Single center prospective cohort study on the histopathology, genotype and postsurgical outcomes of patients with primary aldosteronism

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Total word count: 6,000
Tables: 2 tables
Figures: 3 figures

Online-data supplement: 1 table, 2 figures

Running title: Histopathology and persistence of aldosteronism

Abstract

Unilateral forms of primary aldosteronism (PA) are usually surgically treated to remove the source of aldosterone excess. After adrenalectomy, aldosteronism persists in some patients indicating abnormal aldosterone production from the unresected gland. Our objective was to investigate histopathology, genotype and postsurgical outcomes in a 3-year prospective cohort of surgically treated patients for PA (from 2016 to 2018). The cohort comprised 60 consecutively operated patients categorized with classical or nonclassical histopathologic findings of unilateral PA. In the classical group were 45 solitary aldosterone-producing adenomas or dominant aldosterone-producing nodules; in the nonclassical group, 15 cases of multiple aldosterone-producing micronodules or nodules (12 cases), or aldosterone-producing diffuse hyperplasia (3 cases). The classical group displayed higher baseline plasma aldosterone concentrations (262 *versus* 155 pg/mL, P=0.008) and an increased aldosterone-to-renin ratio (81 *versus* 42, P=0.002). A high proportion of the classical group achieved complete biochemical success (97.6% *versus* 66.7% in the nonclassical group, P=0.002). The nonclassical *versus* classical group displayed an increased ratio of absolute aldosterone concentration in the contralateral adrenal vein to peripheral vein at adrenal venous sampling (3.8 *versus* 2.0, P=0.004). Variants in aldosterone-driver genes were identified in 85% of 41 APAs and were excluded in the remaining 15% by CYP11B2 guided next generation sequencing. There were no differences in clinical or biochemical outcomes in patients with a solitary APA categorized by *KCNJ5* mutation status. In conclusion, adrenals with a nonclassical histopathology of unilateral PA are associated with a higher incidence of disease persistence and increased aldosterone production from the unresected adrenal.

Introduction

Unilateral primary aldosteronism (PA) is the most common surgically correctable form of hypertension[1,2] usually caused by aldosterone overproduction from an adrenocortical aldosterone-producing adenoma (APA). Surgical resection of the diseased gland should remove the source of aldosterone excess but postsurgical aldosteronism (partial and absent biochemical success) persists in 6% of patients when unilateral forms are identified by adrenal venous sampling (AVS).[3] When subtype differentiation is performed by AVS, postsurgical persistence is likely accounted for by presurgical highly asymmetrical forms of bilateral PA.

CYP11B2 immunohistochemistry of the resected glands determines the expression of aldosterone synthase and the probable source of aldosterone production.[4,5] We and others have previously reported the utility of routine adrenal CYP11B2 immunostaining in the histopathologic diagnosis of surgically treated patients with PA.[6-10] Recently, an international group of pathologists and adrenal experts published the HISTALDO (histopathology of primary aldosteronism) consensus to standardize the previously discordant nomenclature for histopathologic features of adrenals from patients with PA.[10]

Somatic variants in genes involved in the control of intracellular ion homeostasis have been identified that drive the overproduction of aldosterone in APAs.[11,12] CYP11B2 immunohistochemistry has been used as a guide to identify regions in formalin-fixed paraffin embedded (FFPE) tissue sections for genotyping by targeted next generation sequencing (NGS) panels focusing on selected genes associated with PA.[13-15] This approach of CYP11B2-guided amplicon NGS helped identify mutations in aldosterone-driver genes in 88-96% of

APAs[13-15] and a CYP11B2 guided whole-exome sequencing method also identified somatic variants in additional genes[16,17].

We investigated the molecular and clinical pathology of a 3-year prospective series of surgically treated patients with PA operated from 2016 to 2018 diagnosed at a single tertiary referral center. Our aims were to determine i) the prevalence and clinical presentation of different histopathologic forms of PA; ii) the association of defined histopathologic phenotypes with abnormal aldosterone production from the contralateral adrenal gland at baseline and with postsurgical outcomes; (iii) the prevalence of variants in aldosterone-driver genes in APAs and potential association with postsurgical outcomes.

Materials and Methods

The expanded methods are available in the Data Supplement. The authors declare that all supporting data are available within the article and the Data Supplement.

Patient cohort

The study included all consecutively operated patients for PA who gave written informed consent for use of clinical data and biomaterial in accordance with the local ethics committee over a 3-year period (2016 to 2018) at the Klinikum der Ludwig-Maximilians-Universität München, Munich, Germany. Accordingly, 60 patients were included from a total of 66 patients treated by unilateral laparoscopic adrenalectomy for PA over the same period at the same centre.

The diagnosis of PA and subtype differentiation by adrenal venous sampling (AVS) was based on the Endocrine Society Guideline[18] and by local criteria[19]. Follow-up data were available for 54 of 60 patients at 6-12 months after surgery. Clinical and biochemical outcomes were assessed according to the PASO criteria.[3] There were 3 patients who bypassed AVS for subtype differentiation. Two of these patients met the criteria predictive of cure[20,21] and were classified as complete biochemical success after surgery[3]. The third patient refused AVS but was favourable for surgery despite counselling of 20% probability of failure[20] and the postsurgical outcome was classified as absent biochemical success.

Histopathology

Haematoxylin and eosin (H&E) staining and CYP11B2 immunostaining[4] (clone 41-17B, a kind gift from Celso E. Gomez-Sanchez, University of Mississippi Medical Center, Jackson, MS, USA) was performed on 3 μm thick FFPE adrenal tissue sections as described previously.[7] Tissue sections of all blocks from each resected adrenal were evaluated by H&E and CYP11B2 immunostaining and adrenal specimens were categorized as classical or nonclassical histopathological findings of unilateral PA according to the HISTALDO consensus.[10] The classical group comprised adrenals with solitary APAs (aldosterone-producing adenomas) and dominant APNs (aldosterone-producing nodules). The nonclassical group comprised adrenals without an APA or dominant APN, but with multiple aldosterone-producing nodules or aldosterone-producing micronodules (previously referred to as aldosterone-producing cell clusters) or aldosterone-producing diffuse hyperplasia.[10]

Genotyping

All adrenals with a solitary APA or dominant APN were used for CYP11B2 guided targeted Sanger sequencing using FFPE adrenal samples.[22] The genotype of mutation negative samples was validated by CYP11B2 guided targeted amplicon next generation sequencing.[13,17] For 2 solitary APAs (diameter 28 mm and 10 mm), exome sequencing was performed on fresh frozen tissue (Eurofins Genomics, Ebersberg, Germany). For all mutation-positive samples, leukocyte DNA or a CYP11B2-negative region of adrenal from the same patient was sequenced for the presence of the same mutation.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics 26 or GraphPad Prism version 8.4.2. Clinical and biochemical parameters are shown as medians and interquartile ranges, means ± SD or absolute numbers and percentages. Non-normally distributed data were analysed using a Mann-Whitney U test or Kruskal-Wallis test for two or more groups, respectively. Normally distributed variables were analysed using a t-test for 2 groups or 1-way-ANOVA with Tukey's multiple comparisons test for >2 groups. For categorical variables, a Chi-squared test or Yates's chi-squared test was used. Differences were considered significant with a *P* value <0.05.

Results

Patient cohort and Histopathology

The cohort comprised 60 patients with a mean age of 51 ± 13 years and a median known duration of hypertension of 103 [39-171] months. The patients were categorized into 2 groups according to classical *versus* nonclassical histopathologic findings of unilateral PA according to the HISTALDO consensus.[9] There was no difference in age, sex distribution or duration of

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hypertension between the classical and nonclassical groups. Patients with a solitary APA or dominant APN (classical histopathology group) displayed higher baseline plasma aldosterone concentrations (262 pg/mL [164-356] *versus* 155 pg/mL [129-180], *P*=0.008) and an increased aldosterone-to-renin ratio (81 [45-116] *versus* 42 [28-50], *P*=0.002) compared with the nonclassical group (**Table 1**).

The 60 adrenal samples comprised 45 with the classical histopathology (solitary APA or dominant APN) and 15 with nonclassical findings comprising multiple aldosterone-producing nodules or micronodules (12 cases) or aldosterone-producing diffuse hyperplasia (3 cases) (**Figure 1A**). More than half (58%) of the 45 adrenals with a solitary APA or dominant APN displayed aldosterone-producing lesions in the adjacent cortex (multiple aldosterone-producing micronodules or aldosterone-producing diffuse hyperplasia in 23 and 3 cases, respectively) (**Figure 1B**). The categorization of the resected adrenals according to histopathologic findings is shown in **Figure 2**.

Histopathology and postsurgical outcomes

Follow-up parameters were available for 54 of 60 patients for assessment of clinical and biochemical outcomes according to the international PASO criteria.[3] Postsurgical complete clinical success with normalization of blood pressure was observed in 18.5% of patients, and a further 57.4% displayed partial clinical success with significant clinical improvements (**Figure 3**). Notwithstanding the absence of significant differences in clinical outcomes between the classical *versus* nonclassical histopathology groups (*P*=0.224), the proportion of patients with absent clinical success was around 2-fold higher in the nonclassical compared with the classical group (41.7% *versus* 19%).

In the total cohort, postsurgical complete biochemical success was observed in 90.7% of patients. This proportion increased to 97.6% in patients with the classical histopathology of a solitary APA or dominant APN compared with 66.7% in the nonclassical group (P=0.004). Of note, the presence of aldosterone-producing lesions in the adjacent cortex of 26 of 45 (58%) of the classical cases did not have a negative effect on the biochemical outcomes of these patients.

Histopathology and contralateral suppression

The differences in biochemical outcomes suggested baseline abnormal aldosterone production from the contralateral gland in a significant proportion of patients with the nonclassical histopathology group. Using unstimulated AVS results, we evaluated contralateral suppression of aldosterone production from the ratio of the absolute aldosterone concentration in the contralateral adrenal vein to the peripheral vein (A_{CL}/A_P). Overall, contralateral suppression was observed in 7% (4 of 54) of cases, with all 4 cases occurring in patients with the classical histopathology. Further, the ratio of absolute aldosterone concentration in the contralateral adrenal vein to the peripheral vein was increased in the nonclassical compared with the classical histopathology group (3.8 [1.7-6.5] *versus* 2.0 [1.1-3.1], P=0.004) (**Figure 3**). Restricting the analysis of contralateral suppression (A_{CL}/A_P) to the classical histopathology group alone with categorization of adrenal samples according to the absence or presence of aldosterone-producing lesions in the adjacent cortex did not detect a between-group difference (1.6 [1.1-2.6] *versus* 2.3 [1.2-3.9], P=0.338).

Contralateral suppression indices using cortisol-corrected aldosterone concentrations did not reveal differences between the classical and nonclassical groups. In addition, the lateralization index did not significantly differ between the 2 histopathologic groups (**Table S1**).

Prevalence of variants in aldosterone-driver genes

All solitary APAs from patients who gave consent for genetic analysis (41 of 45 adrenals) were analyzed for the presence of variants in aldosterone-driver genes. A summary of the sequencing approach, the cDNA and corresponding protein sequence variant is shown in **Table 2**. The overall prevalence of variants in aldosterone-driver genes was 85% (35 of 41 samples) with mutations in the *KCNJ5* gene predominating (56%, 23 of 41 samples). The *KCNJ5* mutations included a novel p.L168_L169delinsKR variant, that we found in 2 different APAs, absent from paired leukocyte DNA (**Table 2**). The prevalence of mutations in the different aldosterone-driver genes is shown in **Figure S1**. Of the 41 sequenced APAs, 5 (12%) carried a mutation in *ATP1A1*, 2 (5%) in *ATP2B3* and 4 (10%) in *CACNA1D*. Variants in *CTNNB1* in this series were not identified and 6 APAs were mutation-negative (15% of 41 APAs), an observation determined by CYP11B2 guided amplicon NGS. A variant in a highly conserved region of the chloride voltage-gated channel 2 encoded by *CLCN2* was also identified by exome sequencing (NM_004366.6, c.731G>A, p.C244Y), but did not appear to have a functional effect (**Figure S2**).

KCNJ5 mutations and postsurgical outcomes

Patients with classical histopathologic findings of a solitary APA or dominant APN were stratified into those with and without *KCNJ5* variants. Of these, there were 38 patients with available outcome data, comprising 21 APAs with and 17 without a *KCNJ5* mutation. There

were no differences in clinical or biochemical outcomes according to the presence or absence of a *KCNJ5* mutation (**Figure S1**).

Discussion

The increased use of AVS and intensified screening for PA has increased the detection of milder forms[23] and amplified the detection of diverse histopathologic forms of lateralized PA.[7,24,25] Using nomenclature defined by the HISTALDO consensus[9] with morphologic evaluation and multiple block CYP11B2 immunohistochemistry[4,5], we demonstrate a solitary APA or a dominant aldosterone-producing nodule in ¾ of the cohort (classical histopathologic findings of unilateral PA). This prevalence corresponds to reports in 2 retrospective reviews of 206 and 95 surgically treated patients for unilateral PA diagnosed by AVS that identified solitary adenomas in 74% and 69% of cases.[26,27]

In contrast, aldosterone overproduction was attributed to multiple aldosterone-producing nodules, micronodules or diffuse hyperplasia in the remaining ¼ cases (nonclassical histopathologic findings of unilateral PA). Thus, adrenals without a solitary APA or a dominant APN were not uncommon histopathologic findings in this series of surgical adrenal specimens. The nonclassical histopathologic forms displayed a milder clinical phenotype with significantly lower plasma aldosterone concentrations and aldosterone-to-renin ratios at baseline compared with the classical phenotype. Specifically, the nonclassical group comprised 20% multiple aldosterone-producing micronodules (formerly referred to as aldosterone-producing cell clusters[28]) (or multiple aldosterone-producing nodules), or in 5% of cases, aldosterone-producing diffuse hyperplasia.[9]

Patients with nonclassical histopathologic findings of unilateral PA were often characterized by aldosterone-producing micronodules, which are prevalent in some forms of bilateral PA.[29] We demonstrate that a third of patients (4 of 12) in the nonclassical group with multiple aldosterone-producing micronodules (or nodules) or aldosterone-producing diffuse hyperplasia are not biochemically cured after adrenalectomy, in contrast to 1 of 42 in the classical group. These findings are consistent with observations of a multicenter study that reported an increased incidence of adrenals with adrenocortical hyperplasia in the absence of a solitary APA (nonclassical form) from surgically treated patients with PA who were not biochemically cured after adrenalectomy with age- and sex- matched patients who were biochemically cured (partial + absent *versus* complete biochemical success).[7] These data are in contrast to previous studies that reported no link between adrenal pathology and outcomes.[26,27] However, neither report evaluated CYP11B2 immunohistochemistry for functional histopathologic assessment and outcomes were assessed just after adrenalectomy,[26,27] in contrast to 6-12 months post-adrenalectomy as in the present study.[3]

The presence of aldosterone-producing multiple nodules, micronodules or diffuse hyperplasia in the ipsilateral gland suggests their possible presence in the contralateral gland. An APA produces and secretes high amounts of aldosterone that suppresses the renin-angiotensin system and hence aldosterone production from the contralateral (non-dominant) gland, which is usually calculated using cortisol-corrected aldosterone concentrations in the contralateral adrenal vein compared with the peripheral vein.[30] However, absolute aldosterone concentrations have also been used to evaluate the association of contralateral suppression with postsurgical outcomes.[31,32] We were interested in quantifying aldosterone production from the contralateral gland. Higher absolute aldosterone concentrations in the

contralateral vein compared with the peripheral vein (A_{CL}/A_P ratio) would provide good evidence for inappropriate aldosterone secretion without requiring cortisol-correction for adrenal venous blood dilution during the AVS procedure because in the case of blood dilution, the real adrenal vein aldosterone concentrations would be even higher but not lower. Also, there would be no reason to correct for dilution of aldosterone in peripheral blood which could potentially confound the evaluation in some way. We observed an increased A_{CL}/A_P ratio in patients with the nonclassical histopathology, which suggests elevated aldosterone production from the contralateral gland at baseline compared with patients with the classical histopathology of a solitary APA or dominant APN. Of note, the presence of aldosterone-producing multiple nodules, micronodules or diffuse hyperplasia in the ipsilateral adrenal in the presence of a solitary APA or dominant APN did not adversely influence postsurgical biochemical outcomes.

The finding of higher aldosterone concentrations in the contralateral adrenal vein compared with peripheral blood in the nonclassical histopathology group suggests presurgical asymmetrical bilateral disease in these patients. The AVS procedure in this study was performed under basal conditions. However, comparison of AVS under basal conditions with cosyntropin (synthetic adrenocorticotropic hormone)-stimulated AVS have shown that an initial diagnosis of unilateral PA can switch to a diagnosis of bilateral disease with a cosyntropin-stimulated procedure.[31] A low incidence of discordance between the 2 approaches has been reported[33] potentially explained by the strictness of the AVS criteria.[34] Wannachalee et al.[35] reported different patterns of response to cosyntropin stimulation during AVS, an observation which may be related to baseline aldosterone concentrations and

aldosterone-driver mutations, but histopathological phenotype could also be a contributing factor.

Several studies have reported variants in aldosterone-driver genes which potentially account for over 90% of the aldosterone overproduction from APAs.[13-17] We used a similar approach to these studies and validated mutation-negative status of APAs by CYP11B2 guided NGS and found an 85% prevalence of variants in aldosterone-driver genes. Consistent with other reports, we did not find mutations in *CTNNB1*.[14,15] *CACNA1H* variants were also absent[16]. Sequencing paired blood samples demonstrated the absence of all variants from germline DNA except for the rare *CLCN2* variant which is likely a non-pathogenic variant because we found no functional effect *in vitro*. Despite the high prevalence of variants in aldosterone-driver genes in this and other studies, their functional effect in the majority of cases is unknown and it is likely that in some cases the variants ascribed as aldosterone-driver mutations are non-pathogenic variants as demonstrated previously for p.CACNA1D-M1354I.[36]

We did not find an association of APAs with *KCNJ5* mutations with postsurgical outcomes. We excluded from the analysis patients with nonclassical histopathologic findings of unilateral PA to avoid negative bias of the group without a *KCNJ5* mutation which likely confounded the findings of previous studies. [37,38]

Strengths and Limitations

The strengths of this study include the analysis of a prospective cohort of surgically treated patients for PA with evaluation of histopathology by H&E staining and CYP11B2 immunostaining according to the international HISTALDO consensus. In addition, all FFPE

blocks for each adrenal specimen were examined to assess sections from all fragments of the removed gland. Postsurgical outcomes were assessed by the PASO criteria and adrenal venous sampling results allowed the evaluation of aldosterone production from the unresected glands. A limitation is the heterogeneous approach used for genotype determination, but the mutation negative status of all samples was determined by CYP11B2 guided amplicon NGS.

In conclusion, nonclassical histopathologic findings of unilateral forms of PA are associated with higher baseline aldosterone production from the unresected adrenal gland and a higher incidence of post-surgical persistent aldosteronism.

Perspectives

The application of recently established consensus criteria to assess histopathology and postsurgical outcomes provides relevant information for patient care. It links functional in vivo data of adrenal mineralocorticoid secretion with the newly defined histopathology phenotypes. This enables refined patient selection for surgical management and a better understanding of the pathophysiology of PA.

Conflicts of Interest/Disclosures

None

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Novelty and Significance

What is new?

- The histopathology of adrenals from a 3-year prospective series of surgically treated patients for PA were evaluated according to the international HISTALDO consensus

- A notable proportion displayed nonclassical histopathologic findings of unilateral PA with aldosterone-producing nodules or micronodules (20%) or aldosterone-producing diffuse hyperplasia (5%)

- More than half the adrenals with a solitary APA displayed aldosterone-producing lesions (nodules, micronodules or diffuse hyperplasia) in the adjacent cortex which did not affect postsurgical biochemical outcomes

- Nonclassical histopathology was associated with higher aldosterone production from the contralateral adrenal at AVS and a decreased incidence of postsurgical biochemical remission

What Is relevant?

- Adrenals without a solitary APA are not infrequent histopathologic findings from surgically treated patients for PA

- Patients with classical histopathologic lesions stratified by presence or absence of a *KCNJ5* mutation displayed similar postsurgical outcomes

Summary

Nonclassical histopathologic findings from surgically treated patients for PA are associated with higher baseline aldosterone production from the unresected adrenal and an increased incidence of persistent aldosteronism.
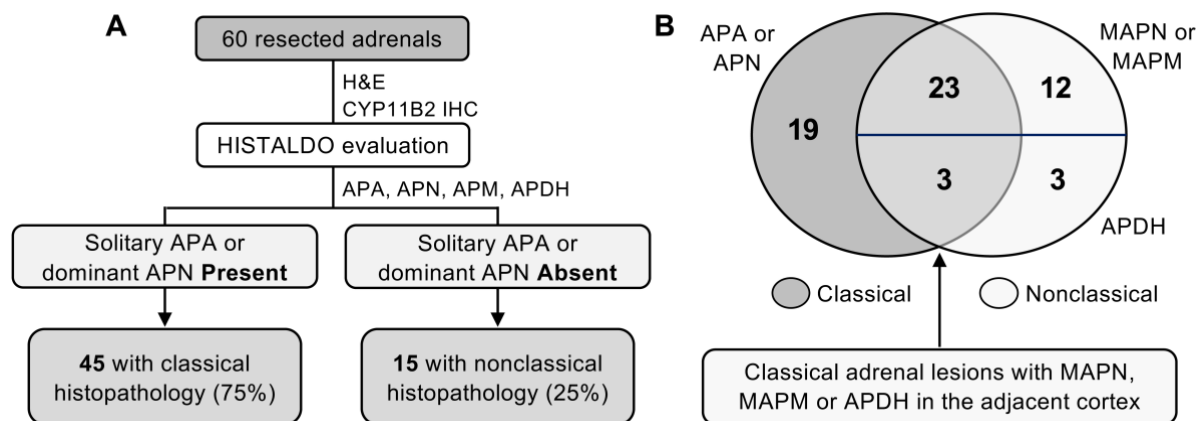
Figure 1. Histopathologic evaluation of adrenals according to HISTALDO consensus

Histopathology of surgically removed adrenals evaluated by H&E and CYP11B2 immunohistochemistry from a 3-year prospective series of surgically treated patients for PA. Histopathologic features were classified according to the HISTALDO consensus[9] and adrenals were categorized into classical and nonclassical forms of unilateral PA based on the source of aldosterone overproduction originating mainly from a solitary APA or a dominant APN *versus* alternative aldosterone-producing lesions (multiple aldosterone-producing nodules or micronodules, or aldosterone-producing diffuse hyperplasia) (**A**). Adrenals with classical histopathologic findings displayed aldosterone-producing lesions in the adjacent adrenal cortex in more than half of cases (**B**, 26 of 45 cases). Adrenals in the nonclassical group displayed multiple aldosterone-producing nodules or micronodules or aldosterone-producing diffuse hyperplasia (**B**). APA, aldosterone-producing adenoma; APM, aldosterone-producing micronodule; APN, aldosterone-producing nodule; APDH, aldosterone-producing diffuse hyperplasia; CYP11B2 IHC, CYP11B2 (aldosterone synthase) immunohistochemistry; H&E, hematoxylin and eosin; HISTALDO, histopathology of primary aldosteronism consensus; MAPM, multiple aldosterone-producing micronodules; MAPN, multiple aldosterone-producing nodules.
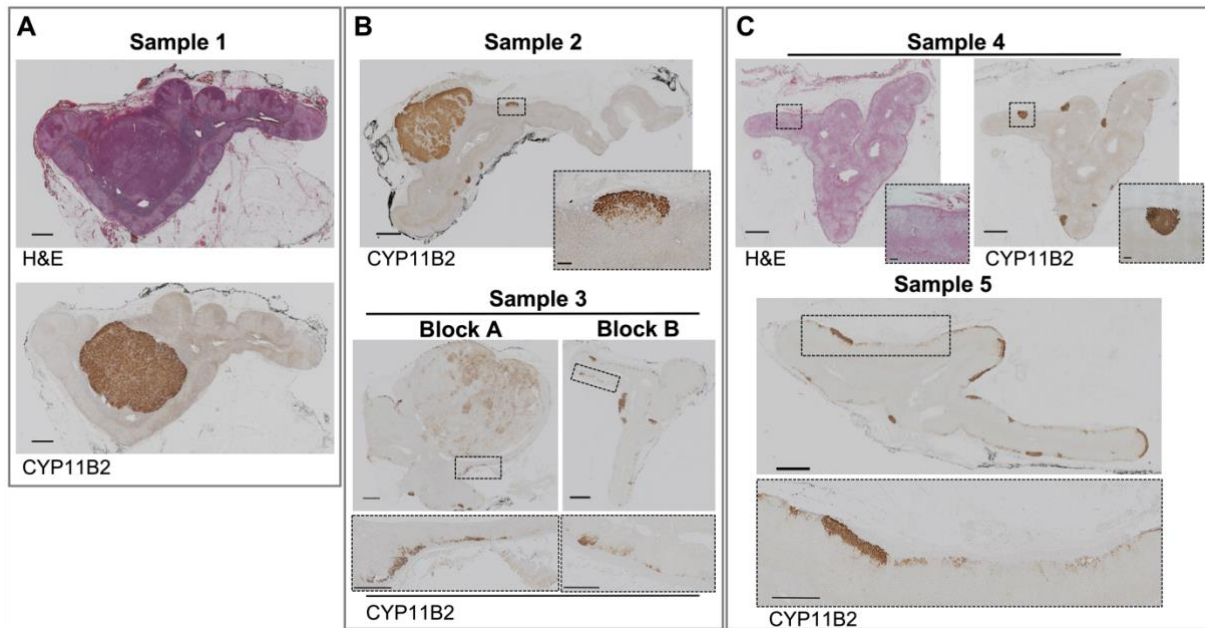
Figure 2. Histopathologic categorization of adrenal sample specimens

Panels **A** and **B** show examples of classical histopathologic findings of unilateral forms of PA, panel **C** shows examples of nonclassical findings. The figure shows an APA without aldosterone-producing lesions in the adjacent cortex (**A, sample 1**); an APA with multiple aldosterone-producing micronodules in the adjacent cortex (**B, upper panel, sample 2**), and below, an APA with aldosterone-producing diffuse hyperplasia in the adjacent cortex in block A, which was clearly visualized in the fragment shown in block B (**B, lower panel, sample 3**). Examples of adrenals in the nonclassical group are also shown with multiple aldosterone-producing micronodules (**C, upper panel, sample 4**), and below, aldosterone-producing diffuse hyperplasia (**C, lower panel, sample 5**). H&E, haematoxylin and eosin, CYP11B2 (aldosterone synthase) immunohistochemistry. Scale bar represents 2 mm for the overview images and 200 µm (samples 2 and 4) or 1 mm (samples 3 and 5) for insets.
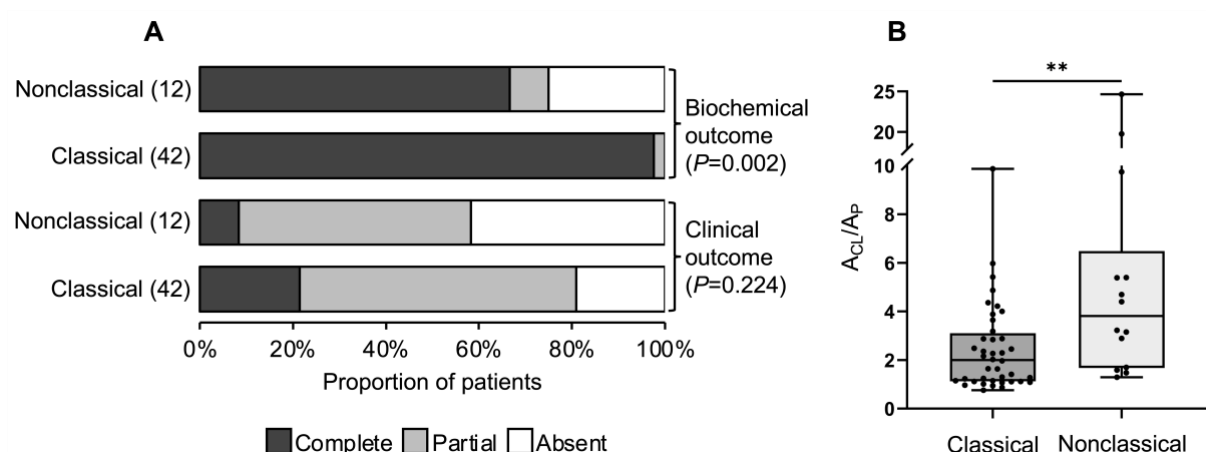
Figure 3. Postsurgical outcomes and baseline contralateral suppression stratified by histopathology

Postsurgical outcomes were assessed as complete, partial and absent clinical and biochemical success and categorized as classical *versus* nonclassical histopathologic findings (**A**). Numbers of patients are indicated in parenthesis; outcome data was missing for 6 patients. A chi-squared test was used for statistical analysis. Contralateral suppression was compared between patients with classical *versus* nonclassical histopathologic findings from the ratio of the absolute aldosterone concentration in the contralateral adrenal vein to the peripheral vein (A_{CL}/A_P) (**B**). Adrenal venous sampling results from the contralateral gland were available for 40 patients in the classical group and 14 patients in the nonclassical group. The box plot represents the median with 25th to 75th percentiles. Group differences were analysed with a Mann-Whitney U test (*P*<0.005). A_{CL}, aldosterone concentration in the contralateral vein; A_P, aldosterone concentration in the peripheral vein.

Variable	N	Total Cohort (n=60)	Classical (n=45)	Nonclassical (n=15)	P value
Age at surgery (y)	60	51 ± 13	50 ± 13	52 ± 13	0.565
Sex (ref. female)	60	31 (51.7)	25 (55.6)	6 (40.0)	0.296
BMI (kg/m^2)	60	27.6 ± 5.2	27.5 ± 5.2	28.0 ± 5.3	0.754
Systolic BP (mmHg)	60	150 ± 17	150 ± 18	149 ± 14	0.810
Diastolic BP (mmHg)	60	94 ± 12	94 ± 12	93 ± 12	0.715
Anti-HTN meds (DDD)	60	2.0 [1.0-3.0]	2.0 [1.0-3.0]	1.3 [0.5-2.0]	0.083
Duration HTN (mo)	60	103 [39-171]	108 [48-180]	78 [24-144]	0.614
PAC (pg/mL)	60	210 [154-350]	262 [164-356]	155 [129-180]	**0.008**
DRC (mU/L)	60	3.1 [2.0-5.6]	2.0 [2.0-5.0]	4.3 [2.0-6.4]	0.101
ARR	60	68 [41-106]	81 [45-116]	42 [28-50]	**0.002**
Serum K$^+$ (mmol/L)	60	3.4 ± 0.5	3.4 ± 0.5	3.6 ± 0.5	0.102
Largest nodule (mm)	60	12 [7-17]	14 [9-18]	5 [0-7]	**<0.001**
Clinical Outcome (n=54)					0.224
Complete success	10	10 (18.5)	9 (21.4)	1 (8.3)	
Partial success	31	31 (57.4)	25 (59.6)	6 (50.0)	
Absent success	13	13 (24.1)	8 (19.0)	5 (41.7)	
Biochemical Outcome (n=54)					**0.002**
Complete success	49	49 (90.7)	41 (97.6)	8 (66.7)	
Partial success	2	2 (3.7)	1 (2.4)	1 (8.3)	
Absent success	3	3 (5.6)	0	3 (25.0)	

Table 1. Clinical characteristics of patients stratified by histopathologic findings.

Quantitative normally distributed variables are shown as means ±SD and nonnormally distributed variables are reported as medians [IQR]. Categorical variables are shown as absolute numbers and percentages in parenthesis. *P* values were calculated using the Chi-squared or Yates's chi-squared test for categorical variables or with a t-test or Mann Whitney U test for quantitative variables as appropriate. *P*<0.05 was considered significant. The defined daily dose (DDD) is the assumed average maintenance dose per day for a drug used for its main indication in adults according to the ATC/DDD Index 2019 (https://www.whocc.no/atc_ddd_index/). Serum K^+ refers to the lowest measured serum potassium concentration. Largest nodule indicates the largest nodule diameter measured at pathology. Clinical and biochemical outcomes were assessed at 6-12 months after surgery. ARR, aldosterone-to-renin ratio; BMI, body mass index; BP, blood pressure; CL, contralateral (*n=39); DDD, defined daily dose; DRC, direct renin concentration; HTN, hypertension; K^+, potassium ions; meds, medications; PAC, plasma aldosterone concentration; ref., reference

Gene	cDNA change	Amino acid change	Sequencing approach	No. samples
KCNJ5	c.451G>A	p.G151R	CYP11B2-guided Sanger seq	6
	c.451G>C	p.G151R	CYP11B2-guided Sanger seq	4
	c.503T>G	p.L168R	CYP11B2-guided Sanger seq	11
	*c.502_506delinsAAGAG	p.L168_L169delinsKR	CYP11B2-guided amplicon NGS Whole exome seq (FF)	2
ATP1A1	c.311T>G	p.L104R	CYP11B2-guided Sanger seq; CYP11B2-guided amplicon NGS	4
	c.995T>G	p.V332G	CYP11B2-guided Sanger seq	1
ATP2B3	c.1272_1277del	p.L425_V426del	CYP11B2-guided Sanger seq	1
	c.1274_1279del	p.V426_V427del	CYP11B2-guided amplicon NGS	1
CACNA1D	c.1207G>C	p.G403R	CYP11B2-guided amplicon NGS	1
	c.2239T>G	p.F747V	CYP11B2-guided Sanger seq; CYP11B2-guided NGS	2
	c.815T>A	p.L272H	CYP11B2-guided amplicon NGS	1

CLCN2	c.731G>A	p.C244Y	Whole exome seq (FF)	1
Mutation negative	-	-	CYP11B2-guided amplicon NGS	6

Table 2. Coding DNA and protein sequence variants in solitary aldosterone-producing adenomas and nodules

Sequencing was guided by CYP11B2 immunohistochemistry from formalin-fixed paraffin-embedded resected adrenals except for the 2 indicated samples that were sequenced using fresh frozen (FF) tissue. ***Novel variant**.

FF, fresh frozen tissue; NGS, next generation sequencing; seq, sequencing