinol* (2011) 164:405–12. doi: 10.1530/EJE-10-0879
41. Lopez AG, Duparc C, Naccache A, Castanet M, Lefebvre H, Louiset E. Role of Mast Cells in the Control of Aldosterone Secretion. *Horm Metab Res* (2020) 52:412–20. doi: 10.1055/a-1119-1063
42. Sonoyama T, Sone M, Miyashita K, Tamura N, Yamahara K, Park K, et al. Significance of Adrenocorticotropin Stimulation Test in the Diagnosis of an Aldosterone-Producing Adenoma. *J Clin Endocrinol Metab* (2011) 96:2771–8. doi: 10.1210/jc.2011-0573
43. El Ghorayeb N, Bourdeau I, Lacroix A. Role of ACTH and Other Hormones in the Regulation of Aldosterone Production in Primary Aldosteronism. *Front Endocrinol (Lausanne)* (2016) 7:72. doi: 10.3389/fendo.2016.00072
44. St-Jean M, Bourdeau I, Martin M, Lacroix A. Aldosterone is Aberrantly Regulated by Various Stimuli in a High Proportion of Patients With Primary

Aldosteronism. *J Clin Endocrinol Metab* (2021) 106:e45–60. doi: 10.1210/clinem/dgaa703
45. Nishimoto K, Nakagawa K, Li D, Kosaka T, Oya M, Mikami S, et al. Adrenocortical Zonation in Humans Under Normal and Pathological Conditions. *J Clin Endocrinol Metab* (2010) 95:2296–305. doi: 10.1210/jc.2009-2010
46. Gomez-Sanchez CE, Qi X, Velarde-Miranda C, Plonczynski MW, Parker CR, Rainey W, et al. Development of Monoclonal Antibodies Against Human CYP11B1 and CYP11B2. *Mol Cell Endocrinol* (2014) 383:111–7. doi: 10.1016/j.mce.2013.11.022
47. Williams TA, Gomez-Sanchez CE, Rainey WE, Giordano TJ, Lam AK, Marker A, et al. International Histopathology Consensus for Unilateral Primary Aldosteronism. *J Clin Endocrinol Metab* (2021) 106:42–54. doi: 10.1210/clinem/dgaa484
48. Nanba K, Yamazaki Y, Bick N, Onodera K, Tezuka Y, Omata K, et al. Prevalence of Somatic Mutations in Aldosterone-Producing Adenomas in Japanese Patients. *J Clin Endocrinol Metab* (2020) 105:e4066–73. doi: 10.1210/clinem/dgaa595
49. Nanba K, Omata K, Gomez-Sanchez CE, Stratakis CA, Demidowich AP, Suzuki M, et al. Genetic Characteristics of Aldosterone-Producing Adenomas in Blacks. *Hypertension* (2019) 73:885–92. doi: 10.1161/HYPERTENSIONAHA.118.12070
50. Nishimoto K, Tomlins SA, Kuick R, Cani AK, Giordano TJ, Hovelson DH, et al. Aldosterone-Stimulating Somatic Gene Mutations Are Common in Normal Adrenal Glands. *Proc Natl Acad Sci USA* (2015) 112:E4591–9. doi: 10.1073/pnas.1505529112
51. Omata K, Anand SK, Hovelson DH, Liu CJ, Yamazaki Y, Nakamura Y, et al. Aldosterone-Producing Cell Clusters Frequently Harbor Somatic Mutations and Accumulate With Age in Normal Adrenals. *J Endocr Soc* (2017) 1:787–99. doi: 10.1210/js.2017-00134
52. Omata K, Satoh F, Morimoto R, Ito S, Yamazaki Y, Nakamura Y, et al. Cellular and Genetic Causes of Idiopathic Hyperaldosteronism. *Hypertension* (2018) 72:874–80. doi: 10.1161/HYPERTENSIONAHA.118.11086
53. Nanba AT, Nanba K, Byrd JB, Shields JJ, Giordano TJ, Miller BS, et al. Discordance Between Imaging and Immunohistochemistry in Unilateral Primary Aldosteronism. *Clin Endocrinol (Oxf)* (2017) 87:665–72. doi: 10.1111/cen.13442
54. Fernandes-Rosa FL, Giscos-Douriez I, Amar L, Gomez-Sanchez CE, Meatchi T, Boulkroun S, et al. Different Somatic Mutations in Multinodular Adrenals With Aldosterone-Producing Adenoma. *Hypertension* (2015) 66:1014–22. doi: 10.1161/HYPERTENSIONAHA.115.05993
55. Fernandes-Rosa FL, Williams TA, Riester A, Steichen O, Beuschlein F, Boulkroun S, et al. Genetic Spectrum and Clinical Correlates of Somatic Mutations in Aldosterone-Producing Adenoma. *Hypertension* (2014) 64:354–61. doi: 10.1161/HYPERTENSIONAHA.114.03419
56. Nanba K, Chen AX, Omata K, Vinco M, Giordano TJ, Else T, et al. Molecular Heterogeneity in Aldosterone-Producing Adenomas. *J Clin Endocrinol Metab* (2016) 101:999–1007. doi: 10.1210/jc.2015-3239
57. Dekkers T, ter Meer M, Lenders JW, Hermus AR, Schultze Kool L, Langenhuijsen JF, et al. Adrenal Nodularity and Somatic Mutations in Primary Aldosteronism: One Node is the Culprit? *J Clin Endocrinol Metab* (2014) 99:E1341–51. doi: 10.1210/jc.2013-4255
58. De Sousa K, Boulkroun S, Baron S, Nanba K, Wack M, Rainey WE, et al. Genetic, Cellular, and Molecular Heterogeneity in Adrenals With Aldosterone-Producing Adenoma. *Hypertension* (2020) 75:1034–44. doi: 10.1161/HYPERTENSIONAHA.119.14177

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

ORIGINAL RESEARCH
published: 16 July 2021
doi: 10.3389/fendo.2021.647184

Evaluation of Abdominal Computed Tomography Scans for Differentiating the Discrepancies in Abdominal Adipose Tissue Between Two Major Subtypes of Primary Aldosteronism

Kuan-Ming Chen[1,2,3,4], *Bo-Ching Lee*[5], *Po-Ting Chen*[5], *Kao-Lang Liu*[5], *Kuan-Heng Lin*[1,2,3,4], *Chin-Chen Chang*[5,6*], *Tung-Hsin Wu*[1,2*], *Jia-Sheng Hong*[1,2] *and Yen-Hung Lin*[7]

[1] *Department of Biomedical Imaging and Radiological Sciences, National Yang-Ming University, Taipei, Taiwan,* [2] *Department of Biomedical Imaging and Radiological Sciences, National Yang Ming Chiao Tung University, Hsinchu, Taiwan,* [3] *Industrial Ph.D. Program of Biomedical Science and Engineering, School of Biomedical Science and Engineering, National Yang-Ming University, Taipei, Taiwan,* [4] *Industrial Ph.D. Program of Biomedical Science and Engineering, School of Biomedical Science and Engineering, National Yang Ming Chiao Tung University, Hsinchu, Taiwan,* [5] *Department of Medical Imaging, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan,* [6] *Department and Graduate Institute of Forensic Medicine, National Taiwan University College of Medicine, Taipei, Taiwan,* [7] *Department of Internal Medicine, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan*

The aim of this study was to analyze the differences in the distribution of abdominal adipose tissue between the two subtypes of primary aldosteronism (PA) using abdominal computed tomography. We retrospectively analyzed patients diagnosed as having essential hypertension (EH) or PA from the prospectively collected Taiwan Primary Aldosteronism Investigation (TAIPAI) database. Patients with PA were divided into the subgroups of idiopathic hyperaldosteronism (IHA) and unilateral aldosterone-producing adenoma (APA). Patients' basic clinicodemographic data were collected, and a self-developed CT-based software program was used to quantify the abdominal adiposity indexes, including visceral adipose tissue (VAT) area, VAT ratio, waist circumference (WC), subcutaneous adipose tissue (SAT) area, and SAT ratio. We included 190 patients with EH and 436 patients with PA (238 with IHA and 198 with APA). The APA group had significantly lower abdominal adiposity indexes than the other groups. We also found negative correlations of aldosterone-to-renin ratio (ARR) with VAT area, VAT ratio, WC, and body mass index (BMI) in the APA group. After propensity score matching (which left 184 patients each in the IHA and APA groups), patients in the APA group still had significantly lower WC, SAT area, SAT ratio, and VAT ratio than those in the IHA group. Furthermore, logistic regression analysis indicated that lower probability of abdominal obesity was significantly related to patients with APA. Our data revealed that the distribution of abdominal adipose tissue was similar in patients with IHA and those with EH, but the abdominal adiposity indexes were significantly lower in patients with APA than in those with IHA and EH.

Keywords: primary aldosteronism, abdominal computed tomography, idiopathic hyperaldosteronism, aldosterone-producing adenoma, abdominal adiposity indexes

OPEN ACCESS

Edited by:
Qiang Wei,
Sichuan University, China

Reviewed by:
Nils Lambrecht,
VA Long Beach Healthcare System, United States
Jinbo Hu,
First Affiliated Hospital of Chongqing Medical University, China
Yan Ren,
Sichuan University West China Hospital, China

****Correspondence:***
Chin-Chen Chang
macotocc@gmail.com
Tung-Hsin Wu
tung7061@gmail.com

Specialty section:
This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Endocrinology

Received: *29 December 2020*
Accepted: *14 May 2021*
Published: *16 July 2021*

Citation:
Chen K-M, Lee B-C, Chen P-T, Liu K-L, Lin K-H, Chang C-C, Wu T-H, Hong J-S and Lin Y-H (2021) Evaluation of Abdominal Computed Tomography Scans for Differentiating the Discrepancies in Abdominal Adipose Tissue Between Two Major Subtypes of Primary Aldosteronism. Front. Endocrinol. 12:647184. doi: 10.3389/fendo.2021.647184

INTRODUCTION

PA is one of the most common types of endocrine hypertension, with a prevalence of 5%–13% in patients with hypertension (1, 2). It is characterized by high plasma aldosterone concentration (PAC) and low plasma renin activity (PRA). PA has been considered a rare disease, but recent studies have determined that up to 20% of patients with resistant hypertension have PA (3–5).

Abdominal obesity is a common risk factor for cardiovascular events in patients with hypertension. Patients with EH have a similar probability of developing lipid metabolism disorder to those with PA (6, 7). In addition, an increased PAC may unstable the metabolic complications associated with abdominal adiposity, leading to a high risk of morbidity and mortality (6, 8–10). This implies that the etiology for developing the metabolic syndromes of obesity, dyslipidemia, and hyperglycemia in patients with PA might differ from that in patients with EH (11, 12).

Among patients with PA, 64% have IHA caused by bilateral adrenal hyperplasia and 27% have APA (13). Moreover, two subtypes of PA (IHA and APA) have different pathogeneses, and patients with APA have much higher levels of aldosterone than do those with IHA (14, 15). Therefore, patients with APA should theoretically experience severer obesity-related disorders than those with IHA due to aldosterone excess. However, studies have reported that patients with IHA have more metabolic disorders and a higher prevalence of obesity than those with APA (16, 17). We accordingly assumed that the distribution and mechanisms of the development of abdominal obesity in the two subtypes of PA should also be different. Therefore, using patients with EH as the control group, we investigated the relationship between abdominal fat tissue distribution and pathophysiology, and the factors affecting them, in the two subtypes of PA.

MATERIALS AND METHODS

Case Collection

This was a retrospective analysis of a prospectively collected database. We enrolled patients diagnosed as having EH and PA from the Taiwan Primary Aldosteronism Investigation (TAIPAI) database from 2010 to 2018. We collected patients' abdominal computed tomography (CT) scans and basic clinicodemographic data, including sex, age, BMI, systolic blood pressure (SBP), diastolic blood pressure (DBP), potassium ion concentration, PAC, PRA, ARR, and estimated glomerular filtration rate (eGFR). Patients' history of hypertension and type 2 diabetes (T2D) were also collected. All patient identifying information was removed from the CT scans before analysis, and the requirement to obtain informed consent was waived by the institutional review board.

Classification of PA Subtypes

PA was diagnosed based on the following biochemical criteria: (a) autonomous excess aldosterone production evidenced with an ARR > 35; (b) a TAIPAI score > 60% (18); and (c) post-saline loading PAC > 10 ng/dL, ARR > 35 in a post-captopril/losartan test, or PAC > 6 ng/dL indicated by a fludrocortisone suppression test.

The classification of PA into subtypes was based on TAIAPI experience (2, 19). IHA was diagnosed according to the following criteria: (a) evidence of bilateral diffuse enlargement on preoperative computed tomography (CT); (b) nonlateralization of aldosterone secretion during adrenal venous sampling; and (c) diffuse cell hyperplasia on biopsy of resected specimen in patients who underwent an operation. APA was diagnosed based on the following criteria: (a) biochemical finding of PA and the evidence of adenoma on preoperative CT; (b) lateralization of aldosterone secretion during adrenal venous sampling; and (c) pathologically proven adenoma after adrenalectomy and (d) the subsequent emergence of either a cure pattern of hypertension without antihypertensive agents and improvement in hypertension, potassium, PAC, and PRA (14).

Quantification of Abdominal Adipose Tissue Using Multidetector CT

CT was used to calculate WC and quantify the parameters of abdominal adipose tissue. The CT scanning parameters were as follows: the tube voltage was 120 kVp, and the tube current was under automated exposure control. Slice thickness was 5 mm, and the scan range went from the upper edge of T12 to S1. A self-developed program written on the MATLAB platform was used to measure abdominal adipose tissue and its relevant indexes. To set the threshold segmentation, the Hounsfield unit (HU) values were set between −190 and −30 for fat and between −29 and 150 for muscle (20). The procedure is depicted in **Figure 1**. First, a slice of the umbilical area was selected (**Figure 1A**) to measure WC (**Figure 1B**). Next, one of the slices at L4 transverse process was selected (**Figures 1C, D**). The regions of interest in the layer of VAT, layer of muscle, and layer of SAT were circled on the slice to calculate the area of the total abdomen (**Figure 1E**). Finally, the set HU values for fat was used for threshold segmentation, and the corresponding SAT and VAT areas were calculated (**Figure 1F**). To eliminate individual differences, the SAT and VAT areas were divided by the total abdomen area to ensure standardized SAT and VAT ratios.

Statistical Analysis

Statistical analysis was performed using SPSS v22.0 (IBM Corp., Armonk, NY, USA). Data were presented as mean ± standard deviation for continuous variables and as percentage for categorical variables. Nonnormally distributed variables, such as PAC, PRA, and ARR, were expressed as median and interquartile range. Significant difference were compared using one-way ANOVA with the Bonferroni post-hoc for continuous variables, Chi-square test for categorical variables and Kruskal–Wallis tests for nonnormally distributed variables among EH, IHA, and APA groups or between any two groups. Spearman's rank correlation was calculated for each of the three groups, and linear correlations among BMI, abdominal adiposity indexes, and ARR were analyzed.

A 1:1 propensity score matching (PSM) analysis was conducted. Propensity scores were estimated to control for

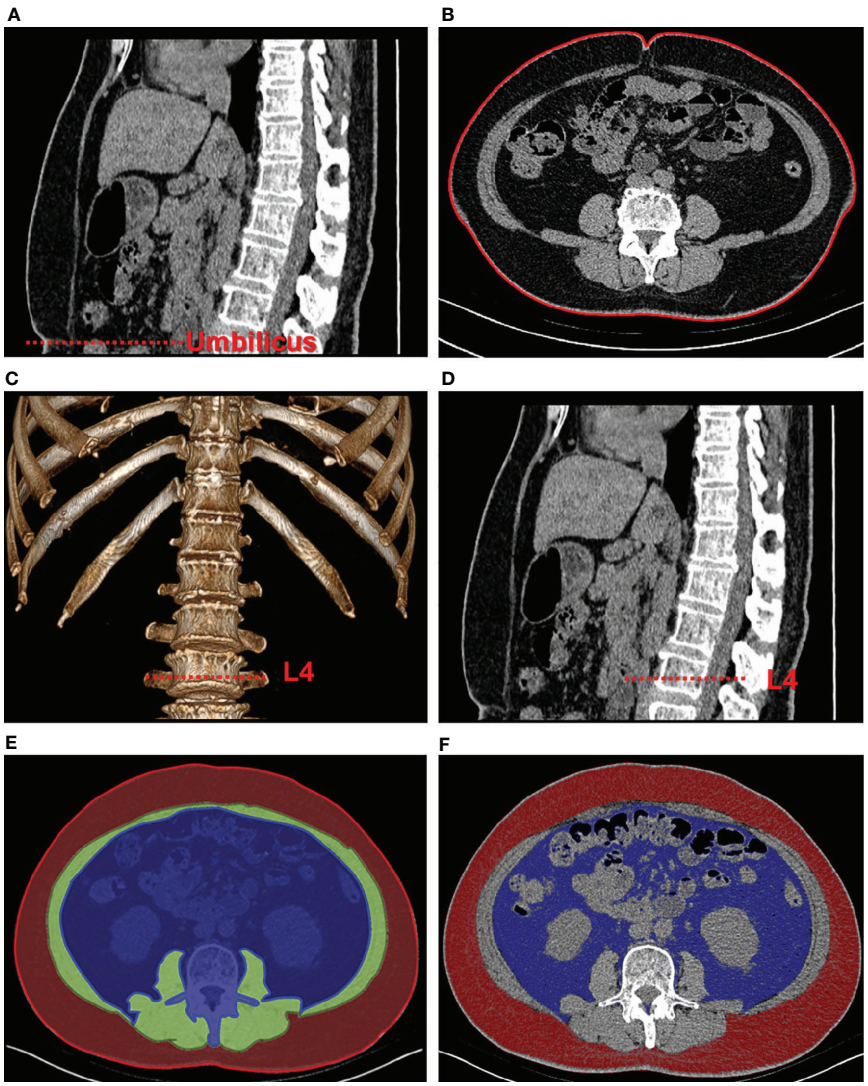

FIGURE 1 | Schematic of abdominal adipose tissue quantification using computed tomographic images. The sagittal plane **(A)** was used to locate the umbilical area, and one of the corresponding transverse planes **(B)** was selected to measure waist circumference. Then, volume rendering **(C)** was used to locate the L4 area **(D)**. The regions of interest of visceral fat (blue), muscular layer (green), and subcutaneous fat (red) were circled in one of the transverse planes **(E)**. Quantification of the fat areas was completed through threshold segmentation **(F)**.

possible confounding bias, such as sex, age, and BMI between any two groups in this study. The independent samples t test was used to compare continuous variables between two groups. We also performed univariate and multivariate logistic regression analysis to detect the relationship between the two subtypes of PA, clinical data and abdominal adiposity indexes.

RESULTS

EH *vs*. PA

We included 190 patients diagnosed with EH and 436 diagnosed with PA. The EH group had a higher proportion of men than the PA group did, but age and BMI were not significantly different between the two groups. The PA group exhibited significantly higher SBP, higher DBP, and longer duration of hypertension compared with the EH group. Significantly higher PAC, higher ARR, lower PRA, and lower potassium ion concentration were observed in the PA group compared with the EH group. Analysis of abdominal adiposity indexes indicated that the PA group had significantly lower WC, total abdomen area, VAT area, and VAT ratio than the EH group. The results of before and after PSM for sex between EH and PA groups are presented in **Table S1**.

EH *vs*. Two Subtypes of PA

In total, 238 patients with IHA and 198 patients with APA who were scheduled to undergo adrenalectomy. **Table 1** compares the clinical and demographic variables between the groups. The EH

TABLE 1 | Comparison of clinicodemographic data and abdominal adiposity indexes among the EH, IHA, and APA groups.

Variables	EH (n = 190)	IHA (n = 238)	APA (n = 198)	Overall *p*-value	*p*-value between each two groups		
					EH *vs*. IHA	EH *vs*. APA	IHA *vs*. APA
Clinicodemographic data							
Sex, male (%)[a]	115 (61%)	110 (46%)	101 (51%)	*<0.05*	*<0.05*	*0.18*	*0.95*
Age, years	54.56 ± 14.85	54.39 ± 11.10	51.29 ± 10.79	*<0.05*	*1.00*	*<0.05*	*<0.05*
BMI, kg/m^2	25.93 ± 4.90	26.03 ± 3.89	24.95 ± 4.11	*<0.05*	*1.00*	*0.08*	*<0.05*
Duration of hypertension, years	5.41 ± 7.92	7.39 ± 8.41	6.56 ± 6.33	*<0.05*	*<0.05*	*0.42*	*0.79*
Presence of type 2 diabetes (%)[a]	25 (13%)	45 (19%)	34 (17%)	*0.26*	*0.33*	*0.87*	*1.00*
SBP, mmHg	146.76 ± 26.29	153.39 ± 18.96	153.78 ± 20.80	*<0.01*	*<0.01*	*<0.01*	*1.00*
DBP, mmHg	86.77 ± 16.49	92.96 ± 13.01	92.71 ± 13.96	*<0.001*	*<0.001*	*<0.001*	*1.00*
Potassium, mmol/L	4.11 ± 0.44	3.83 ± 0.56	3.49 ± 0.61	*<0.001*	*<0.001*	*<0.001*	*<0.001*
PAC[b], ng/dL	31.48 (22.27 to 46.63)	42.20 (31.50 to 61.95)	45.09 (30.78 to 74.80)	*<0.001*	*<0.05*	*<0.001*	*<0.05*
PRA[b], ng/mL/h	1.67 (0.33 to 4.95)	0.31 (0.10 to 0.62)	0.23 (0.10 to 0.56)	*<0.001*	*<0.001*	*<0.001*	*0.67*
ARR[b]	20.95 (9.62 to 99.77)	153.46 (70.34 to 422.84)	233.60 (70.13 to 640.50)	*<0.001*	*0.14*	*<0.001*	*<0.001*
eGFR, mL/min/1.73m^2	85.96 ± 25.12	92.73 ± 32.11	88.80 ± 25.86	*0.11*	*0.16*	*1.00*	*0.47*
Abdominal adiposity indexes							
WC, cm	84.61 ± 10.26	84.17 ± 9.80	80.52 ± 9.84	*<0.001*	*1.00*	*<0.001*	*<0.001*
Total abdomen area, cm^2	624.36 ± 166.42	606.30 ± 146.13	575.39 ± 157.99	*<0.01*	*0.70*	*<0.01*	*0.12*
SAT area, cm^2	172.04 ± 79.00	178.77 ± 71.29	150.21 ± 63.06	*<0.001*	*0.99*	*<0.01*	*<0.001*
VAT area, cm^2	162.85 ± 85.38	156.04 ± 72.06	131.54 ± 82.89	*<0.001*	*1.00*	*<0.001*	*<0.01*
SAT ratio	0.27 ± 0.07	0.29 ± 0.08	0.25 ± 0.07	*<0.001*	*<0.01*	*0.42*	*<0.001*
VAT ratio	0.24 ± 0.08	0.24 ± 0.07	0.21 ± 0.08	*<0.001*	*1.00*	*<0.001*	*<0.001*

Data were presented as mean ± SD, median (interquartile range), or number (%). EH, essential hypertension; IHA, idiopathic hyperaldosteronism; APA, aldosterone-producing adenoma; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PAC, plasma aldosterone concentration; PRA, plasma renin activity; ARR, aldosterone–renin ratio; WC, waist circumference; SAT area, area of subcutaneous adipose tissue; VAT area, area of visceral adipose tissue; SAT ratio was calculated by dividing SAT area by total abdomen area; VAT ratio was calculated by dividing VAT area by total abdomen area.

[a]chi-square test; [b]Kruskal-Wallis test.

group had a significantly higher proportion of men than the IHA group did, but not compared with the APA group. SBP and DBP were higher in the IHA and APA groups than in the EH group, but the duration of hypertension was significantly longer in patients with IHA than in those with EH. Compared with the EH group, the APA group had significantly higher ARR and both the APA and IHA groups had significantly higher PAC, higher ARR, lower PRA, and lower potassium ion concentration. Analysis of abdominal adiposity indexes indicated that the SAT ratio was significantly higher in the IHA group than in the EH group. However, the EH and IHA groups did not differ significantly in terms of WC, total abdomen area, SAT area, VAT area, and VAT ratio. By contrast, the APA group had significantly lower WC, total abdomen area, SAT area, VAT area, and VAT ratio than the EH group did. The results of PSM for sex, age, and BMI for the EH group *vs*. the IHA group and the EH group *vs*. the APA group are presented in **Table S2**.

Relationships of ARR With Factors

No significant correlations were observed between ARR, abdominal adiposity indexes, and BMI in the PA group. However, in the APA group, negative correlations of ARR were identified with VAT area ($r = -0.174$, $p < 0.05$; **Figure 2A**), VAT ratio ($r = -0.177$, $p < 0.05$; **Figure 2B**), WC ($r = -0.182$, $p < 0.05$; **Figure 2C**), and BMI ($r = -0.176$, $p < 0.05$; **Figure 2D**); however, no statically significant correlations were observed with total abdomen area ($r = -0.152$, $p = 0.06$), SAT area ($r = -0.132$, $p = 0.09$), or SAT ratio ($r = -0.038$, $p = 0.63$). By contrast, in the patients with EH or IHA, no statically significant correlations were observed of ARR with any factors.

IHA *vs*. APA

As shown in **Table 1**, age and BMI were significantly higher in the IHA group than in the APA group. PAC and ARR were significantly higher and potassium ion concentration was significantly lower in patients with APA than in those with IHA. Analysis of abdominal adiposity indexes indicated that the APA group had significantly lower WC, SAT area, VAT area, SAT ratio, and VAT ratio than the other groups.

The clinical data and abdominal adiposity indexes after PSM for age and BMI, which led to 184 patients each in IHA and APA groups, are listed in **Table 2**. No significant differences in sex, duration of hypertension, presence of T2D, SBP, DBP, PAC, or PRA were noted. However, as was the case before PSM, ARR remained significantly higher and potassium ion concentration remained significantly lower in patients with APA than in those with IHA. Furthermore, the APA group had significantly lower values of all the abdominal adiposity indexes than the IHA group, including WC, SAT area, SAT ratio and VAT ratio (**Figure 3**).

Univariate and Multivariate Logistic Regression Analysis Between IHA and APA

Logistic regression for WC, SAT ratio, VAT ratio, potassium ion concentration, ARR and eGFR were performed to distinguish APA from IHA after PSM for age and BMI. As shown in **Table 3**,

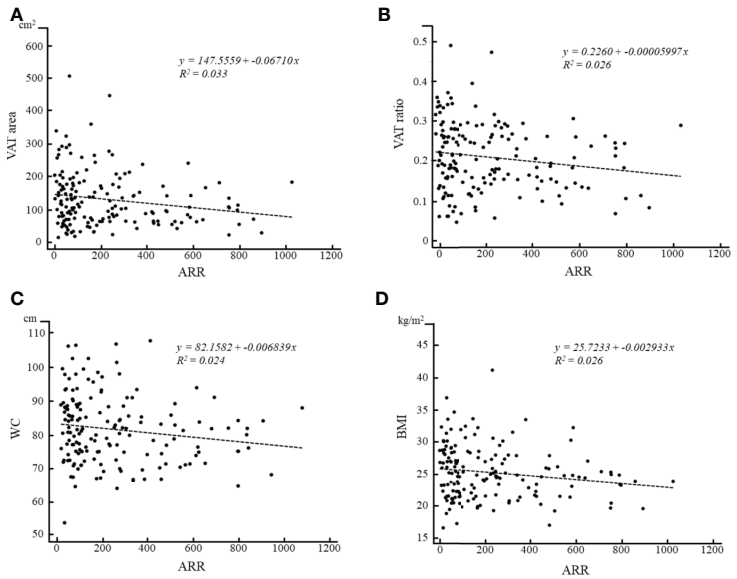

FIGURE 2 | Correlations of ARR with **(A)** VAT area, **(B)** VAT ratio, **(C)** WC and **(D)** BMI in patients with APA.

TABLE 2 | Comparison of clinicodemographic data and abdominal adiposity indexes between the IHA and APA groups after propensity score matching for age and BMI.

Variables	IHA (n = 184)	APA (n = 184)	*p*-value
Clinicodemographic data			
Sex, male (%)[(a)]	96 (52%)	89 (47%)	*0.39*
Age, years	53.35 ± 11.06	52.66 ± 9.72	*0.52*
BMI, kg/m^2	25.96 ± 3.83	25.21 ± 4.08	*0.07*
Duration of hypertension, years	7.08 ± 8.41	6.87 ± 6.43	*0.79*
Presence of type 2 diabetes (%)[(a)]	28 (15%)	33 (18%)	*0.31*
SBP, mmHg	152.32 ± 18.65	153.89 ± 20.56	*0.44*
DBP, mmHg	92.60 ± 12.36	92.22 ± 13.53	*0.77*
Potassium, mmol/L	3.82 ± 0.54	3.51 ± 0.61	*<0.001*
PAC[(b)], ng/dL	42.600 (31.90 to 62.60)	45.500 (30.55 to 74.72)	*0.14*
PRA[(b)], ng/mL/h	0.310 (0.10 to 0.62)	0.220 (0.097 to 0.52)	*0.18*
ARR[(b)]	155.750 (67.81 to 455.86)	235.380 (70.34 to 620.35)	*<0.01*
eGFR, mL/min/1.73m^2	93.84 ± 33.91	87.21 ± 24.23	*<0.05*
Abdominal adiposity indexes			
WC, cm	84.22 ± 9.51	81.39 ± 9.48	*<0.01*
Total abdomen area, cm^2	597.32 ± 140.91	586.31 ± 157.00	*0.48*
SAT area, cm^2	173.62 ± 68.27	153.61 ± 63.28	*<0.01*
VAT area, cm^2	151.73 ± 70.52	137.09 ± 83.04	*0.06*
SAT ratio	0.58 ± 0.15	0.52 ± 0.14	*<0.001*
VAT ratio	0.24 ± 0.07	0.21 ± 0.08	*<0.001*

Data were presented as mean ± SD, median (interquartile range), or number (%). IHA, idiopathic hyperaldosteronism; APA, aldosterone-producing adenoma; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PAC, plasma aldosterone concentration; PRA, plasma renin activity; ARR, aldosterone–renin ratio; WC, waist circumference; SAT area, area of subcutaneous adipose tissue; VAT area, area of visceral adipose tissue; SAT ratio was calculated by dividing SAT area by total abdomen area; VAT ratio was calculated by dividing VAT area by total abdomen area.
[(a)]chi-square test; [(b)]Kruskal-Wallis test.

our multivariate regression analysis data revealed that patients with APA exhibited lower SAT ratio, VAT ratio and potassium ion concentration than patients with IHA. We additionally provide regression data before executing PSM in **Table S3**, the results also show that patients with APA has a lower chance of developing abdominal obesity and more severe hypokalemia, and are irrelevant to age and BMI.

DISCUSSION

This study is unique in that it involves 436 sets of abdominal CT scans and clinical data from patients with PA; in contrast to other studies, we added an additional 190 sets of data from patients with EH for comparison. The strength of our study is the direct use of CT scans to assess the abdominal component, in particular, the SAT and VAT can be measured directly and quantified using the following indexes, area and ratio. Our approach should be more convincing than other previous studies that use weight, BMI, or WC for assessment (16, 17). Our study reveals that the APA group had significantly lower abdominal adiposity indexes than the other groups. We also found negative correlations of ARR with VAT area, VAT ratio, WC, and BMI in the APA group. After PSM, patients in the APA group still had significantly lower WC, SAT area, SAT ratio, and VAT ratio than those in the IHA group. Furthermore, the logistic regression analysis indicated that lower probability of abdominal obesity was significantly related to patients with APA.

Adipose tissue is involved in many physiological and pathological processes. Excessive adipose tissue often causes with excessive PAC, causing the mineralocorticoids continuously activated and strengthened. This finally leads to the inflammation and adipocyte differentiation (21–24). Studies have indicated that

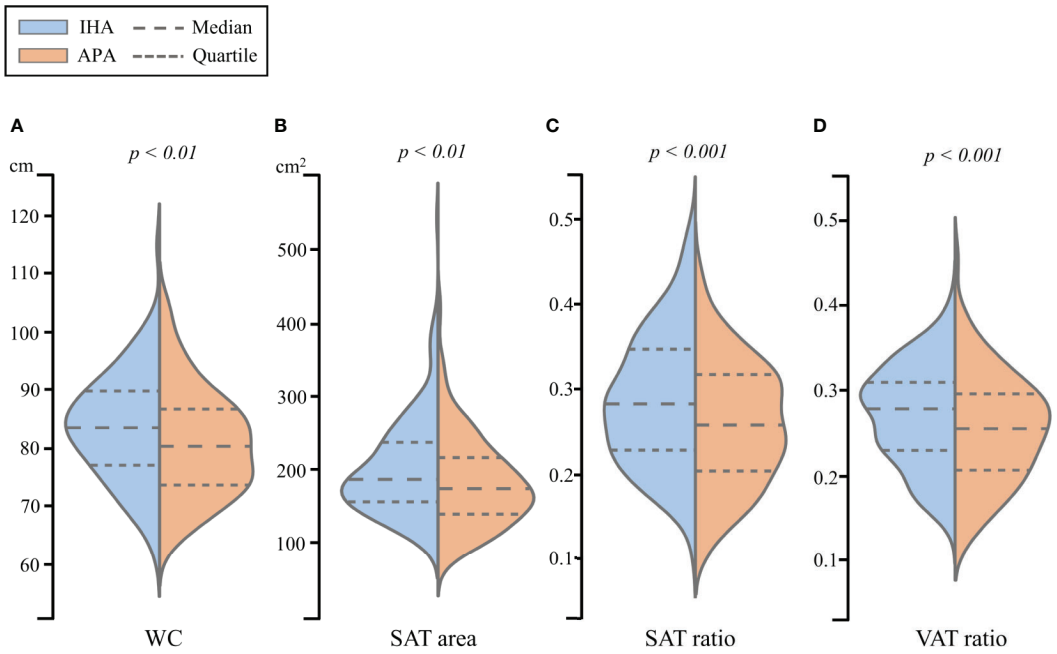

FIGURE 3 | The violin plot of **(A)** WC, **(B)** SAT area, **(C)** SAT ratio and **(D)** VAT ratio for propensity score matched patients between IHA and APA.

TABLE 3 | Logistic regression analysis of WC, SAT ratio, VAT ratio, potassium concentration, ARR and eGFR between IHA and APA group after propensity score matching for age and BMI.

Variables	Univariate Regression Analysis				Multivariate Regression Analysis			
	β	OR	95% CI of OR	*p*-value	*β*	OR	95% CI of OR	*p*-value
WC, cm	-0.032	0.969	0.948 – 0.991	*<0.05*	-0.004	0.996	0.966 - 1.027	*0.793*
SAT ratio	-5.138	0.006	0.000 – 0.096	*<0.01*	-4.559	0.010	0.000 - 0.336	*<0.05*
VAT ratio	-3.834	0.022	0.002 – 0.289	*<0.05*	-5.271	0.005	0.000 - 0.216	*<0.01*
Potassium, mmol/L	-0.930	0.394	0.269 – 0.579	*<0.001*	-0.828	0.437	0.290 - 0.658	*<0.001*
ARR	0.001	1.000	1.000 – 1.000	*<0.01*	0.001	1.000	1.000 - 1.000	*<0.05*
eGFR, mL/min/1.73m^2	-0.008	0.992	0.985 – 0.999	*<0.05*	-0.008	0.992	0.984 - 1.001	*0.073*

IHA, idiopathic hyperaldosteronism; APA, aldosterone-producing adenoma; β, Coefficient of regression equation; OR, Odds ratio; CI, Confidence interval; WC, waist circumference; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; SAT ratio was calculated by dividing SAT area by total abdomen area; VAT ratio was calculated by dividing VAT area by total abdomen area; ARR, aldosterone–renin ratio. For the OR, IHA group was coded as 0, APA group was coded as 1.

the prevalence of obesity or dyslipidemia does not differ significantly between EH and PA groups (6, 25). Although our results indicated that the PA group had significantly lower WC and VAT than the EH group did, these differences disappeared after PSM for sex (**Table S1**).

However, PSM for sex, age, and BMI yielded no significant differences in WC or other abdominal adiposity indexes between patients with EH and IHA, whereas patients with APA had significantly lower WC, total abdomen area, SAT and VAT than those with EH (**Table S2**). This demonstrates that the metabolic phenotypes of obesity are similar in the EH and IHA groups, which is consistent with previous research (7). The present data suggested that, in contrast to patients with IHA, the metabolic profile of patients with APA might be a crucial factor that lowers the obesity prevalence in the overall PA population.

Distribution of abdominal fat is an important predictor of cardiovascular risk, increasing accumulation of SAT and VAT are associated with several cardiac diseases, particularly the VAT is an independent factor for metabolic syndrome (MetS) in patients with obesity (26). Whereas, interesting was that recent studies had indicated that patients with APA had higher risk of developing cardiac events among the PA population (27, 28). And despite having lower PAC than patients with APA, only those with IHA are more likely to develop MetS (16, 17).

Our study at least provides another point of view to support these inconsistent issues. In this study, patients with IHA and APA exhibited different distributions of abdominal adipose tissue. Even after controlling for age and BMI, patients with APA exhibited significant lower WC, SAT and VAT than patients

with IHA. According to our logistic regression analysis, patients with IHA and APA exhibited significant differences in SAT and VAT. Consequently, it can be assumed that the higher incidence of cardiac events in the APAs is not associate with obesity, but may be direct related to the toxicity of chronic excess aldosterone.

ARR is an indicator of disorder in aldosterone activity and is useful for the diagnosis of PA (29). In particular, ARR is highly sensitive and specific in diagnosing APA (30, 31). In the present study, ARR was significantly negatively correlated with VAT area, VAT ratio, WC, and BMI in patients with APA. These findings were consistent with Er et al., who indicated that patients with APA had relatively smaller abdominal fat distribution, but the VAT will be increased after unilateral adrenalectomy (32). However, relevant study is still sparse, and the exact pathomechanisms remain clarified in the future.

No relationship was observed between ARR and other factors in patients with IHA. By contrast, Shibayama et al. (33) had reported that PAC was positively proportional to visceral fat distribution in patients with IHA. Taken together, the findings indicate that the relationship of abdominal obesity with aldosterone activity may differ between IHA and APA. These findings are in line with the notion that long-term aldosterone excess in patients with APA may lead to inflammation and fibrosis of perirenal fat tissue, eventually resulting in reduced abdominal adiposity indexes (34).

This retrospective study had the following limitations. First, we did not exclude autonomous cortisol secretion in patients with APA. Cortisol hypersecretion can be associated with obesity. Second, we did not evaluate laboratory data, such as lipid profile and HOMA-IR index, but these data have been proven to be irrelevant to abdominal fat accumulation (32). Third, this retrospective study did not take the effects of relevant medication history into account, such as antihypertensives or statins.

In conclusion, our data revealed that the abdominal adipose tissue indexes were similar in patients with IHA and those with EH but were markedly lower in patients with APA. Furthermore, ARR was negatively correlated with VAT area, VAT ratio, WC, and BMI in patients with APA but not in patients with IHA. These findings provide new insights into the relationship between adipose tissue and aldosterone excess in patients with APA and IHA. This suggests that patients with APA could be relatively lean during clinical practice, but still need to beware of higher risk of cardiac disease, such as heart failure, atrial fibrillation, ischemic heart disease, and other vascular events.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by National Taiwan University Hospital Research Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

K-MC and J-SH carried out the experiment. K-MC wrote the manuscript with support from B-CL, P-TC, and K-HL. K-LL and Y-HL conceived the original idea. C-CC and T-HW supervised the project. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by the research grants MOST109-2314-B-010 -023 -MY3 from the Ministry of Science and Technology, Taipei, Taiwan to T-HW.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.647184/full#supplementary-material

REFERENCES

1. Loh KC, Koay ES, Khaw MC, Emmanuel SC, Young WF Jr. Prevalence of Primary Aldosteronism Among Asian Hypertensive Patients in Singapore. *J Clin Endocrinol Metab* (2000) 85:2854–9. doi: 10.1210/jcem.85.8.6752
2. Wu VC, Hu YH, Er LK, Yen RF, Chang CH, Chang YL, et al. Case Detection and Diagnosis of Primary Aldosteronism - The Consensus of Taiwan Society of Aldosteronism. *J Formos Med Assoc* (2017) 116:993–1005. doi: 10.1016/j.jfma
3. Rossi GP, Bernini G, Caliumi C, Desideri G, Fabris B, Ferri C, et al. A Prospective Study of the Prevalence of Primary Aldosteronism in 1,125 Hypertensive Patients. *J Am Coll Cardiol* (2006) 48:2293–300. doi: 10.1016/j.jacc.2006.07.059
4. Douma S, Petidis K, Doumas M, Papaefthimiou P, Triantafyllou A, Kartali N, et al. Prevalence of Primary Hyperaldosteronism in Resistant Hypertension: A Retrospective Observational Study. *Lancet* (2008) 371:1921–6. doi: 10.1016/S0140-6736(08)60834-X
5. Calhoun DA. Hyperaldosteronism as a Common Cause of Resistant Hypertension. *Annu Rev Med* (2013) 64:233–47. doi: 10.1146/annurev-med-042711-135929
6. Matrozova J, Steichen O, Amar L, Zacharieva S, Jeunemaitre X, Plouin PF. Fasting Plasma Glucose and Serum Lipids in Patients With Primary Aldosteronism: A Controlled Cross-Sectional Study. *Hypertension* (2009) 53:605–10. doi: 10.1161/HYPERTENSIONAHA.108.122002
7. Somlóová Z, Widimský J Jr, Rosa J, Wichterle D, Strauch B, Petrák O, et al. The Prevalence of Metabolic Syndrome and Its Components in Two Main Types of Primary Aldosteronism. *J Hum Hypertens* (2010) 24:625–30. doi: 10.1038/jhh.2010.65
8. Krug AW, Ehrhart-Bornstein M. Aldosterone and Metabolic Syndrome: Is Increased Aldosterone in Metabolic Syndrome Patients an Additional Risk Factor? *Hypertension* (2008) 51:1252–8. doi: 10.1161/HYPERTENSIONAHA
9. Wu VC, Chueh SJ, Chen L, Chang CH, Hu YH, Lin YH, et al. Risk of New-Onset Diabetes Mellitus in Primary Aldosteronism: A Population Study Over 5 Years. *J Hypertens* (2017) 35:1698–708. doi: 10.1097/HJH.0000000000001361

10. Beuschlein F, Reincke M, Arlt W. The Impact of Connshing's Syndrome – Mild Cortisol Excess in Primary Aldosteronism Drives Diabetes Risk. *J Hypertens* (2017) 35(12):2548. doi: 10.1097/HJH.0000000000001550
11. Fallo F, Pilon C, Urbanet R. Primary Aldosteronism and Metabolic Syndrome. *Horm Metab Res* (2012) 44:208–14. doi: 10.1055/s-0031-1295412
12. Goodfriend TL, Egan B, Stepniakowski K, Ball DL. Relationships Among Plasma Aldosterone, High-Density Lipoprotein Cholesterol, and Insulin in Humans. *Hypertension* (1995) 25:30–6. doi: 10.1161/01.hyp.25.1.30
13. Monticone S, Burrello J, Tizzani D, Bertello C, Viola A, Buffolo F, et al. Prevalence and Clinical Manifestations of Primary Aldosteronism Encountered in Primary Care Practice. *J Am Coll Cardiol* (2017) 69:1811–20. doi: 10.1016/j.jacc.2017.01.052
14. Chao CT, Wu VC, Kuo CC, Lin YH, Chang CC, Chueh SJ, et al. Diagnosis and Management of Primary Aldosteronism: An Updated Review. *Ann Med* (2013) 45:375–83. doi: 10.3109/07853890.2013.785234
15. Huang KH, Yu CC, Hu YH, Chang CC, Chan CK, Liao SC, et al. Targeted Treatment of Primary Aldosteronism - The Consensus of Taiwan Society of Aldosteronism. *J Formos Med Assoc* (2019) 118:72–82. doi: 10.1016/j.jfma.2018.01.006
16. Ohno Y, Sone M, Inagaki N, Yamasaki T, Ogawa O, Takeda Y, et al. Obesity as a Key Factor Underlying Idiopathic Hyperaldosteronism. *J Clin Endocrinol Metab* (2018) 103:4456–64. doi: 10.1210/jc.2018-00866
17. Zhang Z, Luo Q, Tuersun T, Wang G, Wu T, Zhang D, et al. Higher Prevalence of Metabolic Disorders in Patients With Bilateral Primary Aldosteronism Than Unilateral Primary Aldosteronism. *Clin Endocrinol (Oxf)* (2021) 94:3–11. doi: 10.1111/cen.14318
18. Wu VC, Yang SY, Lin JW, Cheng BW, Kuo CC, Tsai CT, et al. Kidney Impairment in Primary Aldosteronism. *Clin Chim Acta* (2011) 412:1319–25. doi: 10.1016/j.cca.2011.02.018
19. Chang CC, Chen YY, Lai TS, Zeng YH, Chen CK, Tu KH, et al. Taiwan Mini-Frontier of Primary Aldosteronism: Updating Detection and Diagnosis. *J Formos Med Assoc* (2020) 120:121–9. doi: 10.1016/j.jfma.2020.08.001
20. Aubrey J, Esfandiari N, Baracos VE, Buteau FA, Frenette J, Putman CT, et al. Measurement of Skeletal Muscle Radiation Attenuation and Basis of its Biological Variation. *Acta Physiol (Oxf)* (2014) 210:489–97. doi: 10.1111/apha.12224
21. Nagase M, Yoshida S, Shibata S, Nagase T, Gotoda T, Ando K, et al. Enhanced Aldosterone Signaling in the Early Nephropathy of Rats With Metabolic Syndrome: Possible Contribution of Fat-Derived Factors. *J Am Soc Nephrol* (2006) 17:3438–46. doi: 10.1681/ASN.2006080944
22. Colussi G, Catena C, Lapenna R, Nadalini E, Chiuch A, Sechi LA. Insulin Resistance and Hyperinsulinemia Are Related to Plasma Aldosterone Levels in Hypertensive Patients. *Diabetes Care* (2007) 30:2349–54. doi: 10.2337/dc07-0525
23. Yuan Y, Xu X, Zhao C, Zhao M, Wang H, Zhang B, et al. The Roles of Oxidative Stress, Endoplasmic Reticulum Stress, and Autophagy in Aldosterone/Mineralocorticoid Receptor-Induced Podocyte Injury. *Lab Invest* (2015) 95:1374–86. doi: 10.1038/labinvest.2015.118
24. Clemente-Postigo M, Tinahones A, El Bekay R, Malagón MM, Tinahones FJ. The Role of Autophagy in White Adipose Tissue Function: Implications for Metabolic Health. *Metabolites* (2020) 10:179. doi: 10.3390/metabo10050179
25. Pimenta E, Calhoun DA. Aldosterone and Metabolic Dysfunction: An Unresolved Issue. *Hypertension* (2009) 53:585–6. doi: 10.1161/HYPERTENSIONAHA.108.123406
26. Neeland IJ, Ayers CR, Rohatgi AK, Turer AT, Berry JD, Das SR, et al. Associations of Visceral and Abdominal Subcutaneous Adipose Tissue With Markers of Cardiac and Metabolic Risk in Obese Adults. *Obes (Silver Spring)* (2013) 21:E439–47. doi: 10.1002/oby.20135
27. Matsumoto T, Oki K, Kajikawa M, Nakashima A, Maruhashi T, Iwamoto Y, et al. Effect of Aldosterone-Producing Adenoma on Endothelial Function and Rho-associated Kinase Activity in Patients With Primary Aldosteronism. *Hypertension* (2015) 65:841–8. doi: 10.1161/HYPERTENSIONAHA.114.05001
28. Huang WC, Chen YY, Lin YH, Chen L, Lin PC, Lin YF, et al. Incidental Congestive Heart Failure in Patients With Aldosterone-Producing Adenomas. *J Am Heart Assoc* (2019) 8:e012410. doi: 10.1161/JAHA.119.012410
29. Kuo CC, Wu VC, Huang KH, Wang SM, Chang CC, Lu CC, et al. Verification and Evaluation of Aldosteronism Demographics in the Taiwan Primary Aldosteronism Investigation Group (Taipai Group). *J Renin Angiotensin Aldosterone Syst* (2011) 12:348–57. doi: 10.1177/1470320310391329
30. Ducher M, Mounier-Véhier C, Baguet JP, Tartière JM, Sosner P, Régnier-Le Coz S, et al. Aldosterone-to-Renin Ratio for Diagnosing Aldosterone-Producing Adenoma: A Multicentre Study. *Arch Cardiovasc Dis* (2012) 105:623–30. doi: 10.1016/j.acvd.2012.07.006
31. Okamoto R, Taniguchi M, Onishi Y, Kumagai N, Uraki J, Fujimoto N, et al. Predictors of Confirmatory Test Results for the Diagnosis of Primary Hyperaldosteronism in Hypertensive Patients With an Aldosterone-to-Renin Ratio Greater Than 20. The SHRIMP Study. *Hypertens Res* (2019) 42:40–51. doi: 10.1038/s41440-018-0126-1
32. Er LK, Lin MC, Tsai YC, Hsiao JK, Yang CY, Chang CC, et al. Association of Visceral Adiposity and Clinical Outcome Among Patients With Aldosterone Producing Adenoma. *BMJ Open Diabetes Res Care* (2020) 8:e001153. doi: 10.1136/bmjdrc-2019-001153
33. Shibayama Y, Wada N, Baba S, Miyano Y, Obara S, Iwasaki R, et al. Relationship Between Visceral Fat and Plasma Aldosterone Concentration in Patients With Primary Aldosteronism. *J Endocr Soc* (2018) 2:1236–45. doi: 10.1210/js.2018-00187
34. Wu C, Zhang H, Zhang J, Xie C, Fan C, Zhang H, et al. Inflammation and Fibrosis in Perirenal Adipose Tissue of Patients With Aldosterone-Producing Adenoma. *Endocrinology* (2018) 159:227–37. doi: 10.1210/en.2017-00651

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

ORIGINAL RESEARCH
published: 17 August 2021
doi: 10.3389/fendo.2021.667951

Alteration of Gut Microbiota Relates to Metabolic Disorders in Primary Aldosteronism Patients

Yu Liu[†], *Qingyao Jiang*[†], *Zhihong Liu*, *Sikui Shen*, *Jianzhong Ai*, *Yuchun Zhu*[*] *and Liang Zhou*[*]

Department of Urology, Institute of Urology (Laboratory of Reconstructive Urology), West China Hospital, Sichuan University, Chengdu, China

OPEN ACCESS

Edited by:
Vin-Cent Wu,
National Taiwan University, Taiwan

Reviewed by:
Marloes Dekker Nitert,
The University of Queensland, Australia
Silvia Monticone,
University of Turin, Italy
Shenghui Li,
China Agricultural University, China

****Correspondence:***
Yuchun Zhu
mmaalleee@126.com
Liang Zhou
zhouliang5678@foxmail.com

[†]*These authors have contributed equally to this work and share first authorship*

Specialty section:
This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Endocrinology

Received: *15 February 2021*
Accepted: *28 July 2021*
Published: *17 August 2021*

Citation:
Liu Y, Jiang Q, Liu Z, Shen S, Ai J, Zhu Y and Zhou L (2021) Alteration of Gut Microbiota Relates to Metabolic Disorders in Primary Aldosteronism Patients. Front. Endocrinol. 12:667951. doi: 10.3389/fendo.2021.667951

Purpose: This study aimed to determine the relationships among gut microbiota, primary aldosteronism (PA), and related metabolic disorders.

Methods: The study enrolled 13 PA patients, 26 sex-matched primary hypertension patients, and 26 sex-matched healthy controls. Demographic and clinical characteristics such as age, body mass index (BMI), blood aldosterone–renin ratio, blood potassium, blood glucose, blood lipid parameters, and history of diabetes mellitus (DM) were compared between the three groups. The gut microbiota of each participant was examined by 16S rRNA gene sequencing. Spearman correlation analysis was performed to demonstrate the relationship between gut microbiota and clinical characteristics.

Results: BMI and the percentage of DM in PA patients were higher than those in healthy controls ($p < 0.05$), but not higher than those in primary hypertension patients ($p > 0.05$). The gut microbiota of healthy controls and primary hypertension patients had a higher alpha diversity level than that of PA patients. PA patients had fewer short-chain fatty acid (SCFA)-producing genera (*Prevotella*, *Blautia*, *Coprococcus*, *Anaerostipes*, and *Ruminococcus*) and more inflammation-associated genera (*Megamonas*, *Sutterella*, and *Streptococcus*) than healthy controls ($p < 0.05$). The gut microbiota of PA patients was more inclined to encode microbial pathways involved in sugar metabolism, such as starch and sucrose metabolism and fructose and mannose metabolism. Blood potassium was negatively correlated with the relative abundance of *Romboutsia* ($R = –0.364$, $q = 0.023$). Diastolic blood pressure (DBP) was positively correlated with *Romboutsia* ($R = 0.386$, $q = 0.015$). Systolic blood pressure (SBP) was negatively correlated with *Blautia* ($R = –0.349$, $q = 0.030$).

Conclusions: The alteration of gut microbiota in PA patients, especially bacteria and pathways involved in inflammation, SCFAs, and sugar metabolism, may be associated with chronic metabolic disorders.

Keywords: primary aldosteronism, gut microbiota, metabolic disorders, diabetes mellitus, obesity

INTRODUCTION

Primary aldosteronism (PA) is the most common cause of secondary hypertension (1). PA is frequently poorly diagnosed and treated, leading to aldosterone-specific morbidity and mortality. Many observational studies have reported an increased prevalence of metabolic complications [such as diabetes mellitus (DM) and obesity] among PA patients (2, 3). Hyperaldosteronemia, hypokalemia, and cortisol secretion are regarded as risk factors for metabolic disorders (4). However, a clear pathophysiological interrelationship linking the two entities has yet to be established.

The gut microbiome is part of a complex ecosystem. Many studies have shown that the gut microbiota and its metabolites are involved in some metabolic diseases, such as metabolic syndrome, DM, and obesity (5–8). Since the reasons for high prevalence of metabolic diseases in PA patients have not been well described, it will be interesting to explore the interaction between PA-induced metabolic disorders and independent microbial characteristics. To address this problem, we conducted a case–control study with 65 participants in which we analyzed gut microbiota to establish the intestinal microbial profiles of PA patients. We aimed to determine the relationship between gut microbiota, PA, and related metabolic diseases.

MATERIALS AND METHODS

Study Population

A case–control study was performed at West China Hospital, Sichuan University in China from September 2019 to December 2019. We recruited PA patients, primary hypertension patients, and healthy controls. PA patients (PA group) were primarily diagnosed by the aldosterone–renin ratio (cutoff value = 30), and then confirmed by an intravenous saline load test (cutoff value of postsaline aldosterone = 5 ng/dl) and a captopril challenge test (cutoff value of postcaptopril aldosterone = 11 ng/dl). Abdominal computed tomography was performed to determine the location of the adrenal nodules. Adrenal venous sampling was performed to preoperatively distinguish unilateral and bilateral PA. Primary hypertension patients (hypertension group) were defined as patients with hypertension who had systolic blood pressure (SBP) ≥140 mmHg or diastolic blood pressure (DBP) ≥90 mmHg. Blood cortisol, catecholamine and renin–angiotensin–aldosterone, thyroid hormone, thyroid-stimulating hormone, and parathyroid hormone were examined to verify that they did not have endocrine hypertension. Acromegaly and obstructive sleep apnea were also considered if patients reported related symptoms (1). Healthy controls (control group) were defined as participants without hypertension, with SBP ≤139 mmHg and DBP ≤89 mmHg. Blood pressure was measured in a sitting position by nurses or physicians. Three readings were recorded at 5-min intervals with a random-zero mercury column sphygmomanometer, and the ;average was taken as the final measurement. All PA patients received laparoscopic adrenalectomy, with the pathological specimen confirmed as cortical adenoma. This study was approved by the West China Hospital, Sichuan University Medical Research Ethics Committee (IRB approval number 2018182), and informed consent was obtained from each participant.

Participants were excluded if they had the following conditions: the use of antibiotics within 3 months before fecal sampling, inflammatory bowel disease, irritable bowel syndrome, digestive tract infection, tumors of the digestive system, intestinal surgery, and recurrent diarrhea or constipation within 1 month before stool sample collection.

Collection of Demographic and Clinical Characteristics

A questionnaire was designed to query the demographic and clinical information of participants, including sex, age, and history of smoking and drinking. Weight and height were measured by instruments. Body mass index (BMI) was calculated by dividing weight in kilograms by the square of height in meters. Blood pressure, blood aldosterone–renin ratio, blood potassium, blood glucose, blood lipid parameters [triglyceride, cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL)], and history of DM were obtained from medical records. Fecal samples of the study participants were collected at inpatient or outpatient departments when PA and primary hypertension patients visited our hospital. We asked healthy controls from the health examination center of our hospital to provide stool samples voluntarily. The samples were immediately frozen in liquid nitrogen and subsequently stored at −80°C until analysis.

Analyses of Gut Microbiota

Microbial DNA was extracted from fecal samples using a QIAamp Fast DNA Stool Mini Kit. Negative controls were conducted using sterile water to exclude any possible contamination. The V3–V4 regions of the 16S rRNA gene were amplified with primers 338 F (5′-ACTCCTACGGGAG GCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWT CTAAT-3′). We constructed a library using the NEXTFLEX Rapid DNA-Seq Kit. Amplicons were pooled in equimolar amounts and paired-end sequenced (2 × 300) on an Illumina MiSeq platform. After demultiplexing, the resulting sequences were merged by FLASH (version 1.2.11) (9) and quality filtered with fastp (version 0.19.6) (10). Then the sequences were denoised using the DADA2 (11) plugin in QIIME2 (version 2020.2) (12). The denoised sequences are called amplicon sequence variants (ASVs). Taxonomic assignment of ASVs was performed with the SILVA 16S rRNA gene database (version 138).

Based on the sequencing data, the coverage index was first calculated to determine if the sequencing depth covered the whole bacterial diversity. The higher the coverage index is, the higher the probability of the sequence being detected. We compared the alpha diversity indices, such as the Shannon and Simpson indices between the control, hypertension, and PA groups. A higher Shannon index and a lower Simpson index

indicate a higher richness and evenness of the gut microbiota. Interindividual variability (beta diversity) among these three groups was also evaluated with principal coordinates analysis (PCoA) by Bray–Curtis distance, the permutational multivariate analysis of variance (PERMANOVA) test, and the analysis of similarities (ANOSIM) test. The linear discriminant analysis (LDA) effect size (LEfSe) method was used to identify differentially abundant bacteria among the three groups (13). The Kyoto Encyclopedia of Genes and Genomes (KEGG) metabolic pathways were predicted by PICRUSt2 (14). A random forest algorithm was used to determine which bacteria had key roles in distinguishing PA patients from primary hypertension patients and healthy controls. Spearman correlation analysis was performed to demonstrate the relationship between gut microbiota and clinical characteristics.

Statistical Analysis

Qualitative parameters are presented as numbers and percentages. Quantitative parameters were reported as the mean and standard deviation if they were symmetrically distributed. Otherwise, they are shown as the median and interquartile range. Fisher's exact test was used to examine qualitative parameters. Student's *t*-test, analysis of variance (ANOVA), Mann–Whitney *U* test, and Kruskal–Wallis test were applied for testing quantitative parameters. We performed all the statistical analyses in R (version 3.6.3) (R Project for Statistical Computing, www.r-project.org).

RESULTS

General Characteristics of Healthy Controls, Primary Hypertension Patients, and PA Patients

A total of 65 participants were enrolled in our study, consisting of 13 PA patients, 26 sex-matched primary hypertension patients, and 26 sex-matched healthy controls (**Table 1**). The mean ages of the control group (50.1 years) and the PA group (46.4 years) were not significantly different ($p = 0.196$), while they were both lower than that of the hypertension group (56.9 years) ($p < 0.05$). BMI was higher in PA patients (24.5) than in healthy controls (23.5) ($p = 0.043$), while it was not significantly different between the PA group and the hypertension group ($p = 0.126$). PA patients had higher DBP than primary hypertension patients ($p < 0.001$), while their SBPs were not significantly different ($p = 0.151$). The blood potassium level of the PA group was lower than those of the hypertension and PA groups ($p < 0.001$). More PA patients had DM than healthy controls (30.8% *vs.* 3.8%, $p = 0.018$). However, the percentages of DM were not significantly different between PA patients and primary hypertension patients (30.8% *vs.* 26.9%, $p = 0.801$). History of smoking and drinking, blood glucose, and blood lipid parameters were not different between the three groups ($p > 0.05$).

PA patients used many types of antihypertensive drugs before surgery, including angiotensin-converting enzyme inhibitors,

TABLE 1 | Demographic and clinical characteristics of study participants in control, primary hypertension, and PA patients.

Parameters	PA (*n* = 13)	Primary hypertension (*n* = 26)	Control (*n* = 26)	*p*-value (PA *vs.* primary hypertension *vs.* control)	*p*-value (PA *vs.* control)	*p*-value (PA *vs.* primary hypertension)	*p*-value (primary hypertension *vs.* control)
Sex				1.000 (a)	1.000 (a)	1.000 (a)	1.000 (a)
Male	8 (61.5%)	16 (61.5%)	16 (61.5%)				
Female	5 (38.5%)	10 (38.5%)	10 (38.5%)				
Age (years)	46.4 (11.8)	56.9 (6.9)	50.1 (6.0)	<0.001 (b)	0.196 (b)	0.001 (b)	<0.001 (b)
BMI (kg/m^2)	24.5 (6.4)	24.1 (5.1)	23.5 (3.5)	0.124 (c)	0.043 (c)	0.126 (c)	0.558 (c)
SBP (mmHg)	147.9 (13.8)	140.9 (14.3)	122.7 (13.2)	<0.001 (b)	<0.001 (b)	0.151 (b)	<0.001 (b)
DBP (mmHg)	99.2 (12.2)	83.2 (8.2)	77.7 (9.6)	<0.001 (b)	<0.001 (b)	<0.001 (b)	0.031 (b)
Aldosterone–renin ratio	426.2 (709.3)	6.1 (7.6)	–	–	–	<0.001 (c)	–
Blood potassium (mmol/L)	3.20 (0.22)	3.93 (0.22)	4.34 (0.37)	<0.001 (b)	<0.001 (b)	<0.001 (b)	<0.001 (b)
Blood glucose (mmol/L)	5.42 (1.33)	5.35 (0.95)	4.88 (0.65)	0.053 (c)	0.036 (c)	0.964 (c)	0.019 (c)
Triglyceride (mmol/L)	0.96 (2.18)	1.45 (0.96)	1.41 (0.41)	0.231 (c)	0.126 (c)	0.136 (c)	0.641 (c)
Cholesterol (mmol/L)	4.35 (0.81)	4.41 (1.02)	4.92 (0.96)	0.101 (b)	0.077 (b)	0.864 (b)	0.070 (b)
HDL (mmol/L)	1.27 (0.33)	1.25 (0.28)	1.26 (0.23)	0.973 (b)	0.924 (b)	0.839 (b)	0.868 (b)
LDL (mmol/L)	2.62 (0.64)	2.54 (0.89)	2.64 (0.81)	0.899 (b)	0.939 (b)	0.772 (b)	0.671 (b)
DM				0.036 (a)	0.035 (a)	1.000 (a)	0.050 (a)
Yes	4 (30.8%)	7 (26.9%)	1 (3.8%)				
No	9 (69.2%)	19 (73.1%)	25 (96.2%)				
Smoking				0.835 (a)	0.719 (a)	0.714 (a)	1.000 (a)
Yes	3 (23.1%)	9 (34.6%)	8 (30.8%)				
No	10 (76.9%)	17 (65.4%)	18 (69.2%)				
Drinking				1.000 (a)	1.000 (a)	1.000 (a)	1.000 (a)
Yes	4 (30.8%)	9 (34.6%)	8 (30.8%)				
No	9 (69.2%)	17 (65.4%)	18 (69.2%)				

(a) Fisher's exact test, (b) Student's t-test, (c) Mann–Whitney U test.

PA, primary aldosteronism; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; DM, diabetes mellitus.

angiotensin receptor blockers, alpha-blockers, beta-blockers, calcium channel blockers, diuretics, and mineralocorticoid receptor antagonists, where only one PA patient used spironolactone. The aldosterone–renin ratio of PA patients was much higher than that of primary hypertension patients (426.2 *vs.* 6.1, $p < 0.001$).

Diversity of the Gut Microbiota in Healthy Controls, Primary Hypertension Patients, and PA Patients

Healthy controls and primary hypertension patients had a higher Shannon index and a lower Simpson index than PA patients ($p < 0.001$). This result indicated that the within-sample alpha diversity level of the gut microbiota of PA patients was lower than that of healthy controls and primary hypertension patients (**Figures 1A, B** and **Supplementary Table 1**). The rarefaction curves reached the saturation plateau, and the coverage index ranged from 0.9975 to 0.9998, which indicated that the sequencing depth for the Sobs index was sufficient (**Figure 1C**).

PCoA by Bray–Curtis distance revealed an asymmetrical distribution of gut microbiota composition between the control, hypertension, and PA groups (**Figure 1D**). The ANOSIM test ($R = 0.599$, $p = 0.001$) and PERMANOVA test ($R^2 = 0.093$, $p = 0.001$) both verified that there were significant differences in bacterial composition between the three groups.

Taxonomic Analysis of Microbiota Composition Between Healthy Controls, Primary Hypertension Patients, and PA Patients

At the phylum level (**Figure 2A**), Firmicutes (mean relative abundance, 62.4%) was the most abundant bacteria in all analyzed samples, followed by Bacteroidota (22.9%), Proteobacteria (6.7%), and Actinobacteria (4.7%). The Firmicutes/Bacteroidetes ratio of the gut microbiota was higher in healthy controls (3.23) than in primary hypertension patients (2.45) and PA patients (2.48). At the genus level (**Figure 2B**), *Bacteroides* was the most common genus, whose percentage in

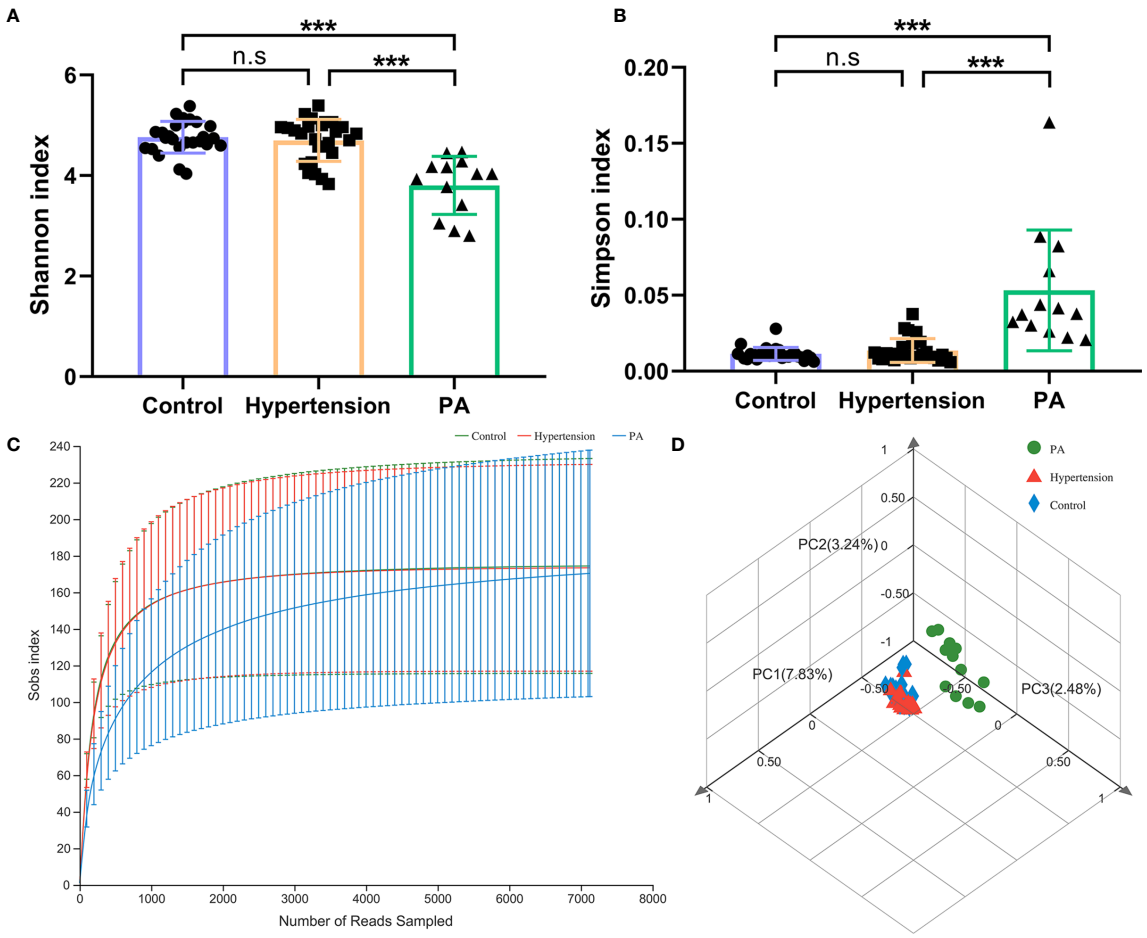

FIGURE 1 | Diversity of the gut microbiota in primary aldosteronism (PA) patients, primary hypertension patients, and healthy controls. **(A)** Shannon index and **(B)** Simpson index of gut microbiota. **(C)** Rarefaction curves of all the samples in the three groups. The horizontal axis shows the number of sequences obtained by sequencing the 16S rRNA gene. The vertical axis shows the number of genera. **(D)** PCoA of gut microbiota by Bray–Curtis distance. ***$p < 0.001$. n.s, not significant.

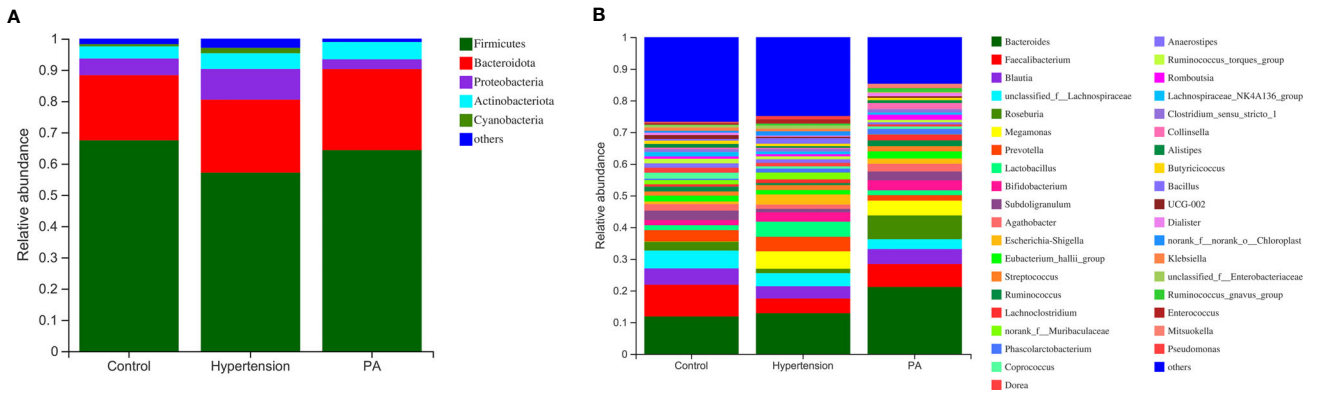

FIGURE 2 | Distribution of gut microbiota between primary aldosteronism (PA) patients, primary hypertension patients, and healthy controls. Relative abundances of the major bacteria at the **(A)** phylum and **(B)** genus levels.

the gut microbiota was much higher in PA patients (21.1%) than in primary hypertension patients (12.9%, $p = 0.043$) and healthy controls (11.8%, $p = 0.032$). The second most common genus was *Faecalibacterium*, whose percentage in the gut microbiota was higher in healthy controls (10.0%) than in primary hypertension patients (4.6%, $p = 0.011$) and PA patients (7.3%, $p = 0.038$).

LEfSe analysis was used to determine the bacterial genera with significant differences between healthy controls, primary hypertension patients, and PA patients. Compared with PA patients, there were 28 and 35 genera with significantly different relative abundances in healthy controls and primary hypertension patients, respectively (**Figures 3A, B**). For example, PA patients had less *Prevotella* ($p = 0.018$), *Blautia* ($p = 0.008$), *Lactobacillus* ($p < 0.003$), *Coprococcus* ($p = 0.007$), *Eubacterium eligens group* ($p = 0.004$), *Anaerostipes* ($p = 0.006$), *Ruminococcus torques group* ($p < 0.032$), and *Enterococcus* ($p = 0.006$) and more *Bacteroides* ($p = 0.032$), *Megamonas* ($p = 0.001$), and *Sutterella* ($p = 0.001$) than healthy controls. Similarly, the relative abundances of *Lactobacillus* ($p < 0.001$), *Prevotella* ($p < 0.001$), *Weissella* ($p < 0.001$), *Lactococcus* ($p < 0.001$), and *Akkermansia* ($p < 0.001$) were higher in primary hypertension patients, while those of *Roseburia* ($p = 0.003$), *Streptococcus* ($p = 0.024$), *Paraprevotella* ($p = 0.011$), and *Sutterella* ($p = 0.016$) were higher in PA patients. Genera with significant differences between the control and hypertension groups are shown in **Supplementary Figure 1**.

We performed a Bray–Curtis distance-based redundancy analysis (dbRDA) to determine the additional effects of age, BMI, and DM on the gut microbiota (**Figure 4**). The envfit function test showed that age ($p = 0.001$), BMI ($p = 0.001$), and DM ($p = 0.001$) were significant explanatory variables (**Supplementary Table 2**).

To eliminate the possible confounding effects of DM and BMI, we excluded participants with DM. BMI and blood glucose were not significantly different between healthy controls, primary hypertension patients, and PA patients after removing DM patients ($p > 0.05$) (**Supplementary Table 3**). LEfSe analysis revealed that the genera with significant differences between the three groups were similar to those before deleting participants with DM (**Supplementary Figure 2**). After deleting DM patients, the relative abundance of *Eubacterium* ($p = 0.017$) was higher in PA patients than in healthy controls. PA patients also had more *Bacteroides* ($p = 0.013$), *Phascolarctobacterium* ($p = 0.024$), *Moryella* ($p = 0.009$), and *Eubacterium fissicatena group* ($p = 0.036$) than primary hypertension patients after deleting DM patients.

Metabolic Function of Gut Microbiota

To evaluate the gene information of gut microbiota, metabolic predictions were achieved using PICRUSt2. The relative abundance of metabolic pathways between the three groups was compared by the Wilcoxon rank sum test. The Benjamini–Hochberg false discovery rate (FDR)-adjusted *p*-values were also calculated. Compared with healthy controls, PA patients had more lipopolysaccharide biosynthesis (FDR-adjusted *p*-value = 0.017), galactose metabolism (FDR-adjusted *p*-value = 0.001), pentose and glucuronate interconversions (FDR-adjusted *p*-value = 0.004), amino sugar and nucleotide sugar metabolism (FDR-adjusted *p*-value = 0.001), fructose and mannose metabolism (FDR-adjusted *p*-value = 0.004), starch and sucrose metabolism (FDR-adjusted *p*-value = 0.002), and arginine and proline metabolism (FDR-adjusted *p*-value = 0.006) (**Supplementary Table 4**). The relative abundance of tryptophan metabolism (FDR-adjusted *p*-value = 0.010) was higher in primary hypertension patients, while those of galactose metabolism (FDR-adjusted *p*-value = 0.005), insulin resistance (FDR-adjusted *p*-value = 0.001), starch and sucrose metabolism (FDR-adjusted *p*-value = 0.003), amino sugar and nucleotide sugar metabolism (FDR-adjusted *p*-value = 0.011), pentose and glucuronate interconversions (FDR-adjusted *p*-value = 0.018), and insulin signaling pathway (FDR-adjusted *p*-value = 0.041) were higher in PA patients (**Supplementary Table 5**). **Supplementary Table 6** shows the metabolic pathways with significant differences between the control and hypertension groups.

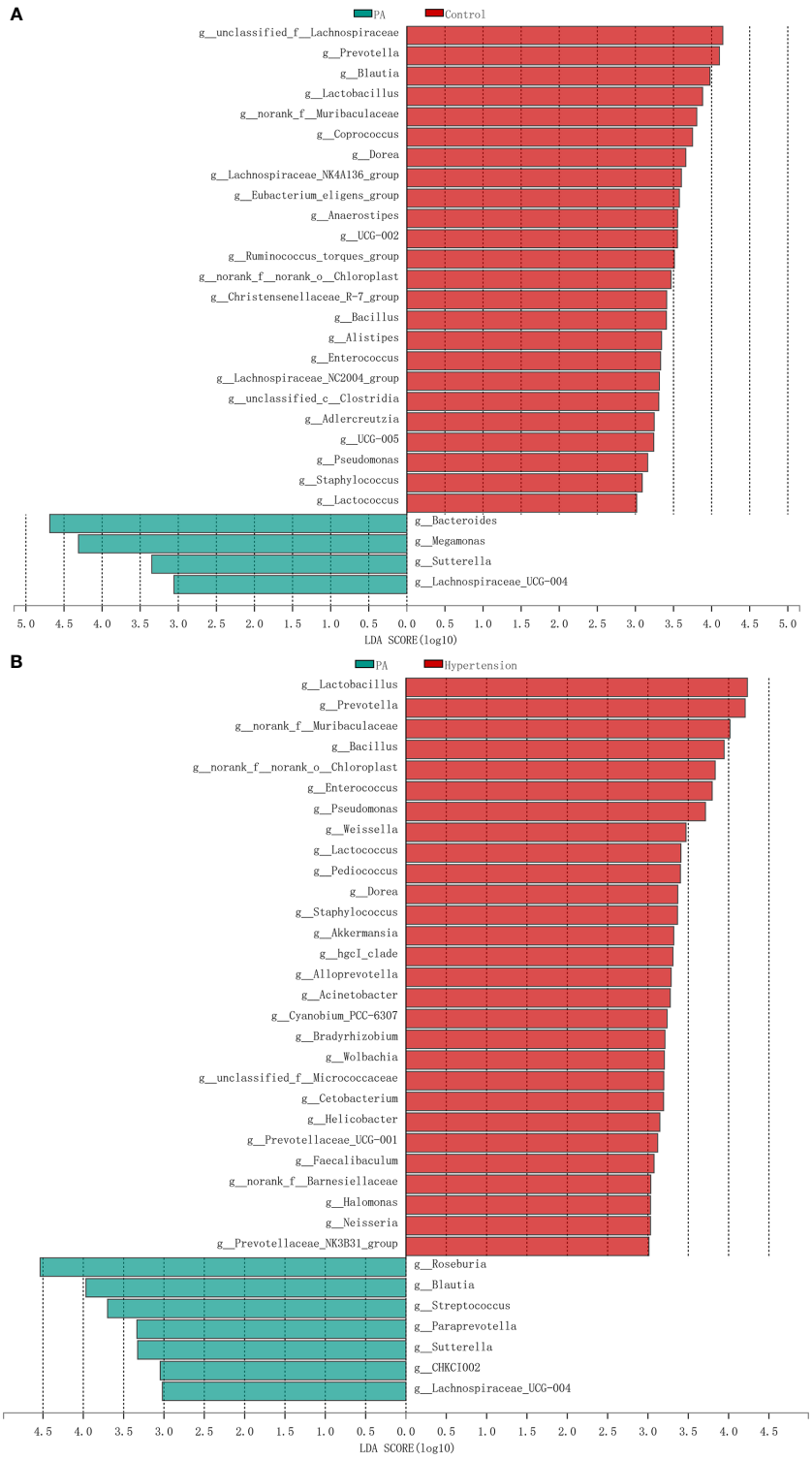

FIGURE 3 | Differences in intestinal bacteria between primary aldosteronism (PA) patients, primary hypertension patients, and healthy controls. **(A)** Significantly different genera between PA patients and healthy controls. Red bars are genera with higher relative abundances in healthy controls. Green bars are genera with higher relative abundances in PA patients. **(B)** Significantly different genera between PA patients and primary hypertension patients. Red bars are genera with higher relative abundances in primary hypertension patients. Green bars are genera with higher relative abundances in PA patients.

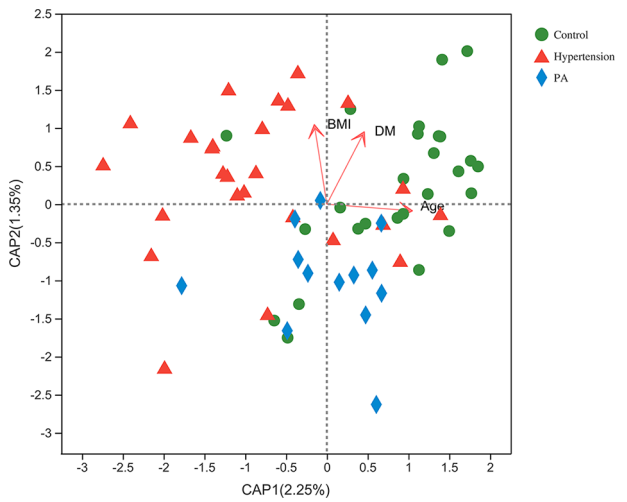

FIGURE 4 | Bray–Curtis distance-based redundancy analysis (dbRDA) of the gut microbiota and explanatory factors in primary aldosteronism (PA) patients, primary hypertension patients, and healthy controls.

Bacteria Distinguishing PA Patients From Primary Hypertension Patients and Healthy Controls

We used random forest algorithm to determine which bacteria had key roles in distinguishing PA patients from healthy controls. **Figure 5A** shows the top 30 genera with the greatest importance, some of which were also screened out by LEfSe analysis, such as *Blautia*, *Anaerostipes*, *Megamonas*, and *Lactobacillus*. The area under the curve (AUC) of the receiver operating characteristic curve was 0.8173 using the random forest model in combination with leave-one-out cross-validation. Similarly, **Figure 5B** presents the top 30 genera with the greatest importance in distinguishing PA patients from primary hypertension patients. The following genera were also detected in the LEfSe analysis: *Pseudomonas*, *Enterococcus*, *Akkermansia*, *Weissella*, and *Pediococcus*. The AUC of the receiver operating characteristic curve was 0.9231.

Association Between Clinical Characteristics and Gut Microbiota

Heatmap analysis (**Figure 6A**) showed that the bacteria with lower relative abundances in the PA group than in the hypertension group were positively correlated with blood potassium and negatively correlated with SBP and DBP. We specifically drew scatter plot graphs of genera with high percentages in the gut microbiota (**Figures 6B–D**). Blood potassium was negatively correlated with the relative abundance of *Romboutsia* ($R = -0.364$, $q = 0.023$). In addition, DBP was positively correlated with *Romboutsia* ($R = 0.386$, $q = 0.015$). SBP was negatively correlated with *Blautia* ($R = -0.349$, $q = 0.030$).

DISCUSSION

This study is the first to demonstrate the characteristics of the gut microbiota in PA patients. The gut microbiota has been reported to be associated with metabolic diseases, such as DM and obesity (6, 7). DM and obesity are more common in PA patients than in healthy people or primary hypertension patients (2, 3). Our study showed that DM was more common in PA patients than in healthy controls. The BMI of PA patients was higher than that of healthy controls. In addition, the composition of the gut

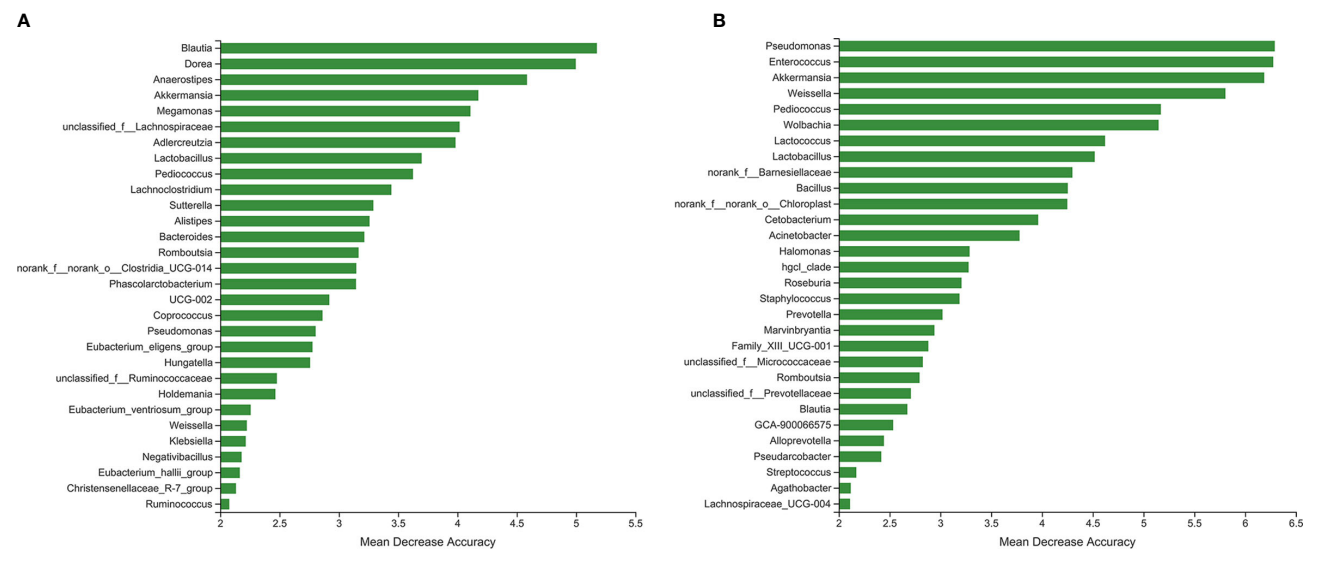

FIGURE 5 | The top 30 genera with great importance in distinguishing primary aldosteronism (PA) patients from **(A)** healthy controls and **(B)** primary hypertension patients.

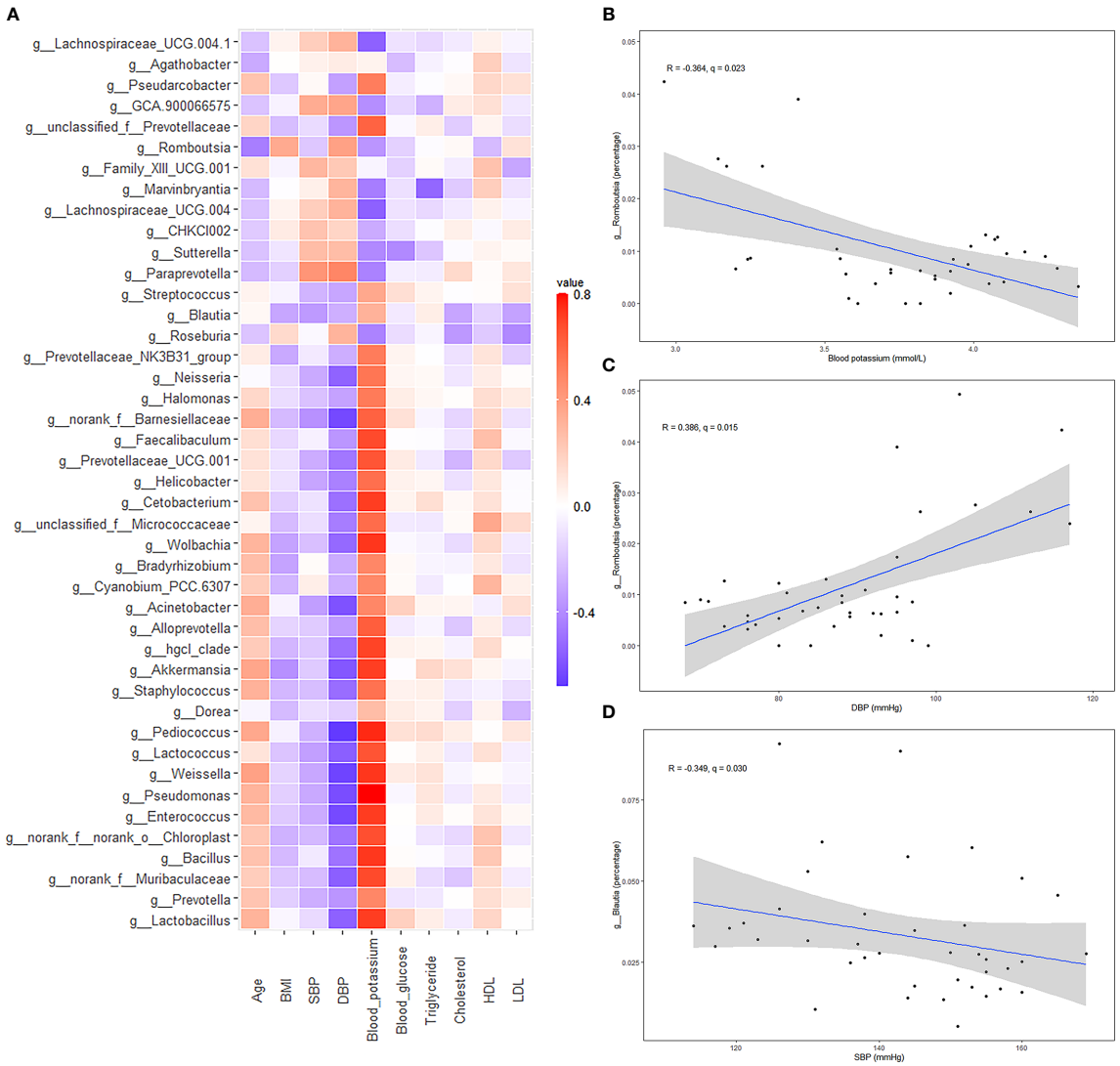

FIGURE 6 | Spearman correlation analysis of different bacteria [primary aldosteronism (PA) patients *vs.* primary hypertension patients] and clinical characteristics. **(A)** Heatmap showing the correlation between different bacteria and clinical characteristics. **(B)** Scatter plot graphs showing the correlation between blood potassium and *Romboutsia*. **(C)** Scatter plot graphs showing the correlation between diastolic blood pressure (DBP) and *Romboutsia*. **(D)** Scatter plot graphs showing the correlation between systolic blood pressure (SBP) and *Blautia*.

microbiota and its metabolic pathways of PA patients were also significantly different from those of healthy controls and primary hypertension patients.

We hypothesized that PA may contribute to the development of DM and obesity *via* gut microbiota. On the one hand, the alpha diversity of the gut microbiota in PA patients was lower than that in primary hypertension patients and healthy controls, which meant that the richness of the gut microbiota of PA patients was disturbed. Folz et al. reported that high-sodium and low-potassium intake could interact with gut microbiota to affect endocrine homeostasis (15). No study has clearly demonstrated the relationship between aldosterone and gut microbiota. It was reasonable to assume that PA is responsible for the changes in gut microbiota. On the other hand, the gut microbiota also affects DM and obesity by regulating inflammation, gut permeability, glucose metabolism, fatty acid oxidation, synthesis and energy expenditure, and the interaction of gut bacteria (6). The richness of gut microbiota in metabolic disease patients decreased (16). The Firmicutes/Bacteroidetes ratio was reported to be lower in individuals with obesity (17).

We found that the relative abundances of *Prevotella*, *Blautia*, *Coprococcus*, *Anaerostipes*, and *Ruminococcus* were lower in the gut microbiota of PA patients than in healthy controls and primary hypertension patients. All these bacteria could produce SCFAs (18–21). SCFAs, the energy sources of enterocytes, could maintain the intestinal epithelial barrier

and decrease the permeability of the gut, circulating lipopolysaccharides, and systemic inflammation. SCFAs could also reduce inflammation *via* GPR41/43 (22, 23). In addition, *Lactobacillus* was more abundant in the gut microbiota of healthy controls and primary hypertension patients than in PA patients. Several studies have already demonstrated that the administration of *Lactobacillus* could alleviate hyperglycemia in diabetic rats (6, 24, 25). We also found that *Lactococcus* and *Enterococcus* were present in lower abundances in PA patients. Lilia et al. reported that consumption of fermented milk with *Lactococcus lactis* had a blood pressure-lowering effect on prehypertensive subjects (26). Anne et al. found that vertical sleeve gastrectomy reduced body mass and blood pressure and increased the relative abundance of *Enterococcus* in the gut microbiota of mice (27). In contrast, PA patients had more *Megamonas*, *Sutterella*, and *Streptococcus* than healthy controls and primary hypertension patients, genera that are associated with inflammation (28–30). *Weissella* and *Akkermansia*, which were present in lower abundances in PA patients than in primary hypertension patients, were shown to have anti-inflammatory potential (31, 32). PA patients had a higher relative abundance of lipopolysaccharide biosynthesis in the gut microbiota than healthy controls. Lipopolysaccharides are bacterial surface glycolipids produced by gram-negative bacteria, which can induce inflammatory reactions (33). It was reported that inflammatory factors could alter glucose tolerance and insulin sensitivity (34). Individuals with obesity had more *Megamonas* (7). Compared with healthy controls and primary hypertension patients, PA patients had more pathways participating in sugar metabolism, such as starch and sucrose metabolism, fructose and mannose metabolism, amino sugar and nucleotide sugar metabolism, pentose and glucuronate interconversions, arginine and proline metabolism, and galactose metabolism. These factors may result in more glucose in the gut and absorption into the blood. In addition, some amino acid metabolism pathways were also significantly different between the PA and hypertension groups. For example, tryptophan metabolism was lower in PA patients. Tryptophan metabolism could produce some metabolites, such as indole-3-ethanol, indole-3-pyruvate, and indole-3-aldehyde, which are essential in maintaining intestinal barrier function (35). This evidence indicated that PA-associated dysbiosis of gut microbiota in SCFAs, sugar and amino acid metabolism, and inflammation were associated with metabolic disorders.

Previous studies regarded hypokalemia as a risk factor for DM. They thought that hypokalemia may decrease insulin secretion and induce insulin resistance, followed by dysbiosis of glucose metabolism (36). We found that *Romboutisa* and *Bacteroides* were more abundant in PA patients than in primary hypertension patients. *Romboutisa* was also negatively correlated with blood potassium. Some studies found that *Romboutsia* was positively associated with obesity and lipid metabolism (37, 38). *Bacteroides* was also found to be increased after the higher-fat diet intervention (39) and to be more abundant in patients with DM among the Chinese population (40). This evidence indicated that gut microbiota may also play an important role in hypokalemia-related disorders of lipid and glucose metabolism, a possibility that needs further exploration.

Our study first revealed the characteristics of the gut microbiota of PA patients and identified some bacteria and metabolic pathways that may be associated with DM and obesity. We believe that the knowledge gained from this study will be important in shaping the framework and platform for broader research on the gut microbiota of PA patients in the future.

The study showed that there was a significant difference in the mean age between PA patients and primary hypertension patients. Several studies have revealed that age is a factor influencing the gut microbiota (41–44). As people age, key changes in gut microbiota include compositional instability, reduced overall diversity, and an increase in proinflammatory opportunistic pathogens. In infants, *Bifidobacterium* is the most dominant genus in the gut microbiota. Biagi et al. examined the fecal microbiome of young adults (22–48 years old), elderly adults (65–75 years old), and semisupercentenarians (105–109 years old) in an Italian population (45). They found that the fecal microbiota in all age groups was dominated by just three families, including Bacteroidaceae, Lachnospiraceae, and Ruminococcaceae. However, the relative abundance of bacteria belonging to these families decreased with age. An independent study of Chinese centenarians reported similar results (46). In addition, they both found that longevity increased microbial community richness and the abundance of subdominant but health-related bacterial genera and families, such as *Oscillospira*, Christensenellaceae, *Akkermansia*, and *Bifidobacterium*. The diversity of gut microbiota continues to increase until the age of 3 years, remains stable afterwards, and then declines during senescence (44). The gut microbiota of elderly people is reduced in beneficial microbes, such as SCFA producers, and enriched in proinflammatory microbes (42, 44).

This study had some limitations. First, almost all PA and primary hypertension patients had received different antihypertensive medications before recruitment. It was unclear whether these drugs affected the composition and metabolism of gut microbiota. Second, the effects of some factors on the gut microbiota could not be ignored, such as DM, aging, use of antihypertensive drugs, and salt intake (47). Third, the sample size of the study was relatively small and some characteristics were heterogeneous between groups, which may contribute to biases. Further case–control studies should have larger sample sizes; match factors that will potentially affect gut microbiota, such as age and dietary habits; and measure the 24-h urinary electrolytes to investigate the role of gut microbiota in metabolic disorders in primary aldosteronism patients and reduce analysis bias.

In conclusion, the alteration of gut microbiota in PA patients, especially bacteria and pathways involved in inflammation, SCFAs, and sugar metabolism, may be associated with chronic metabolic disorders.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: NCBI accession: PRJNA728662.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the West China Hospital, Sichuan University Medical Research Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

YL and QJ conceived and designed the study, collected and analyzed the data, and wrote the manuscript. ZL, SS, and JA analyzed the data. YZ and LZ reviewed and edited the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by 1.3.5 Project for Disciplines of Excellence, West China Hospital, Sichuan University (ZY2016104).

ACKNOWLEDGMENTS

We thank all the doctors, nurses, and laboratorian in the West China Hospital for their assistance and all the patients for their contribution to this study.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.667951/full#supplementary-material

REFERENCES

1. Young WF, Calhoun DA, Lenders JWM, Michael S, Textor SC. Screening for Endocrine Hypertension: An Endocrine Society Scientific Statement. *Endocrine Rev* (2017) 2):103–22. doi: 10.1210/er.2017-00054
2. Akehi Y, Yanase T. High Prevalence of Diabetes in Patients With Primary Aldosteronism (PA) Associated With Subclinical Hypercortisolism and Prediabetes More Prevalent in Bilateral Than Unilateral PA: A Large. *Multicenter Cohort Study Japan* (2019) 42(5):938–45. doi: 10.2337/dc18-1293
3. Adolf C, Asbach E, Dietz AS, Lang K, Hahner S, Quinkler M, et al. Worsening of Lipid Metabolism After Successful Treatment of Primary Aldosteronism. *Endocrine* (2016) 54(1):198–205. doi: 10.1007/s12020-016-0983-9
4. Bothou C, Beuschlein F, Spyroglou A. Links Between Aldosterone Excess and Metabolic Complications: A Comprehensive Review. *Diabetes Metab* (2020) 46(1):1–7. doi: 10.1016/j.diabet.2019.02.003
5. Boulangé CL, Neves AL, Chilloux J, Nicholson JK, Dumas ME. Impact of the Gut Microbiota on Inflammation, Obesity, and Metabolic Disease. *Genome Med* (2016) 8(1):42. doi: 10.1186/s13073-016-0303-2
6. Gurung M, Li Z, You H, Rodrigues R, Jump DB, Morgun A, et al. Role of Gut Microbiota in Type 2 Diabetes Pathophysiology. *EBioMedicine* (2020) 51:102590. doi: 10.1016/j.ebiom.2019.11.051
7. Crovesy L, Masterson D, Rosado EL. Profile of the Gut Microbiota of Adults With Obesity: A Systematic Review. *Eur J Clin Nutr* (2020) 74(9):1251–62. doi: 10.1038/s41430-020-0607-6
8. Wu J, Wang K, Wang X, Pang Y, Jiang C. The Role of the Gut Microbiome and its Metabolites in Metabolic Diseases. *Protein Cell* (2021) 12(5):360–73. doi: 10.1007/s13238-020-00814-7
9. Magoč T, Salzberg SL. FLASH: Fast Length Adjustment of Short Reads to Improve Genome Assemblies. *Bioinf (Oxford England)* (2011) 27(21):2957–63. doi: 10.1093/bioinformatics/btr507
10. Chen S, Zhou Y, Chen Y, Gu J. Fastp: An Ultra-Fast All-in-One FASTQ Preprocessor. *Bioinf (Oxford England)* (2018) 34(17):i884–90. doi: 10.1093/bioinformatics/bty560
11. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. DADA2: High-Resolution Sample Inference From Illumina Amplicon Data. *Nat Methods* (2016) 13(7):581–3. doi: 10.1038/nmeth.3869
12. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. Reproducible, Interactive, Scalable and Extensible Microbiome Data Science Using QIIME 2. *Nat Biotechnol* (2019) 37(8):852–7. doi: 10.1038/s41587-019-0209-9
13. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, et al. Metagenomic Biomarker Discovery and Explanation. *Genome Biol* (2011) 12 (6):R60. doi: 10.1186/gb-2011-12-6-r60
14. Douglas GM, Maffei VJ, Zaneveld JR, Yurgel SN, Brown JR, Taylor CM, et al. PICRUSt2 for Prediction of Metagenome Functions. *Nat Biotechnol* (2020) 38 (6):685–8. doi: 10.1038/s41587-020-0548-6
15. Folz J, Oh YT, Blaženović I, Richey J, Fiehn O, Youn JH. Interaction of Gut Microbiota and High-Sodium, Low-Potassium Diet in Altering Plasma Triglyceride Profiles Revealed by Lipidomics Analysis. *Mol Nutr Food Res* (2019) 63(24):e1900752. doi: 10.1002/mnfr.201900752
16. Sonnenburg ED, Smits SA, Tikhonov M, Higginbottom SK, Wingreen NS, Sonnenburg JL. Diet-Induced Extinctions in the Gut Microbiota Compound Over Generations. *Nature* (2016) 529(7585):212–5. doi: 10.1038/nature16504
17. Schwiertz A, Taras D, Schäfer K, Beijer S, Bos NA, Donus C, et al. Microbiota and SCFA in Lean and Overweight Healthy Subjects. *Obes (Silver Spring Md)* (2010) 18(1):190–5. doi: 10.1038/oby.2009.167
18. Mocanu V, Rajaruban S, Dang J, Kung JY, Deehan EC, Madsen KL. Repeated Fecal Microbial Transplantations and Antibiotic Pre-Treatment Are Linked to Improved Clinical Response and Remission in Inflammatory Bowel Disease: A Systematic Review and Pooled Proportion Meta-Analysis. *J Clin Med* (2021) 10(5):959. doi: 10.3390/jcm10050959
19. Li Y, Liu W, Xu ZZ, Xiao JX, Zong AZ, Qiu B, et al. Research on the Mechanism of Microwave-Toughened Starch on Glucolipid Metabolism in Mice. *Food Funct* (2020) 11(11):9789–800. doi: 10.1039/d0fo02093a
20. Jiang S, Xie S, Lv D, Wang P, He H, Zhang T, et al. Alteration of the Gut Microbiota in Chinese Population With Chronic Kidney Disease. *Sci Rep* (2017) 7(1):2870. doi: 10.1038/s41598-017-02989-2
21. Han SH, Yi J, Kim JH, Lee S, Moon HW. Composition of Gut Microbiota in Patients With Toxigenic Clostridioides (Clostridium) Difficile: Comparison Between Subgroups According to Clinical Criteria and Toxin Gene Load. *PLoS One* (2019) 14(2):e0212626. doi: 10.1371/journal.pone.0212626
22. Koh A, De Vadder F, Kovatcheva-Datchary P, Bäckhed F. From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites. *Cell* (2016) 165(6):1332–45. doi: 10.1016/j.cell.2016.05.041
23. Yao Y, Cai X, Fei W, Ye Y, Zhao M, Zheng C. The Role of Short-Chain Fatty Acids in Immunity, Inflammation and Metabolism. *Crit Rev Food Sci Nutr* (2020) 1–12. doi: 10.1080/10408398.2020.1854675

24. Toejing P, Khat-Udomkiri N, Intakhad J, Sirilun S, Chaiyasut C. Putative Mechanisms Responsible for the Antihyperglycemic Action of Lactobacillus Paracasei HII01 in Experimental Type 2 Diabetic Rats. *Nutrients* (2020) 12 (10):3015. doi: 10.3390/nu12103015
25. Bauer PV, Duca FA, Waise TMZ, Dranse HJ, Rasmussen BA, Puri A, et al. Lactobacillus Gasseri in the Upper Small Intestine Impacts an ACSL3-Dependent Fatty Acid-Sensing Pathway Regulating Whole-Body Glucose Homeostasis. *Cell Metab* (2018) 27(3):572–87.e6. doi: 10.1016/j.cmet.2018.01.013
26. Beltrán-Barrientos LM, González-Córdova AF, Hernández-Mendoza A, Torres-Inguanzo EH, Astiazarán-García H, Esparza-Romero J, et al. Randomized Double-Blind Controlled Clinical Trial of the Blood Pressure-Lowering Effect of Fermented Milk With Lactococcus Lactis: A Pilot Study. *J dairy Sci* (2018) 101(4):2819–25. doi: 10.3168/jds.2017-13189
27. McGavigan AK, Henseler ZM, Garibay D, Butler SD, Jayasinghe S, Ley RE, et al. Vertical Sleeve Gastrectomy Reduces Blood Pressure and Hypothalamic Endoplasmic Reticulum Stress in Mice. *Dis Models Mech* (2017) 10(3):235–43. doi: 10.1242/dmm.027474
28. Ling Z, Jin C, Xie T, Cheng Y, Li L, Wu N. Alterations in the Fecal Microbiota of Patients With HIV-1 Infection: An Observational Study in A Chinese Population. *Sci Rep* (2016) 6:30673. doi: 10.1038/srep30673
29. Lan R, Wan Z, Xu Y, Wang Z, Fu S, Zhou Y, et al. Taurine Reprograms Mammary-Gland Metabolism and Alleviates Inflammation Induced by Streptococcus Uberis in Mice. *Front Immunol* (2021) 12:696101. doi: 10.3389/fimmu.2021.696101
30. Hiippala K, Kainulainen V, Kalliomäki M, Arkkila P, Satokari R. Mucosal Prevalence and Interactions With the Epithelium Indicate Commensalism of Sutterella Spp. *Front Microbiol* (2016) 7:1706. doi: 10.3389/fmicb.2016.01706
31. Yu HS, Lee NK, Choi AJ, Choe JS, Bae CH, Paik HD. Anti-Inflammatory Potential of Probiotic Strain Weissella Cibaria JW15 Isolated From Kimchi Through Regulation of NF-κb and MAPKs Pathways in LPS-Induced RAW 264.7 Cells. *J Microbiol Biotechnol* (2019) 29(7):1022–32. doi: 10.4014/jmb.1903.03014
32. Grajeda-Iglesias C, Durand S, Daillère R, Iribarren K, Lemaitre F, Derosa L, et al. Oral Administration of Akkermansia Muciniphila Elevates Systemic Antiaging and Anticancer Metabolites. *Aging* (2021) 13(5):6375–405. doi: 10.18632/aging.202739
33. Candelli M, Franza L, Pignataro G, Ojetti V, Covino M, Piccioni A, et al. Interaction Between Lipopolysaccharide and Gut Microbiota in Inflammatory Bowel Diseases. *Int J Mol Sci* (2021) 22(12):6242. doi: 10.3390/ijms22126242
34. Scheithauer TPM, Rampanelli E, Nieuwdorp M, Vallance BA, Verchere CB, van Raalte DH, et al. Gut Microbiota as a Trigger for Metabolic Inflammation in Obesity and Type 2 Diabetes. *Front Immunol* (2020) 11:571731. doi: 10.3389/fimmu.2020.571731
35. Scott SA, Fu J, Chang PV. Microbial Tryptophan Metabolites Regulate Gut Barrier Function *via* the Aryl Hydrocarbon Receptor. *Proc Natl Acad Sci* (2020) 117(32):19376–87. doi: 10.1073/pnas.2000047117
36. Liu Y, Zhou L, Liu Z, Ma Y, Lin L, Zhu Y, et al. Higher Blood Urea Nitrogen and Urinary Calcium: New Risk Factors for Diabetes Mellitus in Primary Aldosteronism Patients. *Front Endocrinol* (2020) 11:23. doi: 10.3389/fendo.2020.00023
37. Vazquez-Moreno M, Perez-Herrera A, Locia-Morales D, Dizzel S, Meyre D. Association of Gut Microbiome With Fasting Triglycerides, Fasting Insulin and Obesity Status in Mexican Children. *Pediatr Obes* (2021) 16(5):e12748. doi: 10.1111/ijpo.12748
38. Li TT, Tong AJ, Liu YY, Huang ZR, Wan XZ, Pan YY, et al. Polyunsaturated Fatty Acids From Microalgae Spirulina Platensis Modulates Lipid Metabolism Disorders and Gut Microbiota in High-Fat Diet Rats. *Food Chem Toxicol* (2019) 131:110558. doi: 10.1016/j.fct.2019.06.005
39. Wan Y, Wang F, Yuan J, Li J, Jiang D, Zhang J, et al. Effects of Dietary Fat on Gut Microbiota and Faecal Metabolites, and Their Relationship With Cardiometabolic Risk Factors: A 6-Month Randomised Controlled-Feeding Trial. *Gut* (2019) 68(8):1417–29. doi: 10.1136/gutjnl-2018-317609
40. Qin J, Li Y, Cai Z, Li S, Zhu J, Zhang F, et al. A Metagenome-Wide Association Study of Gut Microbiota in Type 2 Diabetes. *Nature* (2012) 490(7418):55–60. doi: 10.1038/nature11450
41. Bosco N, Noti M. The Aging Gut Microbiome and its Impact on Host Immunity. *Genes Immun* (2021) 1–15. doi: 10.1038/s41435-021-00126-8
42. Ragonnaud E, Biragyn A. Gut Microbiota as the Key Controllers of "Healthy" Aging of Elderly People. *Immun Ageing I A* (2021) 18(1):2. doi: 10.1186/s12979-020-00213-w
43. Yang J, Wu J, Li Y, Zhang Y, Cho WC, Ju X, et al. Gut Bacteria Formation and Influencing Factors. *FEMS Microbiol Ecol* (2021) 97(4):fiab043. doi: 10.1093/femsec/fiab043
44. Walrath T, Dyamenahalli KU, Hulsebus HJ, McCullough RL, Idrovo JP, Boe DM, et al. Age-Related Changes in Intestinal Immunity and the Microbiome. *J Leukocyte Biol* (2021) 109(6):1045–61. doi: 10.1002/jlb.3ri0620-405rr
45. Biagi E, Franceschi C, Rampelli S, Severgnini M, Ostan R, Turroni S, et al. Gut Microbiota and Extreme Longevity. *Curr Biol CB* (2016) 26(11):1480–5. doi: 10.1016/j.cub.2016.04.016
46. Wang N, Li R, Lin H, Fu C, Wang X, Zhang Y, et al. Enriched Taxa Were Found Among the Gut Microbiota of Centenarians in East China. *PLoS One* (2019) 14(10):e0222763. doi: 10.1371/journal.pone.0222763
47. Wilck N, Matus MG, Kearney SM, Olesen SW, Forslund K, Bartolomaeus H, et al. Salt-Responsive Gut Commensal Modulates T(H)17 Axis and Disease. *Nature* (2017) 551(7682):585–9. doi: 10.1038/nature24628

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Endocrinology

ORIGINAL RESEARCH
published: 06 September 2021
doi: 10.3389/fendo.2021.663096

Surgical Outcomes of Aldosterone-Producing Adenoma on the Basis of the Histopathological Findings

Huiping Wang[1,2], Fen Wang[1,3], Yushi Zhang[4], Jin Wen[4], Dexin Dong[4], Xiaoyan Chang[5], Hao Sun[6], Xiaosen Ma[1], Yunying Cui[1], Shi Chen[1], Lin Lu[1], Weidong Ren[2], Anli Tong[1] and Yuxiu Li[1]*

[1] Department of Endocrinology, Key Laboratory of Endocrinology, National Health Commission of the People's Republic of China, Peking Union Medical College Hospital, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing, China, [2] Department of Endocrinology, The First Affiliated Hospital of North University of Hebei, North University of Hebei, Zhangjiakou, China, [3] Department of Endocrinology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, [4] Department of Urology, Peking Union Medical College Hospital, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing, China, [5] Department of Pathology, Peking Union Medical College Hospital, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing, China, [6] Department of Radiology, Peking Union Medical College Hospital, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing, China

OPEN ACCESS

Edited by:
Vin-Cent Wu,
National Taiwan University, Taiwan

Reviewed by:
Kei Omata,
Tohoku University, Japan
Fabrizio Buffolo,
University of Turin, Italy
John Funder,
Hudson Institute of Medical Research, Australia
Koichi Yamamoto,
Osaka University, Japan

***Correspondence:**
Anli Tong
tonganli@hotmail.com

Specialty section:
This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Endocrinology

Received: *02 February 2021*
Accepted: *18 August 2021*
Published: *06 September 2021*

Citation:
Wang H, Wang F, Zhang Y, Wen J, Dong D, Chang X, Sun H, Ma X, Cui Y, Chen S, Lu L, Ren W, Tong A and Li Y (2021) Surgical Outcomes of Aldosterone-Producing Adenoma on the Basis of the Histopathological Findings. Front. Endocrinol. 12:663096. doi: 10.3389/fendo.2021.663096

Introduction: Previous studies on the surgical outcomes of aldosterone-producing adenoma (APA) patients were mainly based on the histopathological diagnosis of HE staining or adrenal venous sampling (AVS) instead of the functional pathology. The aim of the present study was to evaluate the surgical outcomes of APA patients based on the functional pathological diagnosis of APA according to HISTALDO (histopathology of primary aldosteronism) consensus.

Methods: Clinical data of 137 patients with suspected APA were analyzed retrospectively. All patients had hypertension and spontaneous hypokalemia. In all patients, CT showed a unilateral solitary hypodense adrenal lesion, and a contralateral adrenal gland of normal morphology. Tumors were removed and immunostained for CYP11B2, and their pathology were identified based on HISTALDO consensus. Patients were followed up 6 to 24 months after operation.

Results: Among 137 cases of presumptive APA diagnosed by CT, 130 (95%) cases were pathologically diagnosed with classical pathology, including 123 APA(90%) and 7 aldosterone-producing nodule (APN) (5%). 7 cases (5%) had non-functioning adenoma (NFA) with aldosterone-producing micronodule (APM) or multiple aldosterone-producing micronodule (MAPM) in the surrounding adrenal tissue. In all 137 patients, hypertension was complete or partial clinical success postoperatively. Complete clinical success was achieved in 73 (53%), and partial clinical success was achieved in 64 (47%) cases. Serum potassium level recovered to normal in all. In 123 patients with APA, complete clinical success was reached in 67 (54%), and partial clinical success was reached in 56 (46%) cases. Gender, duration of hypertension and the highest SBP were significant independent predictors for cure of APA after surgery. A multiple logistic regression

model integrating the three predictors was constructed to predict the outcome, which achieved a sensitivity of 72.4% and a specificity of 73.1%.

Conclusion: The specificity of CT in the diagnosis of APA and APN patients with hypokalemia was 95%. All patients achieved complete or partial clinical success after surgery. Gender, duration of hypertension and the highest SBP were independent predictors for the postoperative cure of APA.

Keywords: aldosterone-producing adenoma (APA), computed tomography, hypokalemia, surgical outcomes, CYP11B2 (aldosterone synthase), immunohistochemistry (IHC)

INTRODUCTION

Primary aldosteronism (PA) is a common cause of secondary hypertension, with its prevalence standing at about 5-19% (1). PA can be categorized into two major subtypes: unilateral aldosterone-producing adenoma (APA), accounting for about 30%, and bilateral adrenal hyperplasia (BAH), making up about 60% of all PA patients (2). Other less common types are unilateral adrenal hyperplasia (UAH), familial hyperaldosteronism, adrenocortical carcinoma, ectopic tumor, *etc.* PA can be divided into unilateral lesions and bilateral lesions according to the diseased side. Unilateral lesions are mostly seen in unilateral APA and UAH, and bilateral ones in BAH. Unilateral adrenalectomy can cure unilateral disease, whereas pharmacotherapy is the main strategy for bilateral disease (3). Therefore, it is important to distinguish unilateral from bilateral PA.

Adrenal vein sampling (AVS) is at present deemed as the gold standard for the classification of PA. The sensitivity and specificity of AVS for detecting unilateral aldosterone excess are 95% and 100% respectively (3, 4). However, AVS is invasive and brings radiation exposure to patients. In addition, although AVS can distinguish between unilateral and bilateral disease, unilateral aldosterone hypersecretion is not necessarily APA. Unilateral single or multiple APA, UAH and asymmetric BAH all can present lateralized aldosterone secretion on AVS (5–7). In contrast, adrenal computed tomography (CT) is simple and noninvasive, and can directly show adrenal lesions. However, CT may misdiagnose nonfunctional adenoma (NFA) as APA or miss small APA. The sensitivity and specificity of CT for the diagnosis of unilateral disease, with AVS as gold standard, were 78% and 75%, respectively (3, 4, 8, 9).

Histopathology with hematoxylin and eosin (HE) staining cannot distinguish NFA from APA. In 2014, a mouse monoclonal antibody against aldosterone synthase (CYP11B2) was established, which made it possible to study the function of adrenal adenoma (10). In 2021,HISTALDO (histopathology of primary aldosteronism) consensus for unilateral PA, based on HE staining and CYP11B2 immunohistochemistry (IHC), had been achieved (11).

Previous studies on the surgical outcome of APA patients were mainly based on the histopathology of HE staining or AVS (7, 12–14). NFA could be misdiagnosed as APA by HE staining, while lateralization on AVS, indicative of APA, could not exclude UAH or BAH with lateralization. In both cases, the surgical remission rate of APA might well be underestimated.

The aim of the present study was to evaluate the surgical outcome of APA patients based on functional pathological diagnosis of APA according to HISTALDO consensus.

METHODS AND MATERIALS

Patients

We studied 137 patients with PA who underwent tumor resection at Peking Union Medical College Hospital between 2016 and 2019. All patients had clinical hypertension and spontaneous hypokalemia. Hypokalemia was defined as serum potassium level lower than 3.5 mmol/L according to its normal range in our hospital, which is also widely accepted cut-off. In all patients, there was no history of gastrointestinal potassium loss such as diarrhea and vomiting, and no history of use of thiazides and loop diuretics or liquorice. All had both CT imaging and histopathology. In all the patients, their plasma renin activity (PRA) was <1 ng/ml/h, plasma aldosterone concentration (PAC) >12 ng/dl (measured by radioimmunoassay), and ALD to PRA ratio (ARR)>30. Fifth-three patients had a PAC over 20 ng/dl plus PRA below detection levels, and were diagnosed as PA according to PA guideline (15). These patients did not need further confirmatory testing. In the other 84 patients, 45 patients were confirmed to be PA by Captopril suppression test, and 39 patients did not undergo confirmatory testing. These 39 patients were suspected as PA preoperatively and were confirmed by histopathology. Cushing syndrome was excluded in all the patients by measuring plasma adrenocorticotropic hormone (ACTH) level, serum cortisol level, and urinary free cortisol excretion.

The following scan parameters of CT were used: 120 kVp; variable tube current with automatic tube current modulation activated; collimation =128*0.6 mm; rotation time=0.28 s; filter kernel=B30f (medium smooth); pitch=0.9; reconstructed slice thickness and intervals=3 mm. Theoretically, a tumor with size larger than 3 mm could be detected on CT scan. A tumor with size larger than 6 mm, which can be found in at least two slices, definitely can not be missed. Diagnostic criteria of APA on adrenal CT scan was that it showed a unilateral solitary

hypodense adrenal lesion with a CT value no higher than 20 which was consistent with the radiological feature of cortical adenoma, and a contralateral adrenal gland of normal morphology.

Written informed consent was obtained from each patient, and the study was approved by the Ethics Committee on Human Research of Peking Union Medical College Hospital, Beijing, China. Standard biosecurity and institutional safety procedures were adhered in the study.

Retrospectively studied were clinical data of the patients, including blood pressure and serum potassium, duration of hypertension, family history, history of diabetes and cardiovascular diseases, and laboratory results involving serum potassium, PAC, PRA, and imaging and pathological characteristics of the tumors.

Patients were followed up 6 to 24 months after operation. Blood pressure (including office and home blood pressure), serum potassium, antihypertensive medication and potassium supplementation were recorded. Complete clinical success and partial clinical success were defined according to PASO (16). Cure of hypokalemia was defined as serum potassium level over 3.5mmol/L without potassium supplementation.

Immunohistochemistry and Pathological Classification

IHC of aldosterone synthase CYP11B2 was performed in tumor tissues, by using EnVision detection kit (Dako). Dilution of the monoclonal antibody against CYP11B2 was 1:200. The mouse monoclonal CYP11B2 antibody was kindly provided by Dr Celso E. Gomez-Sanchez (Department of Medicine, University of Mississippi Medical Center, Jackson, Mississippi).Adrenal cortical lesions were classified based on HE staining and CYP11B 2 immunostaining according to HISTALDO consensus. The histopathologic results were independently reviewed by two experienced pathologists.

Statistical Analysis

All values were described as mean ± standard deviation or median (the 25th and 75th percentiles) for continuous variables with or without normal distribution, respectively. Student's t-test of two independent samples was used for the data of normal distribution, and the Mann-Whitney test of two independent samples was used for the data of non-normal distribution. Chi-square test was employed for evaluating the association between two categorical variables. Binary logistic regression analysis was utilized to identify predictors of outcome of APA after surgery. The receiver operating characteristic (ROC) curve was used to assess the efficacy of each method and to establish a prediction model. Hosmer–Lemeshow test was performed to assess the goodness of fit of the logistic model. All the aforementioned statistical analyses were carried out by using SPSS 23 software package. Madcalc software was used to compare the areas under the ROC curve (AUC) between two groups. A $P< 0.05$ was considered statistically significant.

RESULTS

Specificity of Adrenal CT Imaging in APA Diagnosis

Among 137 cases of presumptive APA diagnosed by CT, 130 (95%) cases were pathologically diagnosed with classical PA, including 123 APA(90%) and 7 aldosterone-producing nodule (APN)(5%). 7 cases (5%) had non-functioning adenoma (NFA) with aldosterone-producing micronodule(APM) or multiple aldosterone-producing micronodule(MAPM) in the surrounding adrenal tissue, which attributed to nonclassical PA. Therefore, the specificity of CT imaging in the diagnosis of classical PA was 95% (**Figure 1**).

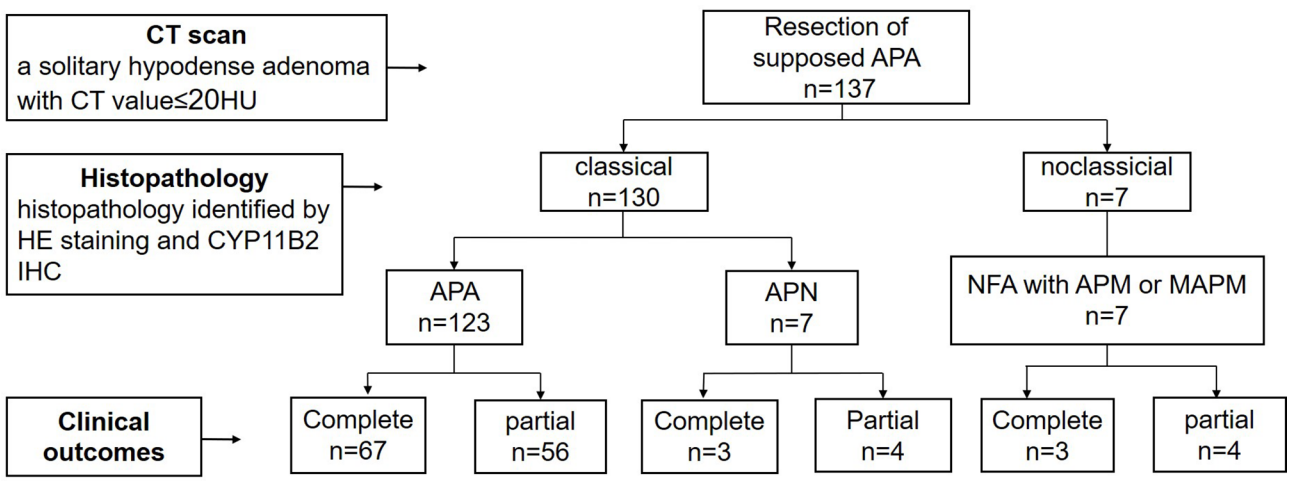

FIGURE 1 | The procedure about preoperative diagnosis of APA, histopathology diagnosis and clinical outcomes. APA, aldosterone producing adenoma; APN, aldosterone-producing nodule; NFA, non-functional adenoma; APM, aldosterone-producing micronodule; MAPM, Multiple aldosterone-producing micronodules.

Clinical Characteristics of PA

The clinical characteristics of 123 cases of APA, 7 cases of APN and 7 cases of nonclassical PA (NFA combined with AMP or MAPM) were compared. Duration of hypertension was different in the three groups. Nonclassical patients had the longest course of hypertension, followed by APA patients, and APN patients had the shortest duration. Compared with nonclassical patients, APA patients had a higher PAC and a lower LDL level. Blood pressure and serum potassium level did not show any difference among the three groups (**Table 1**). NFA patients were between 34 and 72 years old, and APA patients were between 21 and 69 years old. 34 years old was the cutoff to distinguish APA with 100% specificity.

Surgical Outcomes

All 137 patients achieved complete or partial clinical success postoperatively. Seventy-three cases (53%) achieved complete clinical success, and 64 cases (47%) achieved partial clinical success. Serum potassium level recovered to normal in all. In APA patients, complete clinical success was achieved in 67 cases (54%),and partial clinical success in 56 cases (46%), while in APN patients and nonclassical PA patients, only 43% patients reached complete clinical success (**Figure 1** and **Table 1**).

Factors Affecting Surgical Outcomes and the Prediction Model

In 130 classical patients (including APA and APN), 70 patients had complete clinical success, and 60 patients had partial clinical success. 38 out of the 60 patients (63%) still needed antihypertensive medication. Compared to those with postoperative hypertension, postoperative normotensive patients had a shorter duration of hypertension, a lower maximal SBP and maximal DBP, a lower computed tomography (CT) value, and a tendency of higher proportion of females (**Table 2** and **Figure 2**).

Binary logistic regression analysis revealed that gender, duration of hypertension and the highest SBP were significant independent predictors for the postoperative cure of APA (**Table 3**). A multiple logistic regression model integrating these three predictors was constructed to predict the outcome. AUC of the multiple logistic regression prediction model (0.762) was significantly higher than that of gender, duration of hypertension and the highest SBP alone ($P<0.05$). The prediction model had a sensitivity of 72.4% and a specificity of 73.1%, and the positive predictive value was 70% and the negative predictive value was 75% (AUC = 0.762; 95% confidence interval = 0.678–0.846) (**Figure 3** and **Supplement 1**).The Hosmer-Lemeshow test indicated that the model fits the data well ($P>0.1$).

DISCUSSION

In our cohort, based on pathological diagnosis of APA (11), the specificity of CT imaging for APA diagnosis was up to 90%. Although 10% of the patients were preoperatively suspected of

TABLE 1 | Clinical characteristics of patients with aldosterone-producing adenoma, aldosterone-producing nodule and non-functioning adenoma.

Variable	APA (N = 123)	APN (N = 7)	NFA with APM or MAPM (N = 7)
Age (years)	45 ± 10$^{\&\&}$	47 ± 7	57 ± 14
Gender (M/F)	54/69	3/4	2/5
Duration of hypertension (months)	60 (24,120)$^{*\&}$	12 (6,32)$^{\#\#}$	126 (72,180)
Duration of hypokalemia (months)	6 (3,24)	6 (5,67)	19 (1,36)
Preoperatively			
The highest SBP (mmHg)	182 ± 23	189 ± 27	179 ± 21
The highest DBP (mmHg)	112 ± 13	108 ± 17	106 ± 10
Serum potassium (mmol/L)	2.5 ± 0.5	2.9 ± 0.3	2.8 ± 0.5
PRA (ng/ml/h)	0.01 (0.01,0.03)	0.01 (0.01,0.01)	0.02 (0.01,0.02)
PAC (ng/dl)	20.3 ± 7.0$^{\&}$	15.4 ± 3.6	14.4 ± 2.8
LDL (mmol/L)	2.59 ± 0.69$^{\&\&}$	2.57 ± 0.71$^{\#}$	3.51 ± 0.65
Family history of hypertension	22% (27/122)	0% (0/7)	0% (0/7)
Diabetes	10% (12/120)	0% (0/7)	29% (2/7)
Smoker	16% (20/122)	29% (2/7)	29% (2/7)
Cardiac complications	34% (34/99)	25% (1/4)	33% (2/6)
Renal complication	0% (0/123)	0% (0/7)	0% (0/7)
Tumor size (mm)	17 (13,22)**	8 (7,9)$^{\#\#}$	23 (15,23)
Tumor location (Left/Right)	67/56$^{\&}$	5/2	7/0
CTvalue (HU)	2.0 (-5.0,7.3)	5.2 (-9.2,12.4)	3.3 (-5.2,5.1)
Postoperatively			
SBP (mmHg)	128 ± 12$^{\&}$	130 ± 10	140 ± 29
DBP (mmHg)	84 ± 8	83 ± 6	86 ± 12
Serum potassium (mmol/L)	4.2 ± 0.4	4.1 ± 0.3	4.0 ± 0.1
Cure of hypertension	54% (67/123)	43% (3/7)	43% (3/7)
Antihypertensive medications	28% (34/123)	57% (4/7)	57% (4/7)
Potassium supplementation	0% (0/123)	0% (0/7)	0% (0/7)

*$P < 0.05$ **$P < 0.01$ APA vs.APN; $^{\#}P < 0.05$ $^{\#\#}P < 0.01$ APN vs. NFA; $^{\&}P < 0.05$ $^{\&\&}P < 0.01$ APA vs. NFA.

APA, aldosterone-producing adenoma; APN, aldosterone-producing nodule; NFA, non-functioning adenoma; MAPM, multiple aldosterone-producing micronodule; APM, aldosterone-producing micronodule; PRA, plasma renin activity; PAC, plasma aldosterone concentration.

TABLE 2 | Comparison of clinical characteristics between 130 patients with classical histopathologic findings with complete and partial clinical success.

Variable	Complete (N = 70)	Partial (N = 60)	P value
Age (years)	44 ± 10	46 ± 10	0.195
Gender (M/F)	26/44	31/29	0.096
Duration of hypertension (months)	48 (11,120)	72 (39,120)	0.005
Duration of hypokalemia (months)	6 (3,22)	9 (4,24)	0.153
Preoperatively			
The highest SBP (mmHg)	174 ± 18	192 ± 26	<0.001
The highest DBP (mmHg)	107 ± 10	117 ± 15	<0.001
Serum potassium (mmol/L)	2.5 ± 0.5	2.5 ± 0.5	0.915
PRA (ng/ml/h)	0.01 (0.01,0.01)	0.01 (0.01,0.06)	0.446
PAC (ng/dl)	19.7 ± 7.0	20.5 ± 6.9	0.535
LDL (mmol/L)	2.62 ± 0.70	2.55 ± 0.68	0.600
Family history of hypertension	24% (17/70)	17% (10/59)	0.330
Diabetes	4% (3/68)	15% (9/59)	0.103
Smoker	13% (9/70)	22% (13/59)	0.214
Cardiac complications	32% (18/56)	36% (17/47)	0.887
Renal complication	0% (0/70)	0% (0/60)	——
Tumor size (mm)	15.0 (12.3,20.0)	16.5 (13.0,21.5)	0.780
Tumor location (Left/Right)	35/35	37/23	0.182
CTvalue (HU)	2.0 (-5.5,5.0)	5.0 (-4.7,8.8)	0.041
Postoperatively			
SBP (mmHg)	122 ± 9	136 ± 11	<0.001
DBP (mmHg)	79 ± 5	89 ± 7	<0.001
Serum potassium (mmol/L)	4.3 ± 0.4	4.2 ± 0.3	0.045
Antihypertensive medications	0% (0/70)	63% (38/60)	<0.001
Potassium supplementation	0% (0/70)	0% (0/60)	——

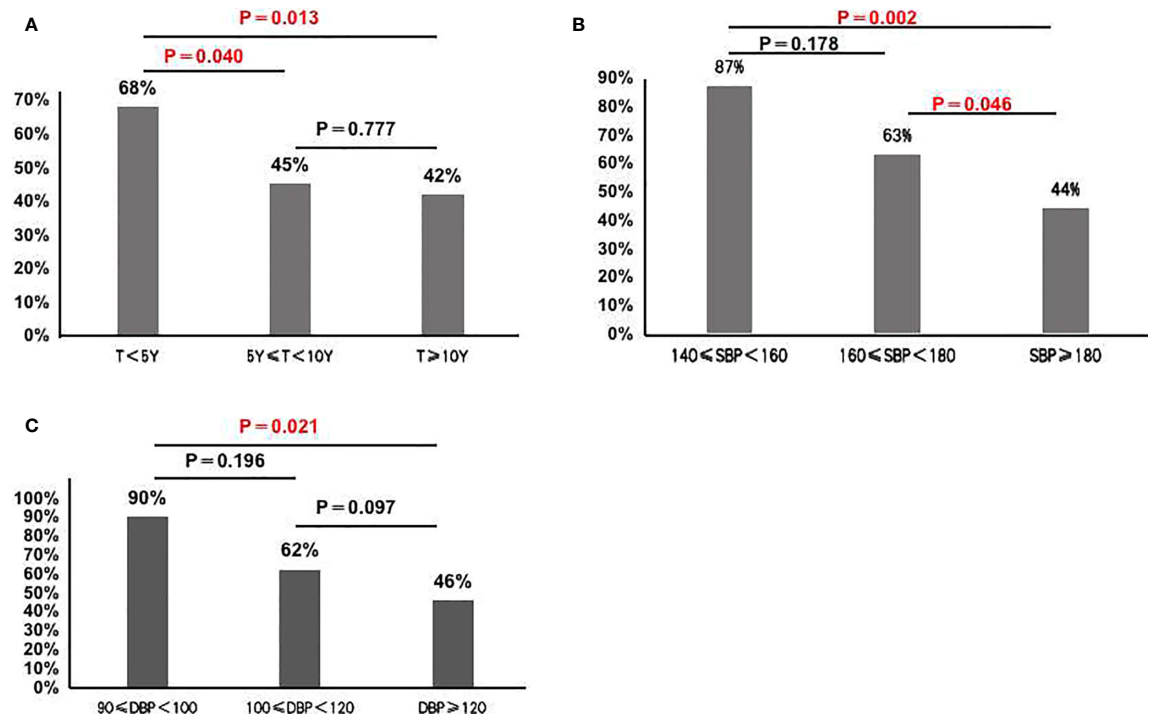

FIGURE 2 | **(A)** Cure rate of hypertension in the group with hypertension during less than 5 years, more than 5 years and 10 years (68% *vs.* 45% *vs* 42%); **(B)** Cure rate of hypertension in the group with SBP<160 mmHg group, 160 mmHg≤SBP<180 mmHg group and SBP≥180 mmHg group (87% *vs*. 63% *vs* 44%); **(C)** Cure rate of hypertension in the group with DBP<100mmHg group, 100mmHg≤DBP < 120mmHg group and DBP≥120mmHg group(90% *vs*. 62% *vs*. 46%).

TABLE 3 | Estimates of parameters of logistic regression model for complete clinical success of patients with classical histopathologic findings.

Variables	Coefficient	SE	Wald	P-value	OR (95% CI)
Gender	1.137	0.435	6.828	0.009	3.118 (1.329 to 7.31)
Duration of hypertension (month)	0.006	0.003	4.916	0.027	1.006 (1.001 to 1.01)
The highest SBP (mmHg)	0.042	0.011	14.714	0.000	1.043 (1.021 to 1.066)
Constant	-8.909	2.104	17.924	0.000	0.000

CI, confidence interval.

APA by CT, and finally diagnosed as APN and NFA based on their pathological characteristics, the blood pressure of all patients in our cohort was relieved, and the cure rate of hypokalemia was 100%. Since all patients in our cohort clinically benefited from the operation, for the PA patients with hypokalemia, if CT reveals a solitary, hypodense adenoma and normal contralateral adrenal morphology, AVS may not be necessary, and surgery can be performed directly. Recently, Burrello et al. proposed the SPACE(Subtyping Primary Aldosteronism by Clinical Evaluation) score for determination of patients that can avoid AVS before surgery. They suggested that patients with a score greater than 16 points can avoid AVS (17). In our study, 117 (85%) patients have a SPACE score greater than 16 points. In fact, a patient with a solitary nodule greater than 1 cm, hypokalemia and normal contralateral adrenal gland should have 16.5 points of SPACE. In our study, patients with NFA with APM or MAPM achieved complete or partial clinical success after adrenalectomy. They would appear to have been not inappropriately treated by unilateral adrenalectomy. Even if the remaining adrenal also has nodules, it may be easier to treat with mineral corticoid receptor antagonists.

In our study, patients with NFA had a longer duration of hypertension than those with APA and APN. They probably had relatively mild symptoms and progress slowly, thus leading to the delay of diagnosis. Patients with APA had a longer duration than those with APN. We speculate that APN may be the precursor of APA, because both APA and APN were dominated by KCNJ5 mutation (unpublished data).

In previous studies, surgical outcomes of APA patients were mainly based on the histopathology of HE staining or AVS instead of functional pathology (7, 12–14, 18, 19). A study from Mayo Clinic observed the outcome of unilateral adrenalectomy in 263 patients with presumptive unilateral disease diagnosed by imaging and AVS. Hypertension was cured in 53patients (41.7%). Whereas 59 patients (46.5%) showed hypertension improvement (12). Recently, CYP11B2 IHC was widely used in studies to examine the functional morphology of normal and pathological adrenals (11, 20) and occasionally in subtyping and outcome prediction in PA (21, 22). Volpe C. et al. examined the clinical outcomes of APA with definite functional, IHC features. In this study, all but one patient (93/94) with APA were biochemically cured after adrenalectomy. Forty-six percent of the APA patients arrived at a complete clinical cure (23). In our study, hypertension was cured in 54% and improved in 46% of the APA patients who were identified based on the new pathological consensus (11).

In our study, the predictive factors of postoperative clinical cure of patients included female gender, shorter duration of hypertension and lower level of blood pressure. Citton et al. also found that female gender, a fewer number of antihypertensive drugs, and a shorter duration of hypertension were the main predictors of hypertension cure (18). In addition, the present study developed a model, integrating multiple predictors to predict the prognosis of surgery, which is easy to use and is more accurate than single clinical parameter for the prediction of surgical outcomes.

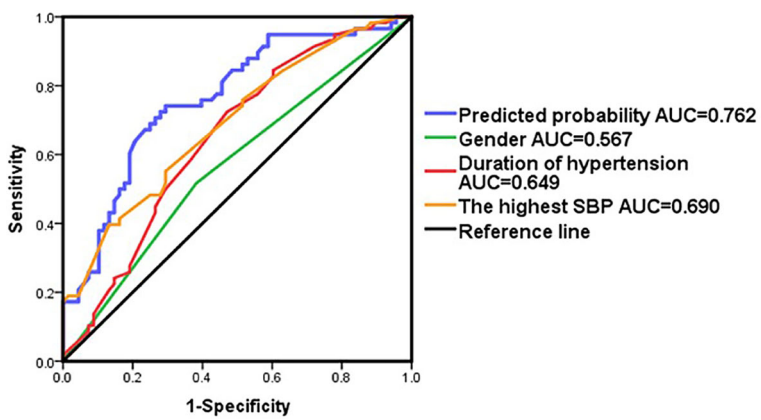

FIGURE 3 | ROC curve in patients by adding variables as gender,duration of hypertension and the highest SBP.

Strengths and Limitations of the Study

The strength of our study lies in that it employed the diagnostic criteria of APA according to HISTALDO consensus, which are superior to pathological examination with only HE staining or AVS alone. Diagnosis by only HE staining cannot exclude NFA, and AVS cannot rule out adrenal nodular hyperplasia with lateralization. In addition, our results support the feasibility to skip AVS in patients with hypokalemic PA, which may simplify the diagnostic procedures and bring benefits to patients.

However, our study had some limitations: (1) Because postoperative data on ALD and PRA were not available in most patients, only clinical parameters were used in the outcome evaluation. Furthermore, lack of AVS for PA classification is another issue. (2) The present paper focused only on the patients with hypokalemia, the severe kind of PA. The PA patients with normokalemia or APA without typical features on CT were excluded, though these patients account for a certain proportion in daily practice. Therefore, our conclusion may not be extrapolated to all PA patients. It is speculated that there will be larger proportion of nodular hyperplasia and NFA in the patients without hypokalemia. (3) The limited number of APN and APM or MAMP is statistically relevant for the comparison. And the limited number of APN is likely due to the fact that indication for surgery was made on the basis of CT alone, and not AVS.(4) The prediction model for clinical outcomes established in our study needs to be verified by a validation cohort.

CONCLUSION

The specificity of CT for the diagnosis of classical PA (APA and APN) with hypokalemia based on functional pathology was 95%. Practically all patients achieved complete or partial clinical success after operation. Gender, duration of hypertension and the highest SBP were independent predictors for the postoperative cure of APA.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: Genome Sequence Archive in National Genomics Data Center, Beijing Institute of Genomics (China National Center for Bioinformation), Chinese Academy of Sciences, under accession number HRA000965.

ETHICS STATEMENT

All procedures performed in studies involving human participants meet the ethical standards of the institutional research committee (Ethics committee of Peking Union Medical College Hospital; S-K431). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: AT and YL. Collected and analyzed clinical data: HW, FW, YZ, JW, DD, SC, LL, WR. Conducted radiological and pathological analysis: XC, HS, XM, YC. Write the manuscript: HW, FW. Revise the manuscript: AT. All authors contributed to the article and approved the submitted version.

FUNDING

This research was supported by National Natural Science Foundation of China (Grant No. 81770427, and 82070822) and CAMS Innovation Fund for Medical Sciences(CIFMS) of China (Grant No. 2017-I2M-1-001).

ACKNOWLEDGMENTS

The authors thank Dr. Zhengpei Zeng in Department of Endocrinology and Dr. Hanzhong Li in Department of Urology in Peking Union Medical College Hospital for providing expert advice. We are also grateful to Dr Celso E. Gomez-Sanchez (Department of Medicine, University of Mississippi Medical Center, Jackson, Mississippi) for kindly providing us antibody against CYP11B2.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.663096/full#supplementary-material

REFERENCES

1. Kline GA, Prebtani APH, Leung AA, Ernesto L, Schiffrin CM. Primary Aldosteronism: A Common Cause of Resistant Hypertension. *Cmaj* (2017) 189(22):E773–e778. doi: 10.1503/cmaj.161486
2. Young WF Jr. Diagnosis and Treatment of Primary Aldosteronism: Practical Clinical Perspectives. *J Intern Med* (2019) 285(2):126–48. doi: 10.1111/joim.12831
3. Funder JW, Carey RM, Mantero F, Murad MH, Reincke M, Shibata H, et al. The Management of Primary Aldosteronism: Case Detection, Diagnosis, and Treatment: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab* (2016) 101(5):1889–916. doi: 10.1210/jc.2015-4061
4. Young WF, Stanson AW, Thompson GB, Grant CS, Farley DR, van Heerden JA. Role for Adrenal Venous Sampling in Primary Aldosteronism. *Surgery* (2004) 136(6):1227–35. doi: 10.1016/j.surg.2004.06.051
5. Shariq OA, Mehta K, Thompson GB, Lyden ML, Farley DR, Bancos I, et al. Primary Aldosteronism: Does Underlying Pathology Impact Clinical Presentation and Outcomes Following Unilateral Adrenalectomy? *World J Surg* (2019) 43(10):2469–76. doi: 10.1007/s00268-019-05059-y
6. Lee JI, Oltmann SC, Woodruff SL, Nwariaku FE, Holt SA, Rabaglia JL. Contralateral Adrenal Abnormalities in Conn's Syndrome. *J Surg Res* (2016) 200(1):183–8. doi: 10.1016/j.jss.2015.07.002

7. Aono D, Kometani M, Karashima S, Usukura M, Gondo M, Hashimoto Y, et al. Primary Aldosteronism Subtype Discordance Between Computed Tomography and Adrenal Venous Sampling. *Hypertens Res* (2019) 42 (12):1942–50. doi: 10.1038/s41440-019-0310-y
8. Nwariaku FE, Miller BS, Auchus R, Holt S, Watumull L, Dolmatch B, et al. Primary Hyperaldosteronism: Effect of Adrenal Vein Sampling on Surgical Outcome. *Arch Surg* (2006) 141(5):497–502. doi: 10.1001/archsurg.141.5.497
9. Sawka AM, Young WF, Thompson GB, Grant CS, Farley DR, Leibson C, et al. Primary Aldosteronism: Factors Associated With Normalization of Blood Pressure After Surgery. *Ann Intern Med* (2001) 135(4):258–61. doi: 10.7326/0003-4819-135-4-200108210-00010
10. Nakamura Y, Maekawa T, Felizola SJA, Satoh F, Qi X, Velarde-Miranda C, et al. Adrenal CYP11B1/2 Expression in Primary Aldosteronism: Immunohistochemical Analysis Using Novel Monoclonal Antibodies. *Mol Cell Endocrinol* (2014) 392(1-2):73–9. doi: 10.1016/j.mce.2014.05.002
11. Williams TA, Gomez-Sanchez CE, Rainey WE, Giordano TJ, Lam AK, Marker A, et al. International Histopathology Consensus for Unilateral Primary Aldosteronism. *J Clin Endocrinol Metab* (2021) 106(1):42–54. doi: 10.1210/clinem/dgaa484
12. Lim V, Guo Q, Grant CS, Thompson GB, Richards ML, Farley DR, et al. Accuracy of Adrenal Imaging and Adrenal Venous Sampling in Predicting Surgical Cure of Primary Aldosteronism. *J Clin Endocrinol Metab* (2014) 99 (8):2712–9. doi: 10.1210/jc.2013-4146
13. Citton M, Viel G, Rossi GP, Mantero F, Nitti D, Iacobone M. Outcome of Surgical Treatment of Primary Aldosteronism. *Langenbecks Arch Surg* (2015) 400(3):325–31. doi: 10.1007/s00423-014-1269-4
14. Takeda M, Yamamoto K, Akasaka H, Rakugi H, Naruse M, Takeda Y, et al. Clinical Characteristics and Postoperative Outcomes of Primary Aldosteronism in the Elderly. *J Clin Endocrinol Metab* (2018) 103 (10):3620–9. doi: 10.1210/jc.2018-00059
15. Williams TA, Reincke M. Management of Endocrine Disease: Diagnosis and Management of Primary Aldosteronism: The Endocrine Society Guideline 2016 Revisited. *Eur J Endocrinol* (2018) 179(1):R19–r29. doi: 10.1530/EJE-17-0990
16. Williams TA, Lenders JWM, Mulatero P, Burrello J, Rottenkolber M, Adolf C, et al. 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):689–99. doi: 10.1016/S2213-8587(17)30135-3
17. Burrello J, Burrello A, Pieroni J, Sconfienza E, Forestiero V, Rabbia P, et al. Development and Validation of Prediction Models for Subtype Diagnosis of Patients With Primary Aldosteronism. *J Clin Endocrinol Metab* (2020) 105 (10):20–6. doi: 10.1210/clinem/dgaa379
18. Grytaas MA, Strømsøy SS, Rørvik JT, Arnes JB, Heie A, Arnesen T, et al. Clinical Characteristics and Long-Term Outcome of Primary Aldosteronism in a Norwegian Population. *Horm Metab Res* (2017) 49(11):838–46. doi: 10.1055/s-0043-118909
19. Rossitto G, Amar L, Azizi M, Riester A, Reincke M, Degenhart C, et al. Subtyping of Primary Aldosteronism in the AVIS-2 Study: Assessment of Selectivity and Lateralization. *J Clin Endocrinol Metab* (2020) 105(6):2042–52. doi: 10.1210/clinem/dgz017
20. Meyer LS, Wang X, Sušnik E, Burrello J, Burrello A, Castellano I, et al. Immunohistopathology and Steroid Profiles Associated With Biochemical Outcomes After Adrenalectomy for Unilateral Primary Aldosteronism. *Hypertension* (2018) 72(3):650–7. doi: 10.1161/HYPERTENSIONAHA.118.11465
21. Holler F, Heinrich DA, Adolf C, Lechner B, Bidlingmaier M, Eisenhofer G, et al. Steroid Profiling and Immunohistochemistry for Subtyping and Outcome Prediction in Primary Aldosteronism-A Review. *Curr Hypertens Rep* (2019) 21(10):77. doi: 10.1007/s11906-019-0985-0
22. Nishimoto K, Umakoshi H, Seki T, Yasuda M, Araki R, Otsuki M, et al. Diverse Pathological Lesions of Primary Aldosteronism and Their Clinical Significance. *Hypertens Res* (2021) 44:498–507. doi: 10.1038/s41440-020-00579-w
23. Volpe C, Hamberger B, Höög A, Mukai K, Calissendorff J, Wahrenberg H, et al. Primary Aldosteronism: Functional Histopathology and Long-Term Follow-Up After Unilateral Adrenalectomy. *Clin Endocrinol (Oxf)* (2015) 82 (5):639–47. doi: 10.1111/cen.12645

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: frontiersin.org/about/contact

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership