Somatic mutations in adrenals from patients with primary aldosteronism not cured after adrenalectomy suggest common pathogenic mechanisms between unilateral and bilateral disease

Inès Hacini, Kelly de Sousa, Sheerazed Boulkroun, Tchao Meatchi, Laurence Amar, Maria-Christina Zennaro, Fabio Fernandes-Rosa

► To cite this version:

Inès Hacini, Kelly de Sousa, Sheerazed Boulkroun, Tchao Meatchi, Laurence Amar, et al.. Somatic mutations in adrenals from patients with primary aldosteronism not cured after adrenalectomy suggest common pathogenic mechanisms between unilateral and bilateral disease. *European Journal of Endocrinology*, 2021, 185 (3), pp.405-412. 10.1530/EJE-21-0338 . inserm-03560062

HAL Id: inserm-03560062

<https://www.hal.inserm.fr/inserm-03560062>

Submitted on 7 Feb 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Somatic mutations in adrenals from patients with primary aldosteronism not cured after adrenalectomy suggest common pathogenic mechanisms between unilateral and bilateral disease

Inès Hacini¹, Kelly de Sousa¹, Sheerazed Boulkroun¹, Tchao Meatchi^{1,2}, Laurence Amar^{1,3}, Maria-Christina Zennaro^{1,4*#}, Fabio L Fernandes-Rosa^{1*#}

1. Université de Paris, PARCC, Inserm. Paris, France
2. Assistance Publique-Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Service d'Anatomie Pathologique, Paris, France
3. Assistance Publique-Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Unité Hypertension artérielle, Paris, France
4. Assistance Publique-Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Service de Génétique, Paris, France

* Equal contribution

Corresponding author

Address correspondence to:

Fabio L Fernandes-Rosa, MD, PhD
Inserm, U970
Paris Cardiovascular Research Center – PARCC
56, rue Leblanc,
75015 Paris – France
Tel : +33 (0)1 53 98 80 43
Fax : + 33 (0)1 53 98 79 52
e-mail : fabio.fernandes-rosa@inserm.fr

Maria-Christina Zennaro, MD, PhD
Inserm, U970
Paris Cardiovascular Research Center – PARCC
56, rue Leblanc,
75015 Paris – France
Tel : +33 (0)1 53 98 80 42
Fax : + 33 (0)1 53 98 79 52
e-mail : maria-christina.zennaro@inserm.fr

Abstract

Objective: Primary aldosteronism (PA) is the most common form of secondary and curable hypertension. Different germline and somatic mutations are found in aldosterone producing adenoma (APA) and familial forms of the disease, while the causes of bilateral adrenal hyperplasia (BAH) remain largely unknown. Adrenalectomy is the recommended treatment for patients with APA; however, 6% of patients are not cured and show persistent PA after surgery suggesting BAH. The objective of this study was to analyze clinical data of patients with APA without biochemical success after adrenalectomy as well as the histological and genetic characteristics of their adrenal glands. **Design and Methods:** Clinical data of 12 patients without biochemical cure were compared to those from 39 PA patients with hormonal cure after surgery. Histological, morphological and genetic characterization of the adrenals was carried out by CYP11B2 and CYP11B1 immunostaining and by CYP11B2-guided NGS. **Results:** Patients with absent hormonal cure displayed longer duration of arterial hypertension and lower lateralization index of aldosterone production. In 10 patients, APA expressing CYP11B2 were identified. No difference in histological and morphological characteristics was observed between patients with and without hormonal cure. Somatic mutations in APA driver genes were identified in all CYP11B2 positive APA; *CACNA1D* mutations were the most frequent genetic abnormality. **Conclusions:** Patients with absent biochemical cure were diagnosed later and exhibited lower lateralization index of aldosterone production, suggesting asymmetric aldosterone production in the context of BAH. Somatic mutations in adrenal glands from those patients indicate common mechanisms underlying BAH and APA.

1 **Introduction**

2 Primary aldosteronism (PA) is the most frequent cause of endocrine hypertension, with
3 a prevalence of $\approx 5\%$ of hypertensive patients in primary care and 10% to 20% in reference
4 centers (1-3). PA patients exhibit hypertension associated with high levels of plasma
5 aldosterone, low levels of plasma renin and in some cases hypokalemia. The excessive
6 aldosterone production is attributable in the majority of cases to an unilateral aldosterone
7 producing adenoma (APA) or to a bilateral adrenal hyperplasia (BAH) (4).

8 In the last decade, different studies identified somatic and germline mutations in genes
9 coding for ion channels and ATPases as responsible for APA and familial forms of PA (5-12).
10 The discovery of these mutations highlighted the central role of calcium signaling in the
11 pathogenesis of the disease (13). Recently, the use of next generation sequencing (NGS)
12 performed on DNA from CYP11B2 positive nodules extracted from formalin fixed paraffin
13 embedded (FFPE) tissues has allowed the identification of somatic heterozygous mutations in
14 *KCNJ5* (coding for the potassium channel GIRK4), *ATP1A1* (coding for the $\alpha 1$ subunit of the
15 Na^+/K^+ -ATPase), *ATP2B3* (coding for the plasma membrane Ca^{2+} -ATPase, type 3 PMCA3),
16 *CACNA1D* (encoding the Cav1.3 voltage dependent calcium channel), *CACNA1H* (encoding
17 the Cav3.1 voltage dependent calcium channel), *CLCN2* (coding for the chloride channel ClC-
18 2), and *CTNNB1* (coding for β -catenin) in more than 88% of APA (14-17). However, the genetic
19 causes of BAH remain largely unknown. Analysis of 15 adrenals from patients with BAH has
20 shown that BAH may result from the accumulation or enlargement of aldosterone producing
21 cell clusters (APCC) harboring somatic mutations, particularly in the *CACNA1D* gene (18).

22 The goal of PA treatment is the normalization of blood pressure, together with correction
23 of hypokalaemia and biochemical abnormalities (aldosterone and renin) in unilateral forms, or
24 the efficient blockade of the mineralocorticoid receptor, since PA is associated with increased
25 risk of cardiovascular complications independently of blood pressure levels (19-23). Surgical

26 adrenalectomy is the recommended treatment for lateralized PA (4, 24) . For this purpose,
27 correct subtyping of PA is mandatory to identify patients with unilateral disease who can be
28 cured after adrenalectomy. Many studies have shown an improvement of hypertension, a
29 decrease in cardiovascular and metabolic risk factors, and a reduction in mortality after
30 adrenalectomy compared with mineralocorticoid receptor antagonist treatment alone (25) (26).
31 Despite the recommendations, approximately 6% of PA patients classified pre-surgery as
32 having unilateral PA do not achieve complete biochemical cure after adrenalectomy (27). These
33 patients exhibit lower lateralization index on adrenal vein sampling (AVS), suggesting bilateral
34 but asymmetric aldosterone production and a misdiagnosed BAH (28).

35 The objective of this study was to analyze clinical data of patients with APA without
36 biochemical success after adrenalectomy and to investigate the histological and genetic
37 characteristics of their adrenal glands.

METHODS

Patients

Among patients who underwent adrenalectomy between 2008 and 2018 at the Hôpital Européen Georges Pompidou recruited within the COMETE-HEGP protocol, 12 patients with partial or absent biochemical cure after adrenalectomy using the PASO criteria (29) were analyzed. Partial biochemical success: correction of hypokalaemia (if present pre-surgery) and a raised aldosterone-to-renin ratio with $\geq 50\%$ decrease in baseline plasma aldosterone concentration and/or abnormal but improved post-surgery confirmatory test result. Absent biochemical success: persistent hypokalaemia or persistent raised aldosterone-to-renin ratio, or both, with failure to suppress aldosterone secretion with a post-surgery confirmatory test. Data from 39 PA patients with biochemical cure after adrenalectomy and complete clinical, biochemical, histological and genetic exploration (16) were used as controls. The term “non-cured group” used in this article corresponds to patients with partial or absent biochemical success. Seven patients were classified as having absent biochemical success after adrenalectomy and five patients exhibited partial biochemical success (Table S2). All patients exhibited visible adrenal adenoma or adrenal enlargement on CT scan. The study was approved by the French Research ethics committee (Comité de Protection des Personnes, CPP) under authorization number CPP 2012-A00508-35. Written consent has been obtained from each patient or subject after full explanation of the purpose and nature of all procedures used. Methods used for screening and subtype identification of PA were performed according to institutional and Endocrine Society guidelines (4, 30-32). All patients underwent adrenal vein sampling (AVS) to differentiate between unilateral and bilateral aldosterone hypersecretion. AVS sampling was performed simultaneously in both adrenal veins and without pharmacologic stimulation (32, 33); the complete protocol is described in the supplementary data. Baseline and follow up clinical and

biochemical characteristics of PA patients without biochemical success after adrenalectomy are described in supplementary tables S1 and S2.

Immunohistochemistry and pathological analysis

Each paraffined adrenal block from the non cured patients (absent biochemical success) was analysed entirely to have a precise morphological and cellular analysis. Sections (4- μ m thick) were deparaffinised in xylene and rehydrated through graded ethanol. Hematoxylin/Eosin, 11 β -hydroxylase (CYP11B1) and aldosterone synthase (CYP11B2) immunohistochemistry were performed as previously described (16). Images were acquired in a Lamina Slide Scanner from Perkin Elmer and analysed on the Cochin Image Database (Institut Cochin, France). Zona glomerulosa (ZG) hyperplasia was defined as the presence of a continuous character of the ZG, or, in case of discontinuity of the ZG, ZG thickness $\geq 200 \mu\text{m}$ (16, 34). CYP11B1 and CYP11B2 staining was quantified as previously described (16). Percentages of aldosterone-synthase and 11 β -hydroxylase expressing cells are reported as: 0, absent; 1, 1%-33%; 2, 34%-66%; 3, 67%-100%.

DNA isolation and sequencing

Somatic DNA of APA was extracted from fresh frozen tissue (FFT) using QIamp DNA midi kit (Qiagen, Courtaboeuf Cedex, France) and Sanger sequencing targeting hot spot regions of *KCNJ5*, *CACNA1D*, *ATP1A1*, *ATP2B3* and *CTNNB1* genes was performed as previously described (35). For negative samples on Sanger sequencing and samples not sequenced previously, CYP11B2 immunohistochemistry-guided NGS (CYP11B2 IHC-guided NGS) was performed as previously described (16). Before DNA/RNA extraction of FFPE tissue, APA and aldosterone producing nodules were identified by CYP11B2 IHC. Based on the CYP11B2 IHC, the areas of interest were delimited and isolated for DNA/RNA extraction by scraping unstained FFPE sections guided by the CYP11B2 IHC slide using a scalpel under a Wild Heerbrugg or

Olympus microscope. DNA was extracted from FFPE sections using AllPrep DNA/RNA FFPE kit (Qiagen). NGS was performed using a NGS kit, covering all coding exons and intron-exon junctions of the *KCNJ5* (NM_000890), *ATP1A1* (NM_000701), *ATP2B3* (NM_0010001344), *CTNNB1* (NM_001904), *CACNAID* (NM_001128839.2 and NM_000720), *APC* (NM_000038.5), *CACNAIH* (NM_021098 and NM_001005407), *PRKACA* (NM_002730) and *ARMC5* (NM_002730) genes (MASTR_PA kit, Multiplicom/Agilent, Santa Clara, CA USA) (16).

Statistical analyses.

Quantitative variables are reported as medians and interquartile range. Comparisons between groups were done with unpaired t-test when Gaussian distribution or the Mann-Whitney test when no Gaussian distribution. Categorical variables are reported as numbers and compared with Fisher's exact test. A p value < 0.05 was considered significant for comparisons between 2 groups.

Results

Comparison of clinical data between patients with or without biochemical success after adrenalectomy

Baseline and follow up clinical and biochemical data are summarized in Table 1. At follow up (8.5 (interquartile range 4.2,15) months), patients with partial or absent biochemical success exhibited higher systolic ($p=0.003$) and diastolic blood pressure ($p=0.008$), higher plasma aldosterone levels ($p<0.0001$), lower renin levels ($p=0.007$), lower plasma K^+ concentration ($p<0.0001$) and higher number of anti hypertensive drugs ($p=0.003$) compared with patients with complete biochemical success. The proportion of female patients was lower, but not significant, in the biochemically non-cured group compared with the cured group (16.7% vs 33%, $p=0.47$). The duration of hypertension before PA diagnosis was longer in patients with absent biochemical success compared with patients with complete biochemical success (11 (6,15) years vs 3 (1,8) years, $p=0.002$). While no differences were observed in pre-operative plasma aldosterone and plasma renin levels, patients with absent biochemical success displayed a lower lateralization index at AVS ($p=0.02$). No significant differences were found in the other baseline parameters.

Histological analysis

No difference in the adenoma size was observed between patients with complete biochemical success (10.5 (7,14) mm) versus absent biochemical success (14.5 (10,15) mm) after adrenalectomy ($p=0.09$) (Table S3). Zona glomerulosa hyperplasia was observed in 7 out of 12 adrenals from biochemically non-cured PA patients (Table 2). CYP11B2 staining was performed on the entire FFPE blocs of adrenals from biochemically non-cured patients in order to identify aldosterone producing nodules. 10 adrenals showed one CYP11B2 positive adenoma of at least 5 mm (Table 2). Micronodular hyperplasia was observed in two of these adrenals

(patients 1 and 4) and CYP11B2 positive secondary micronodules were observed in three adrenals (patients 2, 8 and 9). Aldosterone producing cell clusters (APCCs) were observed in 8 out of 12 adrenals, ranging from 1 to 4 APCCs per adrenal (Table 2). In two adrenals, no adenomas expressing CYP11B2 were observed. The first adrenal (patient 6) exhibited CYP11B2 negative micronodular hyperplasia and three APCCs. In the second adrenal (patient 7) one adenoma non expressing CYP11B2 and two APCCs were observed. Given the previously documented intratumoral heterogeneity of APA, the number of cells expressing CYP11B2 and CYP11B1 was quantified in each APA (Table 2). Five APAs showed between 67% and 100% of cells expressing CYP11B2, two exhibited 33% to 66% of cells expressing CYP11B2, while in three APA, 1% to 33% CYP11B2 expressing cells were observed. Concerning CYP11B1 expression, two adenomas were composed of 33% to 66% of cells expressing CYP11B1 and in eight adenomas 1 to 33% of CYP11B1 expressing cells were observed. In comparison with APA from PA patients with complete biochemical success after adrenalectomy, there was no difference in the number of cells expressing CYP11B2 and CYP11B1 (Table S3).

Identification of somatic mutations in the absent biochemical success group.

Among the 10 CYP11B2 positive adenomas from the absent biochemical success group, analysis of hot spot mutations in APA driver genes was performed by Sanger sequencing in fresh frozen adenoma tissue (FFT) from four patients. One somatic *KCNJ5* mutation (c.451G>C/p.Gly151Arg) was identified in one adenoma and somatic *CACNA1D* mutations were identified in three patients: p.Arg990Gly (c.2968C>G), p.Gly403Arg (c.1207G>C), and p.Val1373Asp (c.4117G>A). CYP11B2 IHC-guided NGS was performed in CYP11B2 positive FFPE samples from five adenomas without previous sequencing. *CACNA1D* mutations were identified in three patients (c.1207G>C/p.Gly403Arg, c.2968C>G/p.Arg990Gly, and

c.3458T>A/p.Val1153Asn), the same *KCNJ5* mutation (c.451G>C/P.Gly151Arg) was identified in two patients, and the *ATP1A1* mutation p.Leu104Arg ((c.311T>G) was identified in one patient. Targeted NGS was also performed in one CYP11B2 negative adenoma and no mutations in APA driver genes were observed. In total, somatic mutations in APA driver genes were observed in all CYP11B2 expressing adenomas from 12 patients (Table 3). Differently from patients with complete biochemical success after adrenalectomy with a higher prevalence of somatic *KCNJ5* mutations (38.4% within the 39 patients analyzed), *CACNA1D* mutations were the most prevalent genetic abnormality in APA from patients without biochemical success (60.0%). Adenomas harbouring *CACNA1D* mutations (median 9 mm) were smaller than adenomas harbouring *KCNJ5* mutations (median 14 mm) (Table S4). No correlation was observed between the number of CYP11B2 or CYP11B1 expressing cells and the mutation status (Table S4).

Discussion

In the present study, we report for the first time the presence of somatic mutations in aldosterone producing nodules from patients with absent biochemical success after adrenalectomy. Analysis of clinical data from 12 PA patients who had undergone adrenalectomy with partial or absent biochemical success after surgery also showed a decreased lateralization index of aldosterone production on AVS but no differences were observed in the histological analysis of CYP11B2 positive adenomas and adrenals from patients completely cured or non-cured after adrenalectomy.

Patients were recruited within a single referral center for hypertension in France and performed histological and genetic characterization of their adrenals. The patients were selected accordingly to an international multicentric consensus for classifying surgical outcome and follow-up of patients with unilateral primary aldosteronism (PASO) (29). This consensus has provided the uniformity of clinical and biochemical criteria of PA outcome in different specialized centres, allowing to identify factors influencing the success of adrenalectomy. We focused this study on patients with unilateral PA who did not reach complete biochemical success after adrenalectomy. These patients could represent a group of patients with a bilateral form of PA misdiagnosed during the AVS, explaining the absence of PA cure after surgery, as suggested by some studies (28, 29).

It has been previously shown that younger patients and women displayed a more favourable outcome after adrenalectomy (29, 36-38). In the present study, we observed a higher percentage of men among patients with absent biochemical success compared with a previous published cohort of 39 patients with unilateral PA with complete biochemical success after surgery, confirming previous studies. In addition to female sex, other variables were described as predictors for favourable clinical outcome, including short term hypertension (≤ 7 years),

189 absence of overweight, low number of antihypertensive drugs, higher baseline blood pressure
190 and no history of diabetes mellitus (29, 37). Although we did not observe differences in blood
191 pressure at baseline, number of hypertensive drugs or BMI between patients with or without
192 biochemical success after surgery, biochemically non-cured patients were diagnosed later and
193 presented a longer duration of hypertension. The duration of hypertension may indicate not only
194 a predictor for clinical but also for biochemical outcome of unilateral PA. In the present study,
195 we observed lower lateralization index of aldosterone at AVS in patients without biochemical
196 success. This finding was previously observed in a larger cohort of non-cured PA patients (28),
197 and together with the longer duration of hypertension before PA diagnosis suggests a bilateral
198 disease with asymmetric aldosterone production.

199 Remarkably, in 10 out of 12 cases, one functional adenoma expressing CYP11B2 was observed
200 in the resected adrenal of patients without biochemical success after adrenalectomy. This
201 finding confirms lateralization of aldosterone production at AVS in the majority of the
202 investigated patients, suggesting asymmetric bilateral aldosterone production; for two patients,
203 no adrenal adenoma expressing CYP11B2 was identified and the source of autonomous and
204 excessive aldosterone production was not identified, suggesting a misdiagnosis of PA subtyping
205 before surgery. Zona glomerulosa hyperplasia as well as heterogeneous CYP11B2 and
206 CYP11B1 expression in APA were observed in the adrenals from non-cured patients,
207 accordingly to previously described adrenals from lateralized PA (16, 39). In contrast to a
208 multicentric study of patients with lateralized PA biochemically not-cured after adrenalectomy
209 (28), we did not observe histological findings associated with biochemical success. The absence
210 of relationship between histopathology and biochemical cure was also observed in an Italian
211 cohort of PA patients (40). The difference between these studies may imply differences between
212 patients recruited in different centers, an observation that has already been reported for clinical

characteristics of patients with PA (35), or may represent less power to identify these differences due to a smaller sample size.

Remarkably, we report a high prevalence of somatic mutations in APA driver genes in adenomas from patients with absent biochemical cure. *CACNA1D* mutations were the most frequent genetic abnormality in APAs from patients with partial or absent success after adrenalectomy, which were not associated with ethnic background as previously reported (15). A higher frequency of somatic *CACNA1D* mutations was observed previously in the context of bilateral adrenal hyperplasia, with the accumulation or enlargement of APCCs harbouring somatic mutations in APA-driver genes (18), suggesting common pathogenic mechanisms at the origin of BAH and APA. Our data showing the presence of somatic mutations (in particular *CACNA1D* mutations) in APA from patients not cured after adrenalectomy, also presenting with lower lateralization index and longer duration of HT, support this hypothesis.

Only a small number of APA harboured somatic *KCNJ5* mutations, in contrast with what observed in patients with complete biochemical cure in this study and studies analysing a large number of patients from French or European cohorts (16, 35, 41-44). While the small number of *KCNJ5* mutations may be associated with the higher number of male patients without biochemical success, the association of *KCNJ5* mutations and favourable outcome was described previously, independently from the favourable impact of female sex (45, 46). While associations between the mutation status and histological findings are well characterized in APA (16, 47), no associations were observed in the present study, probably due to the small number of adenomas within each genotype.

In conclusion, 10 out of 12 adrenals from PA patients without biochemical success after adrenalectomy exhibited *CYP11B2* expressing adenomas carrying somatic mutations, confirming the diagnosis of APA. However, these patients were diagnosed later and exhibited

lower lateralization index of aldosterone production at AVS, suggesting asymmetric aldosterone production in the context of BAH. The identification of somatic mutations in adrenal glands from those patients suggests a possible continuum between bilateral and unilateral disease in a subset of patients, supporting common mechanisms underlying BAH and APA.

Declaration of interest

The authors have nothing to disclose

Acknowledgments

The authors wish to thank CE Gomez-Sanchez (University of Mississippi Medical Center, Jackson, MS) for providing antibodies against CYP11B1 and CYP11B2.

Sources of Funding

This work was funded through institutional support from INSERM, the Agence Nationale de la Recherche (ANR-15-CE14-0017-03), and the Fondation pour la Recherche Médicale (DEQ20140329556).

References

1. Monticone S, Burrello J, Tizzani D, Bertello C, Viola A, Buffolo F, Gabetti L, Mengozzi G, Williams TA, 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.
2. Hannemann A, Wallaschofski H. Prevalence of primary aldosteronism in patient's cohorts and in population-based studies--a review of the current literature. *Horm Metab Res.* 2012;44(3):157-62.
3. Brown JM, Siddiqui M, Calhoun DA, Carey RM, Hopkins PN, Williams GH, Vaidya A. The Unrecognized Prevalence of Primary Aldosteronism. *Ann Intern Med.* 2020.
4. Funder JW, Carey RM, Mantero F, Murad MH, Reincke M, Shibata H, Stowasser M, Young WF, Jr. 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.
5. Choi M, Scholl UI, Yue P, Bjorklund P, Zhao B, Nelson-Williams C, Ji W, Cho Y, Patel A, Men CJ, et al. K⁺ channel mutations in adrenal aldosterone-producing adenomas and hereditary hypertension. *Science.* 2011;331(6018):768-72.
6. Beuschlein F, Boulkroun S, Osswald A, Wieland T, Nielsen HN, Lichtenauer UD, Penton D, Schack VR, Amar L, Fischer E, et al. Somatic mutations in ATP1A1 and ATP2B3 lead to aldosterone-producing adenomas and secondary hypertension. *Nat Genet.* 2013;45(4):440-4, 4e1-2.
7. Scholl UI, Goh G, Stolting G, de Oliveira RC, Choi M, Overton JD, Fonseca AL, Korah R, Starker LF, Kunstman JW, et al. Somatic and germline CACNA1D calcium channel mutations in aldosterone-producing adenomas and primary aldosteronism. *Nat Genet.* 2013;45(9):1050-4.

- 279 8. Azizan EA, Poulsen H, Tuluc P, Zhou J, Clausen MV, Lieb A, Maniero C, Garg S,
280 Bochukova EG, Zhao W, et al. Somatic mutations in ATP1A1 and CACNA1D underlie a
281 common subtype of adrenal hypertension. *Nat Genet.* 2013;45(9):1055-60.
- 282 9. Fernandes-Rosa FL, Daniil G, Orozco JJ, Goppner C, El Zein R, Jain V, Boulkroun S,
283 Jeunemaitre X, Amar L, Lefebvre H, et al. A gain-of-function mutation in the CLCN2 chloride
284 channel gene causes primary aldosteronism. *Nat Genet.* 2018;50(3):355-61.
- 285 10. Scholl UI, Stolting G, Schewe J, Thiel A, Tan H, Nelson-Williams C, Vichot AA, Jin
286 SC, Loring E, Untiet V, et al. CLCN2 chloride channel mutations in familial
287 hyperaldosteronism type II. *Nat Genet.* 2018;50(3):349-54.
- 288 11. Dutta RK, Arnesen T, Heie A, Walz M, Alesina P, Soderkvist P, Gimm O. A somatic
289 mutation in CLCN2 identified in a sporadic aldosterone-producing adenoma. *Eur J Endocrinol.*
290 2019;181(5):K37-K41.
- 291 12. Nanba K, Blinder AR, Rege J, Hattangady NG, Else T, Liu CJ, Tomlins SA, Vats P,
292 Kumar-Sinha C, Giordano TJ, et al. Somatic CACNA1H Mutation As a Cause of Aldosterone-
293 Producing Adenoma. *Hypertension.* 2020;75(3):645-9.
- 294 13. Zennaro MC, Boulkroun S, Fernandes-Rosa FL. Pathogenesis and treatment of primary
295 aldosteronism. *Nat Rev Endocrinol.* 2020;16(10):578-89.
- 296 14. Nanba K, Omata K, Else T, Beck PCC, Nanba AT, Turcu AF, Miller BS, Giordano TJ,
297 Tomlins SA, Rainey WE. Targeted Molecular Characterization of Aldosterone-Producing
298 Adenomas in White Americans. *J Clin Endocrinol Metab.* 2018;103(10):3869-76.
- 299 15. Nanba K, Omata K, Gomez-Sanchez CE, Stratakis CA, Demidowich AP, Suzuki M,
300 Thompson LDR, Cohen DL, Luther JM, Gellert L, et al. Genetic Characteristics of Aldosterone-
301 Producing Adenomas in Blacks. *Hypertension.* 2019;73(4):885-92.

302 16. De Sousa K, Boulkroun S, Baron S, Nanba K, Wack M, Rainey WE, Rocha A, Giscos-
303 Douriez I, Meatchi T, Amar L, et al. Genetic, Cellular, and Molecular Heterogeneity in
304 Adrenals With Aldosterone-Producing Adenoma. *Hypertension*. 2020;75(4):1034-44.

305 17. Nanba K, Yamazaki Y, Bick N, Onodera K, Tezuka Y, Omata K, Ono Y, Blinder AR,
306 Tomlins SA, Rainey WE, et al. Prevalence of Somatic Mutations in Aldosterone-Producing
307 Adenomas in Japanese Patients. *J Clin Endocrinol Metab*. 2020;105(11).

308 18. Omata K, Satoh F, Morimoto R, Ito S, Yamazaki Y, Nakamura Y, Anand SK, Guo Z,
309 Stowasser M, Sasano H, et al. Cellular and Genetic Causes of Idiopathic Hyperaldosteronism.
310 *Hypertension*. 2018;72(4):874-80.

311 19. Catena C, Colussi G, Nadalini E, Chiuch A, Baroselli S, Lapenna R, Sechi LA.
312 Cardiovascular outcomes in patients with primary aldosteronism after treatment. *Arch Intern*
313 *Med*. 2008;168(1):80-5.

314 20. Milliez P, Girerd X, Plouin PF, Blacher J, Safar ME, Mourad JJ. Evidence for an
315 increased rate of cardiovascular events in patients with primary aldosteronism. *J Am Coll*
316 *Cardiol*. 2005;45(8):1243-8.

317 21. Savard S, Amar L, Plouin PF, Steichen O. Cardiovascular complications associated with
318 primary aldosteronism: a controlled cross-sectional study. *Hypertension*. 2013;62(2):331-6.

319 22. Reincke M, Fischer E, Gerum S, Merkle K, Schulz S, Pallauf A, Quinkler M, Hanslik
320 G, Lang K, Hahner S, et al. Observational study mortality in treated primary aldosteronism: the
321 German Conn's registry. *Hypertension*. 2012;60(3):618-24.

322 23. Wu VC, Wang SM, Chang CH, Hu YH, Lin LY, Lin YH, Chueh SC, Chen L, Wu KD.
323 Long term outcome of Aldosteronism after target treatments. *Sci Rep*. 2016;6:32103.

324 24. Amar L, Baguet JP, Bardet S, Chaffanjon P, Chamontin B, Douillard C, Durieux P,
325 Girerd X, Gosse P, Hernigou A, et al. SFE/SFHTA/AFCE primary aldosteronism consensus:
326 Introduction and handbook. *Ann Endocrinol (Paris)*. 2016;77(3):179-86.

327 25. Sukor N, Kogovsek C, Gordon RD, Robson D, Stowasser M. Improved quality of life,
328 blood pressure, and biochemical status following laparoscopic adrenalectomy for unilateral
329 primary aldosteronism. *J Clin Endocrinol Metab.* 2010;95(3):1360-4.

330 26. Lin YH, Wu XM, Lee HH, Lee JK, Liu YC, Chang HW, Lin CY, Wu VC, Chueh SC,
331 Lin LC, et al. Adrenalectomy reverses myocardial fibrosis in patients with primary
332 aldosteronism. *J Hypertens.* 2012;30(8):1606-13.

333 27. Muth A, Ragnarsson O, Johannsson G, Wangberg B. Systematic review of surgery and
334 outcomes in patients with primary aldosteronism. *Br J Surg.* 2015;102(4):307-17.

335 28. Meyer LS, Wang X, Susnik E, Burrello J, Burrello A, Castellano I, Eisenhofer G, Fallo
336 F, Kline GA, Knosel T, et al. Immunohistopathology and Steroid Profiles Associated With
337 Biochemical Outcomes After Adrenalectomy for Unilateral Primary Aldosteronism.
338 *Hypertension.* 2018;72(3):650-7.

339 29. Williams TA, Lenders JWM, Mulatero P, Burrello J, Rottenkolber M, Adolf C, Satoh
340 F, Amar L, Quinkler M, Deinum J, et al. Outcomes after adrenalectomy for unilateral primary
341 aldosteronism: an international consensus on outcome measures and analysis of remission rates
342 in an international cohort. *Lancet Diabetes Endocrinol.* 2017;5(9):689-99.

343 30. Letavernier E, Peyrard S, Amar L, Zinzindohoue F, Fiquet B, Plouin PF. Blood pressure
344 outcome of adrenalectomy in patients with primary hyperaldosteronism with or without
345 unilateral adenoma. *J Hypertens.* 2008;26(9):1816-23.

346 31. Funder JW, Carey RM, Fardella C, Gomez-Sanchez CE, Mantero F, Stowasser M,
347 Young WF, Jr., Montori VM, Endocrine S. Case detection, diagnosis, and treatment of patients
348 with primary aldosteronism: an endocrine society clinical practice guideline. *J Clin Endocrinol*
349 *Metab.* 2008;93(9):3266-81.

350 32. Baron S, Amar L, Faucon AL, Blanchard A, Baffalieu L, Faucard C, Travers S, Pagny
351 JY, Azizi M, Houillier P. Criteria for diagnosing primary aldosteronism on the basis of liquid

352 chromatography-tandem mass spectrometry determinations of plasma aldosterone
353 concentration. *J Hypertens*. 2018;36(7):1592-601.

354 33. Kupers EM, Amar L, Raynaud A, Plouin PF, Steichen O. A clinical prediction score to
355 diagnose unilateral primary aldosteronism. *J Clin Endocrinol Metab*. 2012;97(10):3530-7.

356 34. Lack EE. Tumors of the adrenal gland and extra-adrenal paraganglia. In: Pathology
357 ARO, editor. *Atlas of Tumor Pathology*. Series 42007.

358 35. Fernandes-Rosa FL, Williams TA, Riester A, Steichen O, Beuschlein F, Boulkroun S,
359 Strom TM, Monticone S, Amar L, Meatchi T, et al. Genetic spectrum and clinical correlates of
360 somatic mutations in aldosterone-producing adenoma. *Hypertension*. 2014;64(2):354-61.

361 36. Steichen O, Zinzindohoue F, Plouin PF, Amar L. Outcomes of adrenalectomy in patients
362 with unilateral primary aldosteronism: a review. *Horm Metab Res*. 2012;44(3):221-7.

363 37. Morisaki M, Kurihara I, Itoh H, Naruse M, Takeda Y, Katabami T, Ichijo T, Wada N,
364 Yoshimoto T, Ogawa Y, et al. Predictors of Clinical Success After Surgery for Primary
365 Aldosteronism in the Japanese Nationwide Cohort. *J Endocr Soc*. 2019;3(11):2012-22.

366 38. Rossi GP, Rossitto G, Amar L, Azizi M, Riester A, Reincke M, Degenhart C, Widimsky
367 J, Jr., Naruse M, Deinum J, et al. Clinical Outcomes of 1625 Patients With Primary
368 Aldosteronism Subtyped With Adrenal Vein Sampling. *Hypertension*. 2019;74(4):800-8.

369 39. Boulkroun S, Samson-Couterie B, Dzib JF, Lefebvre H, Louiset E, Amar L, Plouin PF,
370 Lalli E, Jeunemaitre X, Benecke A, et al. Adrenal cortex remodeling and functional zona
371 glomerulosa hyperplasia in primary aldosteronism. *Hypertension*. 2010;56(5):885-92.

372 40. Citton M, Viel G, Rossi GP, Mantero F, Nitti D, Iacobone M. Outcome of surgical
373 treatment of primary aldosteronism. *Langenbecks Arch Surg*. 2015;400(3):325-31.

374 41. Boulkroun S, Beuschlein F, Rossi GP, Golib-Dzib JF, Fischer E, Amar L, Mulatero P,
375 Samson-Couterie B, Hahner S, Quinkler M, et al. Prevalence, clinical, and molecular correlates
376 of KCNJ5 mutations in primary aldosteronism. *Hypertension*. 2012;59(3):592-8.

- 377 42. Akerstrom T, Willenberg HS, Cupisti K, Ip J, Backman S, Moser A, Maharjan R,
378 Robinson B, Iwen KA, Dralle H, et al. Novel somatic mutations and distinct molecular signature
379 in aldosterone-producing adenomas. *Endocr Relat Cancer*. 2015;22(5):735-44.
- 380 43. Scholl UI, Healy JM, Thiel A, Fonseca AL, Brown TC, Kunstman JW, Horne MJ,
381 Dietrich D, Riemer J, Kucukkoylu S, et al. Novel somatic mutations in primary
382 hyperaldosteronism are related to the clinical, radiological and pathological phenotype. *Clin*
383 *Endocrinol (Oxf)*. 2015;83(6):779-89.
- 384 44. Lenzini L, Rossitto G, Maiolino G, Letizia C, Funder JW, Rossi GP. A Meta-Analysis
385 of Somatic KCNJ5 K(+) Channel Mutations In 1636 Patients With an Aldosterone-Producing
386 Adenoma. *J Clin Endocrinol Metab*. 2015;100(8):E1089-95.
- 387 45. Vilela LAP, Rassi-Cruz M, Guimaraes AG, Moises CCS, Freitas TC, Alencar NP,
388 Petenuci J, Goldbaum TS, Maciel AAW, Pereira MAA, et al. KCNJ5 Somatic Mutation Is a
389 Predictor of Hypertension Remission After Adrenalectomy for Unilateral Primary
390 Aldosteronism. *J Clin Endocrinol Metab*. 2019;104(10):4695-702.
- 391 46. Kitamoto T, Omura M, Suematsu S, Saito J, Nishikawa T. KCNJ5 mutation as a
392 predictor for resolution of hypertension after surgical treatment of aldosterone-producing
393 adenoma. *J Hypertens*. 2018;36(3):619-27.
- 394 47. Ono Y, Yamazaki Y, Omata K, Else T, Tomlins SA, Rhayem Y, Williams TA, Reincke
395 M, Carling T, Monticone S, et al. Histological Characterization of Aldosterone-producing
396 Adrenocortical Adenomas with Different Somatic Mutations. *J Clin Endocrinol Metab*.
397 2020;105(3).

Table 1. Baseline and follow up clinical and biochemical data of PA patients with or without biochemical success after adrenalectomy.

	Non biochemical success (12)	Biochemical success (39)	p value
Age (ys)	47 (39,51)	44 (35,50)	0.90
Gender F/M (F%)	2/10 (16.7%)	13/26 (33.3%)	0.03
BMI (kg/m2)	31.2 (29,35)	28.5(25,33)	0.182
HT duration (ys)	12 (6,15)	3 (1,8)	0.0022
Pre-op parameters			
Aldosterone (pmol/L)	1 049 (530,1436)	883.5 (569,1254)	0.71
Renin (mU/L)	1.15 (1,2.75)	1.0 (1,2.075)	0.39
ARR	209 (106,287)	168 (109,234)	0.58
K+ (mmol/L)	2.8 (2.5,3.1)	2.8 (2.5,3.1)	0.95
SBP (mmHg)	157 (144,163)	146 (138,152)	0.06
DBP (mmHg)	94 (88,98)	90 (83,96)	0.51
Lateralization Index	6.3 (5,10)	15 (8,26)	0.02
Antihypertensive drugs (N)	2.5 (2,3)	2 (2,3)	0.37
Follow up			
Aldosterone (pmol/L)	445.8 (333,487)	124.9 (80,168)	<0.0001
Renin (mU/L)	1.8 (1.1,3.7)	9.6 (7,13)	<0.0001
ARR	73 (67,103)	16 (9.4,23)	<0.0001
K+ (mmol/L)	3.4 (3.1,3.9)	4.1 (3.7,4.3)	<0.0001
SBP (mmHg)	147 (142,157)	129 (116,134)	0.003
DBP (mmHg)	93.9 (89,96)	83.7 (79,86)	0.008
Antihypertensive drugs	1 (1,3)	0 (0,1)	0.003

Ys: years; F: female, M: male; BMI: body mass index; HT: hypertension; ARR; Aldosterone to renin ratio; SBP: systolic blood pressure, DBP: diastolic blood pressure. Values are expressed as median (interquartile range).

Table 2 Histological characteristics of adrenals from non-cured PA patients

Patient	CYP11B2 ⁺ adenoma size (mm)	APCC (N)	ZG hyperplasia	CYP11B2 expression (% of positive cells)‡	CYP11B1 expression (% of positive cells) ‡
1	25	0	Y	1	2
2	7	0	Y	3	1
3	8	0	Y	3	1
4	11	3	Y	1	1
5	5	0	Y	1	2
6*	No	3	Y	-	-
7**	No	2	N	-	-
8	10	3	N	2	1
9	14	2	N	3	1
10	10	4	N	2	1
11	15	1	Y	3	1
12	11	4	N	3	1

* Micronodular hyperplasia non expressing CYP11B2. **nodule 12mm non expressing CYP11B2. ‡ 1: 1%-33%; 2: 34%-66%; 3: 67%-100%. APCC: aldosterone producing cell clusters; ZG: zona glomerulosa; Y: yes; N: no.

Table 3. Genetic analysis of CYP11B2 positive APAs from PA patients without biochemical success after adrenalectomy

APA	Gene mutated Sanger sequencing	Gene mutated NGS	Read depth	VAF	Nucleotide	Protein
1	<i>KCNJ5</i>	-	-	-	c.451G>C	p.Gly151Arg
2	<i>CACNAID</i>	-	-	-	c.2968C>G	p.Arg990Gly
3	<i>CACNAID</i>	-	-	-	c.1207G>C	p.Gly403Arg
4	-	<i>KCNJ5</i>	1044	28%	c.451G>C	p.Gly151Arg
5	<i>CACNAID</i>	-	-	-	c.4117G>A	p.Val1373Asp
8	-	<i>CACNAID</i>	2384	15%	c.1207G>C	p.Gly403Arg
9	-	<i>KCNJ5</i>	1044	28%	c.451G>C	p.Gly151Arg
10	-	<i>ATP1A1</i>	625	17%	c.311T>G	p.Leu104Arg
11	-	<i>CACNAID</i>	1871	33%	c.3458T>A	p.Val1153Asn
12	-	<i>CACNAID</i>	646	27%	c.2968C>G	p.Arg990Gly

NGS; next generation sequencing. VAF, Variant Allele Frequency. *KCNJ5* (NM_000890), *ATP1A1* (NM_000701), *CACNAID* (NM_001128839.2 and NM_000720).