11C-metomidate PET CT versus Adrenal Vein Sampling for diagnosing surgically curable primary aldosteronism: prospective test validation, and impact of somatic genotype and ethnicity on outcomes

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¹¹C-metomidate PET CT versus Adrenal Vein Sampling for diagnosing surgically curable primary aldosteronism: prospective test validation, and impact of somatic genotype and ethnicity on outcomes

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Abstract

Primary aldosteronism (PA) due to a unilateral aldosterone-producing adenoma (APA) is a common, curable cause of hypertension, but invasive methods of diagnosis and treatment contribute to <1% of patients being offered the chance of cure. The primary objective of our prospective within-patient study in 143 patients with PA was to compare accuracy of ¹¹C-metomidate (MTO) PET-CT scanning with adrenal vein sampling (AVS) in predicting biochemical cure from PA and resolution of hypertension. Secondary outcomes addressed heterogeneity of underlying pathogenesis and prediction of patients most likely to achieve complete cure of hypertension. 128 patients reached 6-9 month follow-up. 77/78 surgical patients achieved one or more of the four hierarchically analysed Primary Aldosteronism Surgical Outcome (PASO) criteria for biochemical and clinical success. MTO was not superior to AVS but all four differences in accuracy favored MTO, with 95% CIs >-17%, the pre-specified margin of non-inferiority. The best univariate predictors of complete clinical cure were home systolic blood pressure (SBP) ≤ 135 mmHg after one month of spironolactone 100 mg daily (odds ratio 13.0 (3.72, 45.24) $p < 0.001$) and *KCNJ5* genotype of the APA (odds ratio 10.37 (2.50, 42.99) $p = 0.001$). The latter remained significant in logistic regression on age, gender, ethnicity, and was itself predicted by elevated urine 18-hydroxycortisol:cortisol ratio. Our findings validate ¹¹C-metomidate PET-CT for accurate, non-invasive detection of patients with unilateral PA, and identify patients most likely to benefit from adrenalectomy.

Introduction

Primary aldosteronism (PA) is a high morbidity cause of hypertension, resulting from excessive, renin-independent aldosterone secretion from one or both adrenal glands. Atrial fibrillation (AF), stroke, heart failure and renal insufficiency are approximately twice as likely in PA compared to age- and sex-matched individuals with essential hypertension.¹⁻⁴ PA can often be surgically cured. Traditionally, patients are stratified into those in whom excess aldosterone is consequent upon a unilateral aldosterone-producing adrenal adenoma (APA) and those with bilateral production (often termed idiopathic hyperaldosteronism, IHA). The binary distinction underpins clinical decision making despite the more recent appreciation that a continuum of disease is more likely;^{5,6} current international consensus is that patients with APAs should be offered a laparoscopic adrenalectomy and patients with IHA should be treated with aldosterone antagonist drugs.⁷

Despite the prevalence and penalty of PA, currently fewer than 1% of patients are identified and fully investigated.⁸ Many reasons exist for this shortfall in clinical care, including low levels of clinical suspicion; time-consuming and imperfect confirmatory biochemical tests which require manipulation of many commonly prescribed anti-hypertensive medications; difficulties with patient selection for surgical intervention; and uncertainty about outcomes in patients hoping for surgical cure. The paradoxical consequence is patient and physician reluctance to embark on a diagnostic and

therapeutic process with a high likelihood of prolonging life while reducing need for medication.

The 'MATCH' ('Is Metomidate superior to Adrenal vein sampling in predicting outcome from adrenalectomy in primary Hyperaldosteronism') study, addresses selection of patients for surgery, and the reasons for variable outcomes from intervention. The investigation and treatment of apparent APAs have not substantially changed in the past 30 years.^{9,10} The two invasive interventions, with limited availability, namely AVS and surgery, might have been appropriate when PA was considered rare. PA is now known to cause 5-10% of unselected cases of hypertension,¹¹ which necessitates alternative strategies. Molecular imaging has the potential both to increase the number of PA patients who can be fully investigated and to elucidate which patients might benefit most from surgical intervention. ¹¹C-metomidate (MTO) was developed as a tracer in the 1990s, after the anesthetic agent etomidate, the ethyl analogue of MTO, was discovered to inhibit cortisol production.^{12,13} MTO was readily C¹¹H₃-labelled and in pre-clinical and clinical studies, the tracer concentrated in the adrenal.¹⁴⁻¹⁶ However, *ex vivo* it bound, with similar potency, to the enzymes CYP11B1 (11beta-hydroxylase) and CYP11B2 (aldosterone synthase).¹⁷ This discouraged evaluation in PA, where selective binding to CYP11B2 is required, in order to detect a higher signal from an APA than from either the normal adrenal, or an incidental non-aldosterone secreting adenoma. In 2012 we reported that *in vivo* selectivity could be achieved, if patients were pre-treated with dexamethasone for 3 days, which suppresses adrenal CYP11B1 (but not CYP11B2) protein expression. In 35 patients with PA, in whom a diagnosis of unilateral or

bilateral PA had been reached by AVS, dexamethasone-suppressed MTO had 73% sensitivity and 87% specificity; assuming AVS was correct, and using a ratio of SUVmax between adenoma and contralateral adrenal of $>1.25:1$.¹⁸ MTO has been adopted by some international centers, but its evaluation without dexamethasone suppression may have limited its applicability.¹⁹ In order for MTO to enter routine clinical practice, as an alternative to AVS, a definitive, adequately-powered study was required in which the primary objective was to prospectively compare the accuracy of AVS and MTO PET-CT scanning in predicting the outcome of adrenalectomy, in patients with PA.

Guidelines to clinical practice in PA are based largely on expert opinion and retrospective analyses. The one randomised control trial to date, comparing AVS with CT, has not substantially changed practice, possibly because neither study group was deemed to follow standard guidance.²⁰ The MATCH study protocol mandated that every patient be investigated identically, according to international guidelines, with a pre-specified follow up schedule. Patients were clear of interfering medications at baseline and primary outcome visits, so that all outcomes of intervention could be accurately quantified, e.g. fall in aldosterone, and N-terminal pro-B-type natriuretic peptide (BNP) as a measure of cardiac function. In addition to standard investigations, every patient had a dexamethasone-suppressed MTO PET-CT, and a nested 'study-within-study' prospective evaluation of spironolactone response as predictor of the clinical outcome from surgery.

The higher likelihood of biochemical than clinical cure after adrenalectomy²¹ enabled the adoption of hierarchical primary endpoints, as used in previous clinical trials.²²⁻²⁴

Biochemical outcome is a purer measure of accuracy in diagnosing unilateral disease, whilst cure of hypertension is of more concern to patients. Most patients with a unilateral APA will not be able to discontinue all antihypertensive medication, the mean reduction in pill burden being 50% at six months post-operatively. We explored whether, in addition to secondary, irreversible vascular changes,⁶ undiagnosed contralateral disease may contribute to incomplete cure.^{25,26} Clinical conservatism in recent decades has contrasted with advances in the molecular analyses of APAs. MATCH, therefore, included pre-specified evaluations of whether variability in outcomes are related to genotype or transcript variations among APAs, and whether informative variations can be predicted in advance of surgery.

Methods

The study design is represented graphically in Fig. 1a and detailed in the supplementary methods. In summary, participants were recruited from the routine referral practices of the principal investigators, representing a mixture of tertiary endocrine and complex hypertension clinical services. All participants satisfied the Endocrine Society consensus guidelines for the diagnosis of PA.⁷

All participants underwent both AVS and MTO PET-CT scanning. The decision for recommending adrenalectomy was made at a monthly multi-disciplinary team (MDT) meeting. The results of the MTO scan were always interpreted first, blinded to the results of AVS. Each investigation was graded a 'low', 'intermediate' or 'high'

probability of unilateral PA, with the additional category of 'failed' for AVS results, if successful cannulation of the adrenal vein(s) was not achieved. Patients with one or two 'high' scores were recommended for surgery. The detailed criteria for surgical referrals are provided in the online supplementary methods.

After the second investigation participants attended a 'baseline' visit for measurements of all clinical and biochemical parameters, whose response to treatment would assess the accuracy of MTO and AVS. Spironolactone therapy (or, if previously not tolerated, eplerenone) was initiated at 50 mg daily, and up-titrated to 100 mg daily after 2 weeks if tolerated. The home blood pressure response to spironolactone was measured 4 weeks after initiation of therapy. Participants who underwent adrenalectomy were followed up at 1, 3, 6, 12 and 24 months post-surgery while those managed medically were followed up at 9-12 and 24 months after initiation of spironolactone. The primary endpoint for the surgical group was assessed at 6 months post-surgery, and at 9-12 months after initiation of spironolactone for the medical group.

The primary objective of MATCH was to compare the accuracy of MTO and AVS in diagnosing unilateral PA. Accuracy was defined as each investigation's ability to successfully predict biochemical and clinical cure, as defined by the PASO consensus (Supplementary Table 1a),²¹ at 6 months post-surgery. Since the rank order of partial and complete cures of PA and hypertension could be assumed (with more patients being biochemically than clinically cured), prospective studies in PA lend themselves to hierarchical analysis of each PASO outcome, in which all four are primary outcomes

without needing to adjust for multiple statistical comparisons (Fig. 1b). Details of the study design, statistical plan, primary and secondary analyses are described in the online supplementary methods.

Results

146 patients were recruited between 2nd December 2016 and 11th September 2020. The final study visit was completed on 19th February 2021. Patient flow is shown in the Consort diagram (Fig. 1c). Baseline characteristics are shown for all 143 patients in the full analysis set Supplementary Table 2, and stratified by subsequent treatment allocation (surgical or medical). 66% (95/143) of participants were male. The median age was 53 years (43-60, interquartile range). They self-identified as of the following ethnicities: 58% White, 30% Black, 11% Asian and 1% Other. Complete baseline data for 128 patients who completed their allocated treatment and follow-up is shown in Table 1. 80/143 (56%) patients were recruited meeting the Endocrine Society trinity of plasma aldosterone >550 pmol/L, suppressed renin, and spontaneous hypokalemia,⁷ ascertained by measurement at referral or by searching of previous records.

Primary Outcomes

In the full analysis set, MTO and AVS were graded 'high' probability in, respectively, 74/141 (52%) and 63/141 (45%) patients (Fig. 2a). Unilateral PA was diagnosed by one or both investigations in 86/134 patients (64%) (Fig. 2b). MTO and AVS were concordant in 38/89 (43%) of these unilateral cases, and agreed also in 20/45 (44%)

of the bilateral cases. It is apparent that the higher than usual proportion of patients being diagnosed with unilateral PA is due to the large number in whom MTO is positive, over and above those diagnosed by AVS. Thus, 27/89 (30%) and 21/89 (24%), respectively, of the unilateral cases were diagnosed by MTO or AVS alone (Fig. 2b).

The patients who lateralized on these investigations are not completely identical to the 78 evaluable surgical patients. The reasons for a reduced number evaluable after surgery are shown in the Consort diagram (Fig. 1c) – principally 5 patients awaiting surgery, or sufficient period of follow-up for evaluation, and 5 patients in whom, after discussion with the patient and/or local team, personal, practical or medical objections to surgery arose. Conversely, three patients proceeded to surgery on a clinical indication, usually intractable hypertension, and 'intermediate' scores on both MTO and AVS. For analysis of outcome, these patients were deemed to have negative investigations as they would not have been recommended for surgery based on one investigation alone.

Outcomes from adrenalectomy

78 patients had evaluable data 6 months after surgery. Only one patient failed to achieve at least one of the primary outcomes. 69/78 (88%) achieved complete biochemical cure of PA and 24/78 (31%) achieved complete clinical cure (Fig. 2c). For all four PASO measures of cure, these were as likely to be achieved whether patients were diagnosed by one or both of the investigations. This is apparent from comparing the proportion of patients predicted to have unilateral disease, in Fig. 2c, with the proportion of patients achieving cure, in Fig. 2d. For the primary analysis of the

accuracy of each investigation, a point was assigned to one or both investigations if they correctly predicted cure, and to the other or neither investigation if they wrongly predicted cure (Supplementary Table. 3). The Forest plot for the four sequentially analysed primary outcomes shows that MTO correctly predicted nominally more biochemical and clinical cures than AVS (Fig. 2e., Supplementary Fig.1). The difference in accuracy between tests was lower than the hypothesized 25%, and the upper bounds of the confidence intervals around the differences exceeded zero, meaning that MTO was not significantly superior to AVS. The lower bounds of the confidence intervals around the differences (between accuracy of MTO and AVS in predicting the four outcomes) all exceeded -17%, meaning that MTO meets the pre-specified limit for recognizing non-inferiority.

A supplementary analysis found a small impact of AVS 'failures' on the relative accuracy of MTO and AVS in predicting partial or complete biochemical success, but not on the other three PASO outcomes (Supplementary Table 4).

Secondary Outcomes

The quantitative data, at baseline and primary follow-up, used to calculate the binary success outcomes in Figure 2 are shown in Table 2, together with the comparable data for patients in the medically-treated group. As per study design and clinical requirements, the comparison of groups is a non-randomized annotation of outcomes which may respond differently to removal of aldosterone excess and mineralocorticoid receptor blockade.

Biochemically there were large and sustained changes in aldosterone, renin and their ratio (ARR) (Fig. 3a-c). Plasma aldosterone fell in all patients after surgery, by a mean of 83%, but rose in the medical group (Table 2). While renin was de-suppressed in both groups, the rise was greater in the surgical patients. Because renin can be estimated as either mass or activity, and the routine assay differed among centers, we took the opportunity, in the largest center, to measure both mass and activity at baseline and primary outcome time points. While generally well correlated, there were some outliers (Supplementary Fig. 2). We therefore adopted a commonly used conversion factor in order to present all results for renin and ARR in Fig. 3, but also show these separately in Supplementary Fig. 3.

Blood pressures in the surgical group were higher at baseline and lower at follow-up than in the medical group (Table 2 and Fig. 3d). These analyses do not include the initial blood pressure responses to spironolactone 100 mg daily, which are reported below.

Cardiovascular outcomes – BNP and cardiovascular magnetic resonance imaging

(CMR) sub-study

Plasma troponin did not change following adrenalectomy. By contrast, plasma BNP fell by a median of 31% (Supplementary Fig. 4a-c). This finding is consistent with the CMR sub-study, in which end diastolic volume fell by almost 29% following surgery, but only by 9% on medical therapy (Supplementary Fig. 4d). By contrast, left ventricular mass fell in both interventions.

Predictors of outcome from surgery

Univariate analysis confirmed that young age, female gender and lower starting blood pressure predicted patients who achieved complete clinical cure (Fig. 4a-c).²¹ However, for SBP, we observed that starting pressure was similar in those with complete or absent cure, but higher in patients with partial cure (Fig. 4c). This paradox is probably due to the PASO definition of complete cure being an absolute BP (≤ 135 mmHg), which is easier to reach from a lower starting point, whereas partial cure is defined by fall in BP ($>20/10$ mmHg), which is easier to achieve from a higher starting pressure. The majority, 7 of 13 (54%), of absent cures (as defined by PASO) were in Black patients, treated with a calcium channel blocker (Fig. 4c-d).

Spironolactone test

Two analyses were planned. The first quantified the correlation between fall in BP on aldosterone blockade with the fall in BP after removal of the source of aldosterone (Fig. 4e). Because changes in a variable correlate with the baseline value, we performed Oldham corrections (see Fig. 4e legend) of the two sets of blood pressure changes, which removes the intrinsic correlation between two changes from a common starting value.²⁷ The two datasets clearly correlate, but for most patients would not accurately predict change in blood pressure following adrenalectomy. Of greater clinical utility is the binary analysis, Fig. 4f, in which patients whose systolic BP fell to ≤ 135 mmHg on spironolactone were always cured by surgery, partially or complete, with 12/18 (67%) able to discontinue all treatment. The BP achieved on spironolactone reflects both the pre-spironolactone BP, previously reported as a prediction of cure, and the fall during spironolactone therapy.

Sub-types of APA – phenotype : genotype correlations, and immunohistochemistry (IHC)

RNA sequencing (RNA seq) detected, in most samples, the probable culprit somatic mutation, absent from the paired sample of adjacent adrenal (Supplementary table 5). The remainder were detected either by whole exome sequencing (WES), or by targeted sequencing of known gene hotspots. 61/70 (87%) samples had a mutation in the known hotspots of *KCNJ5*, *CACNA1D*, *ATP1A1*, *ATP2B3*, *CTNNB1*, *GNAQ* or *CLCN2*. Some of the *ATP1A1* deletions spanned different bases from those previously described, and the single mutation of *CLCN2* is a deletion in four residues prior to three deleted in one of the germline families.^{28,29} In two APAs without a known functional mutation, RNA seq detected somatic mutations predicted to delete function (SIFT = 0.0) of, respectively, *VAPA* and *DPYSL2* (*CRMP2*), and showed both genes to be abundantly expressed in all samples of adjacent adrenal cortex. Both mutations were confirmed on genomic DNA analysis.

There were similar numbers of *KCNJ5* and *CACNA1D* mutations, but with striking ethnic differences (Fig. 5a[i]). *CACNA1D* mutations were much commoner than *KCNJ5* in the APAs of Black participants, as previously reported, though a difference in our cohort was that the rarity of *KCNJ5* mutations was true of both male and female Black participants (Fig. 5a[ii]-[iii]). Almost all participants with *KCNJ5* mutations, and two with our recently reported double-mutation of *GNAQ* and *CTNNB1*³⁰ were women, whereas the reverse was true of all the other mutations.

As is now well-recognized, the expression of CYP11B2 is slightly lower, and CYP11B1 substantially higher, in *KCNJ5*-mutant than other APAs.³¹⁻³⁵ This was apparent at RNA level in 40 adenomas from 39 adrenals (Fig. 5b). Immunohistochemistry (IHC) showed a similar difference, quantified as H-scores, in a subset of APAs stained for both CYP11B1 and CYP11B2 (Fig. 5c). In patients where MTO PET-CT predicted a mixture of functional and non-functional adenomas, with respectively high and low MTO uptake, IHC confirmed large differences in CYP11B2 expression between adjacent adenomas. This is illustrated for a pair of patients for whom CYP11B1 IHC was also available (Fig. 5d).

RNA seq enabled whole transcriptomes and genotypes to be directly correlated, in the same samples, and results are shown for 200 genes which were the most up-regulated in APAs (by at least 2.5 fold) compared to adjacent adrenal. The results for all 200 genes are shown in Supplementary Fig. 5, and nodes of interest in Fig. 5e [i-iii]).

The first part of the heatmap, the *CYP11B2* node, includes several genes previously noted to be upregulated in APAs, e.g. *VSNL1*, *CALN1*,^{32,36-39} but of interest are genes which are significantly upregulated in aldosterone producing cell clusters (APCCs) compared to the zona glomerulosa (ZG).⁴⁰ The second part illustrates genes which we find to be selectively upregulated by somatic genotype. Most of the genes previously noted to differentiate *KCNJ5*-mutant from other, ZG-like APAs, again vary many-fold in their expression between the two types. In particular, *nephronectin* (*NPNT*), a gene which is selectively expressed in normal ZG compared to zona fasciculata (ZF),^{31,34,41}

was highly discriminant between *KCNJ5*-mutant and other APAs, and in addition was twice as abundant in *CACNA1D*- than the *ATP1A1*- or *ATP2B3*-mutant APAs.

Multivariate prediction, including genotype and urine steroid profile data

All 18 participants with *KCNJ5* mutations achieved clinical cure, which was complete in 14/18 (78%) participants, an odds ratio of 10.4 compared to other patients (Fig. 6a). Biochemical cure was complete in 17 patients, the 18th having the rare p.Glu145Gln mutation. By contrast, the odds ratio for complete clinical cure in participants with *CACNA1D* mutations, which occurred in only 3/19 (16%), was 0.38, while they contributed 7/10 (70%) absences of clinical cures, and 3/5 (60%) patients with only partial biochemical cure.

These results posed the question whether the driver of these opposing outcomes for the two commonest genotypes was a difference in the syndrome of PA caused by the genotype, or the differences in age, gender and ethnicity associated with the genotype. In the planned (binary) logistic regression of complete versus partial or absent clinical cure on genotype, age, gender, and ethnic group, *KCNJ5* was the major dependent variable, with an odds ratio of 11.12 (Fig. 6b). Conversely, *CACNA1D* genotype and ethnicity were difficult to separate as primary determinants of absence of cure. In a proportional odds model, in which all three clinical outcomes (complete, partial, absent) were considered against genotype, age, sex and ethnic group, only age was weakly significant, but the highest odds ratio, 7.84 (95% CI [1.24, 55.96] was for the post-hoc comparison of *KCNJ5* vs *CACNA1D* genotype (Supplementary Table 6).

Previous studies have shown that *KCNJ5*-mutant APAs are associated with high serum levels of hybrid steroids, which require a combination of enzymatic steps typically restricted to the ZG (CYP11B2) and ZF (CYP11B1).⁴²⁻⁴⁴ In our study urine multi-steroid profiling identified significantly higher ratios of the hybrid steroid 18-hydroxycortisol to cortisol in patients harboring *KCNJ5* mutations, allowing for an almost complete separation from other PA cases (Fig. 6c). The only borderline-high result among the latter was from the solitary woman with an *ATP1A1*-mutant APA, who was completely cured. Conversely, the lowest 18-hydroxycortisol/cortisol ratio in the *KCNJ5*-mutant group was in the woman with a p.Glu145Lys mutation, who was not completely cured.

Discussion

MTO PET-CT, following pre-treatment with dexamethasone, enables non-invasive detection of unilateral APAs. Its non-inferiority to AVS, with nominal superiority in hierarchical analysis of all four of the PASO outcomes, has the potential to increase substantially the number of patients whose unilateral APAs can be discovered and removed (Fig. 2a,b). The cure of PA (i.e. partial or complete biochemical cure) in 74/78 surgical patients (out of 128 patients with completed outcomes), and >75% mean reduction in aldosterone, suggest that usual measures under-estimate the proportion of unilateral disease, and the value of its detection and treatment (Fig. 2c,d). The reduction in BNP (Supplementary Fig. 4) is further evidence of benefit, and emphasizes the potential importance of linking suppression of aldosterone to reversal of long-term CV risk, rather than to cure of hypertension.

MTO has been available for more than two decades.^{15,16} However, it has not entered routine practice because, in both early and recent studies which did not use dexamethasone, its *in vivo* selectivity for CYP11B2 was not apparent.^{17,19} In MATCH, in which we compared the *in vivo* uptake of MTO into APA and adjacent adrenal, or into non-aldosterone producing adenomas, with the subsequent measurements, by qPCR and RNA seq, of CYP11B1 and CYP11B2, it is clear that uptake of MTO is much lower in CYP11B1 than CYP11B2-rich regions of the adrenal (e.g. Fig. 5d). We did not formally quantify patient preference between the two techniques, but even in the implausible scenario of more patients preferring the invasive to non-invasive investigation, the limited availability of routinely successful AVS contrasts with the potential for PET CT to become available in all PA centers. The main limitation of MTO is its short half-life which requires synthesis by an on-site cyclotron. 18F-ligands are currently under evaluation (ClinicalTrials.gov Identifier: NCT04529018).⁴⁵

According to guidance from the Endocrine Society, the primary goal of adrenalectomy is removal of autonomous aldosterone production, to reduce long-term cardiovascular risk.⁷ Patients with PA lie on a spectrum between those who will do anything to reduce their pill burden, and those who would avoid surgery at any cost. Probably, patients referred to the tertiary clinics which recruited for MATCH incline towards those willing to follow an interventional pathway without guarantee of complete cure. Because the proportion of referrals would rise if cure were more certain, and because the demand for intervention will outstrip supply as the proportion of PA who are diagnosed rises from <1% to 10% and beyond, MATCH had

major secondary objectives of finding improved, prospectively evaluated predictive measures.

In designing MATCH, a key aim was to improve on the predictive power of univariate variables (young age, female gender, lower baseline blood pressure)⁴⁶⁻⁴⁸ and various algorithms incorporating these and other variables. A further aim was to harness improved understanding of the molecular pathogenesis of APAs and their phenotypes into clinical decision-making. These objectives led to the incorporation of spironolactone testing as a study within a study, and to the genotyping of almost all of the APAs, and measurement of particular urine steroid metabolites as predictor of genotype. The 13-fold greater likelihood of complete cure following surgery, if SBP on spironolactone was ≤ 135 mmHg (Fig. 4f), probably reflects both the lower pre-treatment pressure in these patients, and the contribution of aldosterone excess to this pressure. Since there was also a higher likelihood of SBP falling to ≤ 135 mmHg in patients with unilateral than bilateral PA, an interesting possibility would be to reduce radiation exposure by starting patients on spironolactone at an early stage, and dispense with the diagnostic CT, prior to PET CT, in those with high prior probability of unilateral disease.

Our secondary, pre-specified analyses build on our and others' demonstration that the molecular divisions among APAs, by genotype and gene expression, are becoming more distinct, at the same time as the conventional anatomical 'subtype' division into unilateral and bilateral is becoming more blurred.^{6,31-35} We are not the first to associate *KCNJ5*-mutation with better clinical outcome, or with hybrid steroid

excess.⁴⁹⁻⁵¹ However, the observation has not been uniform; and in studies which are retrospective and/or the control group is wild-type APAs, it is harder to eliminate confounders, such as NFAs ‘masquerading’ as APAs, or to collect data of equal weight from most patients and adenomas. The 78% complete clinical cure rate for *KCNJ5*-mutant APAs, compared to 31% overall (Fig. 6a), and only 15% once *KCNJ5*- and *GNAQ/CTNNB1*-mutant APAs are excluded, is dramatic. The complete cures included males and females, older and younger patients. The paucity of young Black women with *KCNJ5*-mutant APAs in MATCH makes it unlikely that good outcome is due to earlier detection in younger women consulting doctors for obstetric or contraceptive reasons.

CACNA1D mutation has not previously been associated with poorer outcome, but has been strongly linked both to a high proportion of somatic mutations in aldosterone producing cell clusters (APCCs), including those which may underpin bilateral ‘hyperplasia’, and to a higher prevalence than *KCNJ5* mutation in Black male patients.⁵²⁻⁵⁴ It is tempting therefore to postulate that the higher likelihood of absence of cure in Black patients reflects a point in the anatomical spectrum that is asymmetric rather than unilateral.⁶ Some evidence in favor of this is that 4/5 of the partial biochemical cures are Black patients, whose renin remained suppressed despite their aldosterone falling by >75%, to <300 pmol/L. Conversely, *KCNJ5* mutations and the *GNAQ/CTNNB1* double-mutations arise typically in solitary adenomas, and are rarely seen in APCCs. To date, 16/16 double-mutant APAs have been completely cured.^{30,31}

In most endocrine conditions, it is normalization of the culprit hormone that is the main goal of intervention, and in MATCH this was achieved irrespective of genotype. Furthermore, the large fall in plasma BNP adds weight to the recommendation for intervention as a means of cardiovascular protection (Supplementary Fig. 4a and Table 2). Because the allocation to surgical and medical management was not randomized, and blood pressure control slightly less effective in the latter group, no definite conclusions can be drawn from the failure of BNP to fall in the medical patients. It could be that aldosterone suppression is more effective than mineralocorticoid receptor (MR) blockade, with aldosterone able to stimulate non-MR mediated effects.^{55,56} But this hypothesis should wait for formal randomized control trials comparing some of the newer drugs in the aldosterone synthase inhibitor and mineralocorticoid antagonist classes.

In conclusion, our study validates dexamethasone-suppressed MTO PET-CT as a CYP11B2-selective investigation for lateralizing PA. MATCH shows that it will now be possible to diagnose unilateral APAs when AVS is either unavailable, unsuccessful, or not desired by the patient. Unlike AVS, MTO PET-CT is non-invasive and is unlikely to be operator-dependent. These advantages may attract more clinicians and patients to seek a diagnosis, especially if the 18F-analogue becomes available, and transportable to most hospitals with PET imaging facilities.⁴⁵

The longer-term impact of MATCH may be a reassessment of who undergoes lateralization and surgery, and a catalysis of decision-making by molecular, rather than anatomical, diagnosis. In this view, either surgery itself – a procedure usually

undertaken for cure rather than prevention – or the counselling of patients to whom it is offered, will emphasize its success rate in patients with elevated hybrid steroid secretion, and normal blood pressure on spironolactone. Conversely, patients advised of a <1/10 likelihood of complete clinical cure based on demographics, steroid secretion, and response to spironolactone, may now prefer optimized medical treatment, and/or to wait for nodular ablation, if/when this is shown to reduce BP, medication, and plasma BNP as much as surgery. When that time arrives, molecular imaging offers the potential advantage over AVS of localization as well as lateralization, given our examples of patients in whom the largest nodule on CT was not the MTO-avid, CYP11B2-positive adenoma.

Online Supplementary Methods

Study Design

MATCH was an observational multicenter prospective study designed to compare the accuracy of MTO PET-CT with AVS in predicting unilateral PA. Participants were recruited from three study sites in the United Kingdom: St Bartholomew's Hospital, Queen Mary University of London (SBH); Addenbrooke's Hospital, University of Cambridge (CUH) and Guy's and St Thomas' Hospital (GSTT). The study was conducted in accordance with principles of the Declaration of Helsinki. The study protocol was approved by the National Research Ethics Service (Cambridgeshire 2 Research Ethics Committee), the Health Research Authority and Administration of Radioactive Substances Advisory Committee.

Participants

Participants were recruited through tertiary endocrinology clinics at SBH and CUH, or complex hypertension clinics at SBH and GSTT. The criteria for inclusion consisted of patients aged 18 years or older with a probable or definite adenoma on CT or MRI scans of the adrenals, and meeting the Endocrinology Society criteria for diagnosis of PA (ARR > 750 pmol/mU or equivalent local value measured off interfering medications AND either (1) a plasma aldosterone >190 pmol/L after a 2L 0.9% saline infusion test, or (2) failure to suppress plasma aldosterone by 30% + persistent plasma renin suppression a 25 mg captopril challenge test, or (3) spontaneous hypokalemia + plasma renin below detection levels + plasma aldosterone > 550 pmol/L). Patients with elevated ARR could be put forward for consideration by the MDT for inclusion in

the study as exceptional cases in whom confirmatory test was not performed if plasma aldosterone > 450 pmol/L AND (1) plasma renin <0.5 pmol/ml/hr (<9 mU/L) if measured on treatment with an ace-inhibitor (Lisinopril $\geq$ 20 mg or equivalent), or (2) an angiotensin receptor blocker (Losartan 100 mg or equivalent); OR if patient was aged <40 years and a definite adrenal adenoma was seen on CT or MRI.

Exclusion criteria included individuals who were (1) unlikely to proceed with surgery if recommended, (2) contraindicated for spironolactone/eplerenone therapy use, (3) unable to stop beta-blockers or direct renin blockers, (4) pregnant women or unable/unwilling to take secure contraceptive precautions whilst undergoing investigations, (5) unable/unwilling to take the dexamethasone required to prepare for a MTO scan (6) unwilling/unable to have both MTO and AVS, or (7) had a condition or drug regimen that was considered a contraindication by the principal investigator.

Adrenal vein sampling (AVS) and scoring

All AVS procedures were performed by one of three experienced interventional radiologists. 50 μ g/h of intravenous synacthen was started 1 hour before the procedure. In line with a consensus statement cannulation was considered successful if the cortisol level in each adrenal vein was $\geq$ 3x greater than that in the iliac and/or infrarenal IVC.⁵⁷

‘High’ probability of unilateral PA was diagnosed if the aldosterone/cortisol (A/C) ratio in one adrenal vein was $\geq$ 4 times that in the contralateral adrenal vein and if the contralateral vein had an A/C ratio less than that of the infrarenal IVC/iliac vein.^{57,58}

'Low' probability of unilateral PA was diagnosed with an A/C ratio <3 , which was indicative of bilateral aldosterone secretion. An A/C ratio of 3-4 was considered 'intermediate'. In the event of one or both adrenal veins not being successfully cannulated, the outcome was declared 'failed'. Although, in clinical practice, it has been suggested that a focal right adrenal lesion in association with an A/C ratio in a unilaterally cannulated left adrenal vein <0.5 of the infrarenal IVC/iliac vein can provide justification for a right adrenalectomy,⁵⁹ this was not adopted in MATCH.

MTO synthesis, scanning and scoring

¹¹C-metomidate was manufactured in compliance with good manufacturing practice using a GEM Medical Systems PETtrace cyclotron (Milwaukee, WI). ¹¹C-methyl iodide was passed through a solution of (R)-methyl 1-(1-phenylethyl)-1H-imidazole-5-carboxylic acid in anhydrous dimethylformamide, containing tetrabutylammonium hydroxide as a catalyst and loaded directly into the injector loop of a GE TracerLab FX-C system. This captive solvent methylation method produced ¹¹C-MTO with a radiochemical purity of $>99\%$ and specific activity 19.8-414.8 GBq/ μmol .

All patients received dexamethasone 0.5 mg po qid for 72 hours prior to MTO scanning. PET-CT imaging was performed on a GE Discovery 690 PET-CT scanner (GE Medical Systems). Non-contrast CT images were acquired over the adrenals (140kV, 64 mA, slice thickness 3.75mm). Following an intravenous injection of ¹¹C-MTO (150-500 MBq), dynamic PET images were acquired for 45 minutes. Attenuation and decay-corrected images were converted to standardised uptake values (SUV) over regions of interest were determined over a 10 minute period starting 35 minutes after the

injection. Attenuation and decay-corrected images were converted to SUV maps through division (injected activity per patient weight).

All MTO scans were analysed and initially scored by a single experienced radiologist blinded to the AVS result. Three key features were used to determine the probability of unilateral PA: a focal adrenal nodule with Hounsfield units in keeping with a benign adrenocortical adenoma; uptake of MTO into the identified nodule; and, in keeping with our previous report, a calculated ratio of tumour SUVmax to normal background SUVmax of >1.25 .¹⁸ An opinion was formed on the basis of the presence or absence each of the three features described and a score of 'high', 'intermediate' or 'low' probability of unilateral PA assigned.

Minimization

The order that each participant underwent AVS or MTO PET-CT was determined by a minimization program within the study data center (Robertson Centre for Biostatistics, University of Glasgow), designed to maintain balance with respect to study site, gender, and age (<55 or ≥ 55 years). Both AVS and MTO scanning were performed with the patient taking non-interfering medications such as doxazosin, verapamil and hydralazine, according to Endocrine Society consensus guidelines.⁷

Clinical decision-making

Each study patient was discussed in a MDT meeting attended by PIs, radiologists and surgeon. The MTO scan was presented and interpreted by the reporting radiologist prior to the AVS result being revealed. For each investigation, the question posed was:

‘in the absence of any other information, would this result provide sufficient evidence for a referral for surgery?’ The MDT decision was not bound unconditionally to the criteria for diagnosis of unilateral PA as outlined above. The probability of unilateral disease could, by consensus, be reduced if data from the contralateral adrenal suggested bilateral disease (e.g. contralateral adenoma with SUVmax higher than adjacent adrenal, or LI>4 but contralateral A/C not suppressed compared to IVC). Importantly, the agreed score for MTO was entered into the case report form (CRF) prior to discussion of the AVS, and could not be revised. The outcome of the MDT was a recommendation for either surgical or medical management. Surgery was recommended if either investigation was scored ‘high’. In a small number of patients where both investigations indicated ‘intermediate’ probability of unilateral PA, surgery was recommended if there was a strong clinical indication to proceed. A decision for recommending surgery was deferred if MTO and AVS showed discordant results (lateralised to opposite sides). Such participants were managed medically with a view to reviewing the results at the end of the study where recommendations for surgery would be based on the outcomes from the study.

Baseline visit and initiation of spironolactone therapy

After the second investigation, patients attended for measurements of all clinical and biochemical parameters whose response to treatment would assess the accuracy of the MTO and AVS. Obtaining the baseline measurements at this point in the study allowed a maximum interval between discontinuation of any interfering medicines being taken at screening, and the opportunity for the blood pressure response to initiation of spironolactone to be assessed as a predictor of response to surgery.

After four days of home blood pressure recordings, 3 readings twice daily (24 in total), blood was drawn for electrolytes, renin activity and/or renin mass, aldosterone, BNP and troponin. In the rare occasion the minimum number of home blood pressure recordings were not available, office blood pressure recordings (average of 3 sequential readings) were used instead. Participants also performed a 24h urine collection for urine steroid profiles. Participants then commenced spironolactone 50 mg once daily for two weeks, increasing to 100 mg once daily depending on side effects and any concern about renal function or potassium homeostasis. In participants referred for surgery, therapy was continued, as tolerated, until the day of operation. Participants unable to tolerate spironolactone were treated with eplerenone 25 mg twice daily for two weeks, increasing to 50 mg twice daily. Further treatment titrations in the medical group were at the discretion of the study or referring physician.

Surgery

Laparoscopic adrenalectomy, using standard operative techniques, was performed by a single endocrine surgeon at two of the participating sites, unless the participant or referring physician preferred local referral. The serum cortisol value at the conclusion of the 72 hours preparatory dexamethasone before the MTO scan was used to guide the clinical concern about potential postoperative adrenal insufficiency. Where appropriate, supplementary hydrocortisone was provided and withdrawn after subsequent clinical and biochemical reassessment. The outcomes from surgery were assessed biochemically and clinically, at 3 and 6 months after surgery, and at 12 and

24 months in patients recruited in earlier years of the study. At each visit, the same measurements were performed as at the baseline visit, except that BNP, troponin, and 24h urine measurements were repeated only once, at the 6-month post-surgery visit, or 9-12 months in medically treated participants.

Outcomes

The primary objective of MATCH was to compare the accuracy of MTO PET-CT and AVS in diagnosing unilateral PA, by measuring normalisation of biochemical and clinical parameters following adrenalectomy. The criteria for normalisation were the PASO criteria, agreed by international consensus in 2017 (Supplementary table 1), for each of partial or complete cure of PA and hypertension.²¹ Since the rank order of partial and complete cures of PA and hypertension could be assumed (with more patients being biochemically than clinically cured), prospective studies in PA lend themselves to hierarchical analysis of each PASO outcome, in which all four are primary outcomes without needing to adjust for multiple statistical comparisons (Fig. 1b). It is important to note that, whereas partial may seem softer versions of complete cure (either clinical or biochemical), they measure different parameters. Partial responses are *changes* in biochemistry, blood pressure, and defined daily dosage (DDD) of medications, whilst complete responses are numbers below an absolute target. Of note, the first outcome in the hierarchy, partial biochemical cure, is the sole outcome driven by reduction in the culprit hormone, aldosterone, to which excess cardiovascular risk in PA is attributed.

Secondary objectives of MATCH included serial measurements, up to two years post-intervention, of the criteria for assessing biochemical and clinical success, analysed separately in the surgical and medically treated participants; and analyses of parameters which may, in the future, enable more reliable prediction of which patients can achieve complete clinical cure adrenalectomy. The main clinical test was a within-trial measurement of participants' response to 4 weeks treatment with spironolactone. Molecular analyses included RNA sequencing of APAs for genotyping and transcriptomics. We also report the left ventricular mass and end-diastolic volume measurements from the cardiovascular magnetic resonance imaging (CMR) sub-study of 50 patients, performed at baseline and 1 year after surgical or medical management.

Statistics and sample size

The primary analysis was a comparison of the accuracy of MTO PET-CT and AVS. Each test was deemed accurate if it recommended surgery and surgery resulted in cure (according to each PASO criterion), or if the test did not indicate surgery and surgery did not result in cure. Since surgery was almost exclusively undertaken when one or both tests indicated surgery was required, for those patients where neither test indicated surgery, both tests were treated in the analysis as having equal accuracy. Only in those patients for whom the two tests gave conflicting recommendations was it possible to determine which of the two test was accurate, and which was not.

The difference in accuracy between the two tests was estimated amongst those who underwent surgery, using the Newcombe-Wilson score method for paired binary data, and tested using an exact McNemar test.

The sample size for the study was estimated from a table of all permutations of outcomes from each investigation and surgery, in which the frequency of each outcome reflected experience at participating centres (Supplementary Table 7). This estimated a need for 128 completed patients, with half undergoing surgery, and half of those (32) doing so as the result of one of MTO or AVS, but not both. This was based on having 90% power at a 5% significance level to detect a difference in accuracy between the two tests, assuming that on those occasions when only one of the tests was accurate, it would be MTO that was accurate 80% of the time. In addition, if the two tests were truly equally accurate, this sample size would have 90% power to demonstrate non-inferiority of MTO versus AVS within a non-inferiority margin of -17%.

The full analysis set was defined as all participants who had both investigations. Because of pandemic-associated interruptions to the availability of laparoscopic adrenalectomy, the study was censored when more than 75 patients had undergone surgery, and at least one evaluable outcome visit. The analysis is reported for the 128 patients who had reached a primary outcome visit, either 6 months post unilateral adrenalectomy (including 5 for whom data from their most recent, 3-month visit was carried forward), or 9-12 months after their baseline visit in the medically-treated patients.

Secondary analyses

Secondary (and Primary) outcomes were compared between participants who underwent surgery and those who did not, and for those who underwent surgery, between those for whom surgery was indicated by AVS only, MTO only, or both tests. Groups were initially compared using Fisher's Exact or Kruskal-Wallis tests, as appropriate. Linear, binary logistic, or proportional odds logistic regression models were then used, adjusted for age and gender. These models were extended to investigate other predictors of outcome after surgery, in the subset of participants who underwent surgery. There was particular interest in blood pressure at baseline and 4 weeks, age, gender, ethnicity, the number of classes/defined daily dose (DDD) of antihypertensive medications at baseline, tumour genotypes and immunohistochemical classification, contralateral suppression, numbers of nodules, blood biomarkers, SUVmax, and 24 hour urine steroid profile.

For each of the primary outcomes, a sensitivity analysis was carried out to assess the impact of including patients for whom AVS was classified as 'failed'. A bootstrap procedure was applied, sampling with replacement from the 78 patients who underwent surgery. Sampling was stratified by whether AVS was successful, or failed. For each sample, the estimated difference in accuracy between MTO PET-CT and AVS was calculated for the combined sample of 78 patients, and for the 67 patients where AVS had not failed, and the difference between these estimated differences was taken as a measure of the sensitivity of the overall result to the inclusion of AVS failures. One million replicates were drawn, and a 95% confidence interval was derived by the

percentile method. If the 95% CI excluded zero, then the overall result was adjudged to be sensitive to the inclusion of AVS failures. If the 95% CI included zero, then the results were judged to be insensitive to the AVS failures.

Cardiovascular Magnetic Resonance Imaging (CMR) Sub-study

A CMR substudy was performed with imaging acquired at baseline prior to treatment allocation in 76 patients, and at follow up at 12.0+/-3.1 months post in 51 patients. All CMR imaging was performed at 1.5 Tesla (Aera, Siemens Healthcare, Erlangen, Germany). Cine Imaging was performed using Steady State Free Precession sequences. Automated contouring of left ventricular (LV) blood and myocardial volumes using a clinically validated artificial intelligence (AI) analysis platform (with verification by two experts) enabled calculation of LV mass, chamber volumes and ejection fraction.

Biochemistry methods

All biochemistry tests were analysed at United Kingdom Accreditation Service accredited clinical laboratories. Serum aldosterone was measured by automated chemiluminescence immunoassay at SBH and GSTT (Diasorin Liaison, Saluggia, Italy) and by tandem mass spectrometry at CUH (in house method adapted from⁶⁰). Direct renin mass was measured by automated chemiluminescence immunoassay at all three centres (Diasorin Liaison, Saluggia, Italy). Prior to 03/02/2017, plasma renin activity was assayed in EDTA plasma, using generation of angiotensin I from endogenous angiotensinogen, during a 90 min incubation at 37 °C, in the presence of angiotensin converting enzyme inhibitors. Angiotensin I generated was measured by

RENCTK 125I competitive radioimmunoassay kit (Diasorin, Saluggia, Italy) with blank subtraction of angiotensin I levels in aliquots incubated at 4 °C. From 03/02/2017, renin activity was analysed by LC-MS/MS (North West London Pathology). Serum cortisol was measured by electrochemiluminescent immunoassay on Modular Analytics E170 or Cobas 8000 e602 platforms (Roche Diagnostics, Burgess Hill, UK) at SBH and by chemiluminescent immunoassay on the ADVIA Centaur platform (Siemens, Erlangen, Germany) at GSTT and CUH.

Adrenal tissue collection

Adrenal tissue was collected directly from surgery. Once the adrenal was removed this was dissected into 5mm serial slices by the surgeon or histopathologist. All adrenal adenomas were identified and 10-200mg of tissue was removed from each APA and adjacent normal adrenal gland (AAG) for storage in separate tubes in RNALater[®] solution (AM7020, Invitrogen). Slices were sent to pathology for routine clinical hematoxylin and eosin staining and immunohistochemistry.

Immunohistochemistry (IHC)

IHC was performed on 3-µm sections cut from paraffin blocks using a fully automated systems. The CYP11B1 primary antibody clone RAT-87 (MABS502, Merck) was used at a dilution of 1:100 after heat-induced epitope retrieval at pH 9.0 (BOND Epitope Retrieval Solution 2, AR9640, Leica) for 20 minutes; and CYP11B2 primary antibody clone EPR10494 (ab168388, Abcam) was used at a dilution of 1:200 after proteolytic-induced epitope retrieval with Proteinase K (BOND Enzyme Pre-treatment kit,

AR9551, Leica) for 10 minutes. The primary antibody binding to tissue sections was visualized using BOND Polymer Refine Detection system (DS9800, Leica).

Histopathological Analysis

For each case, APAs, aldosterone-producing nodules (APNs), aldosterone-producing micronodules (AP micronodules) formerly known as aldosterone-producing cell cluster (APCC), functional and non-functional hyperplasia were characterised based on the international histopathology consensus for unilateral primary aldosterone (HISTALDO).⁶¹ AP micronodule numbers were counted within the adrenal adjacent to the main nodule. The cell types within the main nodules (lipid rich, lipid poor or mixed) were noted. The intensity and proportion (as a percentage) of the staining of tumours with CYP11B1 and CYP11B2 were evaluated semi-quantitatively using a scoring system as follows: 1= weak, 2= intermediate, and 3= strong. From this data, a H-score was assigned using the following formula: $3 \times (\% \text{ cell } 3+) + 2 \times (\% \text{ cells } 2+) + 1 \times (\% \text{ cells } 1+)$. A consensus of either classical or non-classical histopathological findings was made in line with HISTALDO criteria, where "classical" histopathology associated with unilateral PA included a solitary APA or APN; "nonclassical" histopathology included multiple APMs or multiple APNs (or multiple APMs and multiple APNs together) or aldosterone-producing diffuse hyperplasia.

RNA and DNA extraction

RNA and DNA was extracted from tissue samples stored in RNALater[®] for RNA, whole exome or Sanger sequencing. Tissue homogenisation was performed in Trizol (Life Technology 15596026) using a FastPrep-24 5G Sample Preparation System (MP-

Biomedicals) as per protocol. Genomic DNA (gDNA) was extracted from approximately 25-30mg of normal adrenal or APA tissue using QIAamp DNA Mini kit (51304, Qiagen) according to manufacturer's instructions. If AAG was not available to be used as controls, gDNA was extracted from blood using the salt extraction method. Total RNA was extracted from approximately 50-100mg of AAG or APA tissue using the Trizol method (Life Technology 15596026) followed by on-column DNaseI treatment (Invitrogen) and purification using Invitrogen's PureLink RNA minikit (#12183018A).

Quantitative polymerase chain reaction (qPCR)

qPCR was performed on all adenomas to assess for *CYP11B2* and *CYP11B1* mRNA expression. RNA was reverse transcribed to cDNA using Applied Biosystem's High Capacity RNA-to-cDNA kit. Taqman assay probes were used to quantify the gene of interest. The $\Delta\Delta$ CT method was used to quantify gene expression levels.⁶² Supplementary Table 8 lists the commercially available Taqman Gene Expression Assays used to quantify mRNA expression of the genes of interest.

RNA sequencing and analysis

RNA sequencing was performed on functional adenomas. RNA sequencing was performed by the Barts and the London Genome Centre, Blizard Institute in London, UK. Briefly, quality control was performed using Agilent RNA 6000 nano reagent kit and run on an Agilent 2100 bioanalyzer (Agilent USA). Only samples with a RIN number >0.8 were sequenced. Library preparation using the mRNA library prep method was performed using NEBNext Ultra II RNA Library Prep Kit for Illumina (New England Biolabs, USA). Finally, sequencing was performed on Illumina's NextSeq 500 system,

using the high-output 150 cycle kit v2.5. Partek Flow (Partek, St. Louis, Missouri, United States) was used for RNASeq analysis. Sequences were aligned hg38 with STAR -2.6.1d and annotated genes to Ensembl Transcripts release 93 with Partek's own annotation tool. Partek's GSA tool was used to generate lists of differentially expressed genes.

Whole Exome Sequencing (WES) and analysis.

Approximately 1 mg of gDNA was sent off for WES by the commercial company GENEWIZ LLC. (South Plainfield, NJ, USA). Library preparations, sequencing reactions and bioinformatic analysis were conducted at GENEWIZ, as follows:

DNA Library Preparation and Sequencing

Genomic DNA sample were quantified using Qubit 2.0 Fluorometer (ThermoFisher Scientific, Waltham, MA, USA). Twist Human Core Exome library preparation was performed according to the manufacturer's guidelines (Twist Biosciences, South San Francisco, CA, USA).

Briefly, the genomic DNA was fragmented by acoustic shearing with a Covaris S220 instrument. Fragmented DNAs were cleaned up and end repaired, as well as adenylated at the 3'ends. Adapters were ligated to the DNA fragments, and adapter-ligated DNA fragments were enriched with limited cycle PCR. Adapter-ligated DNA fragments were validated using Agilent TapeStation (Agilent Technologies, Palo Alto, CA, USA), and quantified using Qubit 2.0 Fluorometer. Adapter-ligated DNA fragments were hybridized with biotinylated baits. The hybrid DNAs were captured by streptavidin-coated binding beads. After extensive wash, the captured DNAs were

amplified and indexed with Illumina indexing primers. Post-captured DNA libraries were validated using Agilent TapeStation (Agilent, Santa Clara, CA, USA) and quantified using Qubit 2.0 Fluorometer and Real-Time PCR (KAPA Biosystems, Wilmington, MA, USA).

The sequencing libraries were multiplexed and clustered onto multiple lanes of a flowcell. After clustering, the flowcell was loaded onto the Illumina HiSeq instrument according to manufacturer's instructions. The samples were sequenced using a 2x150bp Paired End (PE) configuration. Image analysis and base calling were conducted by the HiSeq Control Software (HCS). Raw sequence data (.bcl files) generated from Illumina HiSeq was converted into fastq files and de-multiplexed using Illumina bcl2fastq 2.17 software. One mis-match was allowed for index sequence identification.

Data Analysis

Sequence reads were trimmed to remove possible adapter sequences and nucleotides with poor quality using Trimmomatic v.0.38. The trimmed reads were mapped to the reference genome using the Illumina Dragen Bio-IT Platform. BAM files were generated as a result of this step. Somatic variants were called using the Illumina Dragen Bio-IT Platform in somatic mode. Paired normal samples were used in the process, if provided, or a panel of normal (PON) was used to remove technical artifacts. Variants were further filtered and any variants in the follow categories were considered as false positives and removed: (1) marked as common variants in dbSNP build 151 and (2) non_cancer_AC > 5 in gnomad exome database r2.1.1. The filtered

VCF was then annotated with Ensembl Variant Effect Predictor (VEP) v95. For each variant that was mapped to the reference genome, all overlapping Ensembl transcripts were identified, and the effects that each allele of the variant may have on each transcript were predicted by VEP. The set of consequence terms was defined by the Sequence Ontology (SO). The most severe impact was selected for each variant and they are used for downstream cohort analysis. Impact of the variants were classified based on MAF document specifications. Tumor mutation load was calculated based on number of mutations in the genome region that targeted.

Targeted Sanger Sequencing

cDNA from the APA and AAG was sequenced to confirm presence of *KCNJ5* mutations. PCR was performed with the following forward (caacttgctcgtcttcacca) and reverse (gagggtctccgctctcttct) primers, using AmpliTaq Gold™ Fast PCR Master Mix (ThermoFisher, 4390939) as per manufacturer's instructions. 5 μ l of PCR products were then purified using 2 μ l of ExoSAP-IT™ PCR Product Cleanup Reagent (Applied Biosystems, 78201.1.ml) prior to Sanger Sequencing. Sequencing of the PCR products was performed using LIGHTRUN Tube sequencing services from Eurofin (Germany).

Urinary Steroid Profiling by Liquid Chromatography-Tandem Mass Spectrometry

Steroids were extracted from 200 μ L of a 24-hour urine collection after addition of an internal standard mixture, as described previously.^{63,64} In brief steroids were deconjugated from their sulphate and/or glucuronide conjugates through enzymatic hydrolysis with helix pomatia (Sigma Aldrich, Gillingham, UK, 60°C for 3 hours). The steroids were then extracted via solid phase extraction (C18 100mg, Biotage,

Hengoed, UK). The methanol eluent was collected, evaporated and the extract reconstituted and run by liquid chromatography tandem mass spectrometry (LC-MS/MS). The mass spectrometer was a Waters Xevo-XS with an Acquity ultra-high pressure liquid chromatography (uPLC) system, with an electrospray source in positive ionisation mode with a methanol and water gradient system (both with 0.1% formic acid). Gradient started at 30% methanol, linearly increasing to 39% over 6 minutes. Next the column was washed at 98% methanol and re-equilibrated prior to the next injection. Separation was achieved on a Waters HSS T3 1.2 x 50mm 1.8 μ M column. With each batch of samples, a calibration series, (0-1500 ng/mL), three spiked QC samples and seven pooled biological control urines were extracted. Steroids were quantified relative to their deuterated internal standard Cortisol-d4 or 18hydroxycortisol-d4, against this calibration series. In addition to previously published methods 18-hydroxycortisol (18OHF) was included in this method. Bias (% deviation), calculated as the accuracy at three spiked concentrations 20, 300 and 200ng/mL was <5% for cortisol and <10% for 18OHF at all concentrations. Imprecision (% RSD) measured from multiple extractions of the same urine sample (intra-assay (n=7), inter-assay (n=14) was 4.9% and 3.85% for cortisol, and 5.8% and 6.21% for 18OHF respectively.

References

1. Monticone, S., *et al.* Cardiovascular events and target organ damage in primary aldosteronism compared with essential hypertension: a systematic review and meta-analysis. *The Lancet. Diabetes & endocrinology* **6**, 41-50 (2018).
2. Hundemer, G.L., Curhan, G.C., Yozamp, N., Wang, M. & Vaidya, A. Cardiometabolic outcomes and mortality in medically treated primary aldosteronism: a retrospective cohort study. *The Lancet Diabetes & Endocrinology* **6**, 51-59 (2018).
3. Hundemer, G.L., Curhan, G.C., Yozamp, N., Wang, M. & Vaidya, A. Renal Outcomes in Medically and Surgically Treated Primary Aldosteronism. *Hypertension* **72**, 658-666 (2018).
4. Hundemer, G.L., Curhan, G.C., Yozamp, N., Wang, M. & Vaidya, A. Incidence of Atrial Fibrillation and Mineralocorticoid Receptor Activity in Patients With Medically and Surgically Treated Primary Aldosteronism. *JAMA cardiology* **3**, 768-774 (2018).
5. Brown, J.M., *et al.* The Unrecognized Prevalence of Primary Aldosteronism: A Cross-sectional Study. *Annals of Internal Medicine* **173**, 10-20 (2020).
6. Gomez-Sanchez, C.E., Kuppusamy, M., Reincke, M. & Williams, T.A. Disordered CYP11B2 Expression in Primary Aldosteronism. *Horm Metab Res* **49**, 957-962 (2017).
7. Funder, J.W., *et al.* The Management of Primary Aldosteronism: Case Detection, Diagnosis, and Treatment: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab* **101**, 1889-1916 (2016).

8. Funder, J.W. Primary aldosteronism as a public health issue. *The Lancet Diabetes & Endocrinology* **4**, 972-973 (2016).
9. Dunnick, N.R., *et al.* Localization of functional adrenal tumors by computed tomography and venous sampling. *Radiology* **142**, 429-433 (1982).
10. Sardi, A. & McKinnon, W. Laparoscopic adrenalectomy for primary aldosteronism. *Jama* **269**, 989-990 (1993).
11. Monticone, S., *et al.* Prevalence and Clinical Manifestations of Primary Aldosteronism Encountered in Primary Care Practice. *J Am Coll Cardiol* **69**, 1811-1820 (2017).
12. Preda, V.A., Sen, J., Karavitaki, N. & Grossman, A.B. Etomidate in the management of hypercortisolaemia in Cushing's syndrome: a review. *European Journal of Endocrinology* **167**, 137-143 (2012).
13. Fellows, I.W., Byrne, A.J. & Allison, S.P. Adrenocortical suppression with etomidate. *The Lancet* **322**, 54-55 (1983).
14. Bergstrom, M., *et al.* In vitro and in vivo primate evaluation of carbon-11-etomidate and carbon-11-metomidate as potential tracers for PET imaging of the adrenal cortex and its tumors. *Journal of Nuclear Medicine : official publication, Society of Nuclear Medicine* **39**, 982-989 (1998).
15. Bergström, M., *et al.* PET with [11C]-Metomidate for the Visualization of Adrenocortical Tumors and Discrimination from Other Lesions. *Clin Positron Imaging* **2**, 339 (1999).
16. Bergstrom, M., *et al.* PET imaging of adrenal cortical tumors with the 11beta-hydroxylase tracer 11C-metomidate. *Journal of Nuclear Medicine : official publication, Society of Nuclear Medicine* **41**, 275-282 (2000).

17. Abe, T., *et al.* A Novel CYP11B2-Specific Imaging Agent for Detection of Unilateral Subtypes of Primary Aldosteronism. *J Clin Endocrinol Metab* **101**, 1008-1015 (2016).
18. Burton, T.J., *et al.* Evaluation of the sensitivity and specificity of (11)C-metomidate positron emission tomography (PET)-CT for lateralizing aldosterone secretion by Conn's adenomas. *J Clin Endocrinol Metab* **97**, 100-109 (2012).
19. Soinio, M., *et al.* Functional imaging with 11C-metomidate PET for subtype diagnosis in primary aldosteronism. *European Journal of Endocrinology* **183**, 539-550 (2020).
20. Dekkers, T., *et al.* Adrenal vein sampling versus CT scan to determine treatment in primary aldosteronism: an outcome-based randomised diagnostic trial. *The Lancet Diabetes & Endocrinology* **4**, 739-746 (2016).
21. Williams, T.A., *et al.* Outcomes after adrenalectomy for unilateral primary aldosteronism: an international consensus on outcome measures and analysis of remission rates in an international cohort. *The Lancet Diabetes & Endocrinology* **5**, 689-699 (2017).
22. Williams, B., *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. *The Lancet* **386**, 2059-2068 (2015).
23. Brown, M.J., *et al.* Effect of amiloride, or amiloride plus hydrochlorothiazide, versus hydrochlorothiazide on glucose tolerance and blood pressure (PATHWAY-3): a parallel-group, double-blind randomised phase 4 trial. *The Lancet Diabetes & Endocrinology* **4**, 136-147 (2016).

24. MacDonald, T.M., *et al.* Combination Therapy Is Superior to Sequential Monotherapy for the Initial Treatment of Hypertension: A Double-Blind Randomized Controlled Trial. *J Am Heart Assoc* **6**(2017).
25. Intengan, H.D. & Schiffrin, E.L. Structure and Mechanical Properties of Resistance Arteries in Hypertension. *Hypertension* **36**, 312-318 (2000).
26. Rossi, G.P., *et al.* Vascular remodeling and duration of hypertension predict outcome of adrenalectomy in primary aldosteronism patients. *Hypertension* **51**, 1366-1371 (2008).
27. Gill, J.S., Zezulka, A.V., Beevers, D.G. & Davies, P. Relation between initial blood pressure and its fall with treatment. *Lancet (London, England)* **1**, 567-569 (1985).
28. Fernandes-Rosa, F.L., *et al.* A gain-of-function mutation in the CLCN2 chloride channel gene causes primary aldosteronism. *Nat Genet* **50**, 355-361 (2018).
29. Scholl, U.I., *et al.* CLCN2 chloride channel mutations in familial hyperaldosteronism type II. *Nat Genet* **50**, 349-354 (2018).
30. Zhou, J., *et al.* Somatic mutations of GNA11 and GNAQ in CTNNB1-mutant aldosterone-producing adenomas presenting in puberty, pregnancy or menopause. *Nat Genet* **53**, 1360-1372 (2021).
31. Azizan, E.A., *et al.* Somatic mutations in ATP1A1 and CACNA1D underlie a common subtype of adrenal hypertension. *Nat Genet* **45**, 1055-1060 (2013).
32. Azizan, E.A., *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* **97**, E819-829 (2012).
33. Monticone, S., *et al.* Immunohistochemical, genetic and clinical characterization of sporadic aldosterone-producing adenomas. *Mol Cell Endocrinol* **411**, 146-154 (2015).
 34. Akerstrom, T., *et al.* Novel somatic mutations and distinct molecular signature in aldosterone-producing adenomas. *Endocr Relat Cancer* **22**, 735-744 (2015).
 35. De Sousa, K., *et al.* Genetic, Cellular, and Molecular Heterogeneity in Adrenals With Aldosterone-Producing Adenoma. *Hypertension* **75**, 1034-1044 (2020).
 36. Kobuke, K., *et al.* Calneuron 1 Increased Ca(2+) in the Endoplasmic Reticulum and Aldosterone Production in Aldosterone-Producing Adenoma. *Hypertension* **71**, 125-133 (2018).
 37. Williams, T.A., *et al.* Teratocarcinoma-derived growth factor-1 is upregulated in aldosterone-producing adenomas and increases aldosterone secretion and inhibits apoptosis in vitro. *Hypertension* **55**, 1468-1475 (2010).
 38. Boulkroun, S., *et al.* Prevalence, clinical, and molecular correlates of KCNJ5 mutations in primary aldosteronism. *Hypertension* **59**, 592-598 (2012).
 39. Ye, P., Mariniello, B., Mantero, F., Shibata, H. & Rainey, W.E. G-protein-coupled receptors in aldosterone-producing adenomas: a potential cause of hyperaldosteronism. *J Endocrinol* **195**, 39-48 (2007).

40. Nishimoto, K., *et al.* Aldosterone-stimulating somatic gene mutations are common in normal adrenal glands. *Proc Natl Acad Sci U S A* **112**, E4591-4599 (2015).
41. Teo, A.E., *et al.* Physiological and Pathological Roles in Human Adrenal of the Glomeruli-Defining Matrix Protein NPNT (Nephronectin). *Hypertension* **69**, 1207-1216 (2017).
42. Tezuka, Y., *et al.* 18-Oxocortisol Synthesis in Aldosterone-Producing Adrenocortical Adenoma and Significance of KCNJ5 Mutation Status. *Hypertension* **73**, 1283-1290 (2019).
43. Williams, T.A., *et al.* Genotype-Specific Steroid Profiles Associated With Aldosterone-Producing Adenomas. *Hypertension* **67**, 139-145 (2016).
44. Eisenhofer, G., *et al.* Mass Spectrometry-Based Adrenal and Peripheral Venous Steroid Profiling for Subtyping Primary Aldosteronism. *Clin Chem* **62**, 514-524 (2016).
45. Silins, I., *et al.* Para-chloro-2-[(18)F]fluoroethyl-etomidate: A promising new PET radiotracer for adrenocortical imaging. *Int J Med Sci* **18**, 2187-2196 (2021).
46. Zarnegar, R., *et al.* The aldosteronoma resolution score: predicting complete resolution of hypertension after adrenalectomy for aldosteronoma. *Annals of Surgery* **247**, 511-518 (2008).
47. Utsumi, T., *et al.* Development of a novel nomogram to predict hypertension cure after laparoscopic adrenalectomy in patients with primary aldosteronism. *World Journal of Surgery* **38**, 2640-2644 (2014).

48. Burrello, J., *et al.* The Primary Aldosteronism Surgical Outcome Score for the Prediction of Clinical Outcomes After Adrenalectomy for Unilateral Primary Aldosteronism. *Annals of Surgery* (2019).
49. Kitamoto, T., *et al.* Comparison of Cardiovascular Complications in Patients with and without KCNJ5 Gene Mutations Harboring Aldosterone-producing Adenomas. *Journal of Atherosclerosis and Thrombosis* **22**, 191-200 (2015).
50. Mulatero, P., *et al.* 18-hydroxycorticosterone, 18-hydroxycortisol, and 18-oxocortisol in the diagnosis of primary aldosteronism and its subtypes. *J Clin Endocrinol Metab* **97**, 881-889 (2012).
51. Meyer, L.S., *et al.* Single-Center Prospective Cohort Study on the Histopathology, Genotype, and Postsurgical Outcomes of Patients With Primary Aldosteronism. *Hypertension* **78**, 738-746 (2021).
52. Nanba, K., *et al.* Genetic Characteristics of Aldosterone-Producing Adenomas in Blacks. *Hypertension* **73**, 885-892 (2019).
53. Omata, K., *et al.* Aldosterone-Producing Cell Clusters Frequently Harbor Somatic Mutations and Accumulate With Age in Normal Adrenals. *J Endocr Soc* **1**, 787-799 (2017).
54. Omata, K., *et al.* Cellular and Genetic Causes of Idiopathic Hyperaldosteronism. *Hypertension* **72**, 874-880 (2018).
55. Gros, R., Ding, Q., Liu, B., Chorazyczewski, J. & Feldman, R.D. Aldosterone mediates its rapid effects in vascular endothelial cells through GPER activation. *American Journal of Physiology. Cell Physiology* **304**, C532-540 (2013).

56. Funder, J.W. GPR30, mineralocorticoid receptors, and the rapid vascular effects of aldosterone. *Hypertension* **57**, 370-372 (2011).
57. Rossi, G.P., *et al.* An expert consensus statement on use of adrenal vein sampling for the subtyping of primary aldosteronism. *Hypertension* **63**, 151-160 (2014).
58. Young, W.F. & Stanson, A.W. What are the keys to successful adrenal venous sampling (AVS) in patients with primary aldosteronism? *Clinical Endocrinology* **70**, 14-17 (2009).
59. Strajina, V., *et al.* Primary aldosteronism: making sense of partial data sets from failed adrenal venous sampling-suppression of adrenal aldosterone production can be used in clinical decision making. *Surgery* **163**, 801-806 (2018).
60. Hinchliffe, E., Carter, S., Owen, L.J. & Keevil, B.G. Quantitation of aldosterone in human plasma by ultra high performance liquid chromatography tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* **913-914**, 19-23 (2013).
61. Williams, T.A., *et al.* International Histopathology Consensus for Unilateral Primary Aldosteronism. *J Clin Endocrinol Metab* **106**, 42-54 (2021).
62. Livak, K.J. & Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **25**, 402-408 (2001).
63. Bancos, I., *et al.* Urine steroid metabolomics for the differential diagnosis of adrenal incidentalomas in the EURINE-ACT study: a prospective test validation study. *The Lancet Diabetes & Endocrinology* **8**, 773-781 (2020).

64. Sagmeister, M.S., *et al.* Glucocorticoid activation by 11beta-hydroxysteroid dehydrogenase enzymes in relation to inflammation and glycaemic control in chronic kidney disease: A cross-sectional study. *Clinical Endocrinology* **90**, 241-249 (2019).

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Author contributions

M.J.B., M.G. and W.M.D. conceived and designed the study. X.W., E.G., R.S., W.B., G.A., S.M.O., K.L., E.A. and J.S. ran the study and collected the data, under the supervision of M.J.B., M.G. and W.M.D. and K.C. J.S. was Trial Coordinator. AVS was performed by M.M., B.K. and N.H.; N.B., I.B., S.H., V.W., D.G. and F.A. supervised and coordinated MTO synthesis and PET-CT scanning. Adrenalectomies were performed by L.P. and V.K. MTO PET-CT grading was performed by H.C., with further input at MDT from A.S., M.J.B., M.G. and W.M.D. IHC grading was performed by D.M.B. and A.M. RNA sequencing was performed by E.W. and E.S., analysis by C.A.M., C.P.C., W.C. and R.F. 24 hour urine steroid profiling and analysis was performed by A.D., A.P., A.E.T. and W.A. The CMR sub-study was conducted by A.N.B. and supervised by C.M. Statistical analysis was performed by A.M. and A.McC. The manuscript was prepared by X.W., M.J.B., W.M.D. and M.G., with significant input on revisions from E.G., S.M.O., A.P., K.C., W.A. and C.M.

Figures

Figure 1

Study schema, hierarchical co-primary endpoints and consort diagram.

a. Study schema. Participants with confirmed primary aldosteronism (PA) according to the Endocrine Society consensus guidelines underwent both AVS (adrenal vein sampling) and MTO (11 C Metomidate PET CT) in random order. All participants were subsequently reviewed at a 'Baseline Visit' and started on spironolactone 50mg, which was up titrated to 100mg after two weeks. BP response to spironolactone was recorded at two and four weeks from initiation of spironolactone. Eplerenone was used in those previously intolerant of spironolactone. Concurrently, the results of both investigations were reviewed at MDT (multidisciplinary team) meetings where MTO was always reviewed first, followed by AVS. A score of 'high', 'intermediate' or 'low' probability of unilateral PA was assigned to each investigation. Recommendations for unilateral adrenalectomy were made if either investigation indicated 'high' probability of unilateral PA, or in a small number of patients, where both investigations indicated 'intermediate' probability and there was a clinical indication for surgery eg : because of drug intolerance or uncontrolled BP). b. Hierarchical co-primary endpoints. Schema showing definitions of partial and complete biochemical or clinical cure as defined by the PASO consensus, 21 and the hierarchical order in which each definition of cure post adrenalectomy was

assessed. c. Consort diagram showing disposition of study participants. Note adrenalectomy was undertaken in 79 participants, of which 78 had evaluable primary outcome data (assessed at 6 months post surgery or after 9 12 months of medical therapy with spironolactone). Both biochemical and clinical primary outcome data was available in 77/78 participants. Only clinical primary outcome data was available for the final participant.

K, potassium; ARR, aldosterone renin ratio; BP, blood pressure; DDD

defined daily dose

Figure 2

Primary Outcomes

a. Results from MTO and AVS. Number of participants in whom MTO or AVS identified unilateral primary aldosteronism (PA). 'Intermediate' suggests some asymmetry in the adrenals but not meeting criteria for unilateral disease. 'Failed' indicates unsuccessful cannulation of adrenal vein(s) and therefore inability to

interpret AVS result. [i] All participants in full analysis set who had outcomes from MDT discussion n=141, excluding 2 participants who had both investigations but had not yet been discussed at MDT [ii] Participants who reached the primary endpoint time-point (6 or 9-12 months post surgery or medical therapy respectively) n=128. Note proportion of participants in [i] and [ii] are similar. b. MDT decisions. Number of participants recommended for surgery or medical therapy based on outcomes from MTO, AVS, both or neither investigations in [i] full analysis set and [ii] those who reached primary endpoint time-point. Data excludes 7 patients where decision for surgery were 'deferred', due to discordant results from AVS and MTO. c. Outcomes from adrenalectomy. Number of participants achieving each combination of clinical and biochemical outcomes post surgery, n=78. *Note n=78 for clinical cure, n=77 for biochemical cure as post-op biochemical data was not available for one participant.

Complete clinical cure was achieved in this participant. d. Cure rate by investigations. Percentage of participants who underwent surgery who achieved each of the four definitions of cure (as outlined by the PASO consensus) and the proportion of patients where this was based on outcomes from AVS alone (green bar), MTO alone (orange bars) or both investigations (purple bars). e. Forest

plot of hierarchical co primary outcomes. The lower bounds confidence interval for all four definitions of cure are above the pre set inferiority margin of 17%, suggesting MTO is non inferior to AVS in predicting unilateral PA. n=77 for biochemical cure data, n=78 for clinical cure data.

Figure 3.

Figure 3

Secondary Outcomes: Pre- and post-intervention outcomes

a. Reduction in aldosterone levels and b. increase in renin levels is observed post-surgery and with medical therapy c. Effect of each intervention on the ARR (aldosterone renin ratio): ARR is reduced post surgery but remains unchanged with medical therapy. Data are least squares means (95% CI) from mixed

effects models adjusted for baseline covariates. d. Reduction in systolic and diastolic BP post treatment. All effects seen were sustained for at least 2 years. Data are least squares means (95% CI) from mixed effects models adjusted for baseline covariates.

Figure 4

Predictors of clinical outcome from surgery.

Influence of a. age and b. gender on clinical outcomes post adrenalectomy. Younger age and female gender are associated with higher likelihood of complete clinical cure. c. Baseline systolic BP (SBP) and clinical outcomes post adrenalectomy. Each dot represents a single participant, which is colour coded to indicate the individual's ethnicity. d. Number of patients achieving each clinical outcome by ethnicity. An absence of clinical cure (shown in blue) is significantly higher in patients who are Black compared to other ethnicities. e. Correlation of fall in SBP after 4 weeks of spironolactone therapy and fall in SBP at 6 months post surgery. SBP shown as adjusted percentage change, which was calculated with Oldham's correction²⁷ (corrected change in BP [%]=100 x actual change in BP divided by the average of baseline and post-treatment pressure). f. Predictions of clinical outcomes by SBP after 1 month of spironolactone treatment. All participants who's SBP dropped to <135 mmHg after 4 weeks of spironolactone achieved either partial or complete clinical cure. Achieving SBP <135 mmHg after 1 months of spironolactone was the best predictor of complete clinical cure with odds ratio 13.0 (3.72, 45.24) p<0.001.

genotype. Note mRNA expression of APA shown as normalised read count on RNA sequencing. c. Intensity of CYP11B1 and CYP11B2 expression of APAs on IHC staining was quantified using the H-score system.⁶¹ The average score for each

genotype show differences between KCNJ5-mutant and other APAs, n=21. H-scoring only performed on APAs of known gene mutations from Cambridge University Hospital (CUH). d. Two illustrative cases demonstrating selective ¹¹C metomidate uptake in participants with two or more adrenal adenomas. [i] Participant 1 has two adenomas on their adrenal, shown in separate axial MTO images (top panels). The first nodule is MTO avid while the second is 'cold'. IHC staining of the two nodules show strong positive staining for CYP11B2 in nodule 1 while nodule 2 is negative for CYP11B2 (middle panels). IHC staining for CYP11B1 is positive in both nodules (bottom panels). d. Two illustrative cases demonstrating selective ¹¹C-metomidate uptake in patients with two or more adrenal adenomas. [ii] Participant 2 had multiple adrenal nodules, only one of which (nodule 1) demonstrated high MTO uptake on MTO PET-CT (top panels). Left and right middle panels show high power views of participant 2's adrenal with CYP11B2 and CYP11B1 IHC staining respectively. Bottom panel: RNA sequencing results showing mRNA expression for CYP11B2 and CYP11B1 in two of nodules identified. APA, aldosterone producing adenoma; AAG, adjacent adrenal gland; IHC, immunohistochemistry; NFA, nonfunctioning adenoma; APCC, aldosterone producing cell cluster. e. Heat map representation of differentially expressed genes between APAs and NFAs, and between genotypes. Each column represents the expression profile of the APA or NFA. Both genes and individual APAs are hierarchically clustered. Three nodes of interest are shown: shown:[i] node containing CYP11B2 ;[ii] and [iii] nodes showing most upregulated genes in CACNA1D mutant and KCNJ5 mutant APAs respectively. The CYP11B2 nodes includes several genes previously noted to be upregulated in APAs, e.g. VSNL1, CALN1 . Note genes which are significantly upregulated in APCCs are also seen in this node: TMEM266 (TMEM266 (C15ORF27), PPP1R16B, SEMA3D, KIAA1549L (C11ORF41), CFAP221 (PCDP1). Red represents upregulation and blue represents downregulation of genes. APA, aldosterone producing adenoma; AAG, adjacent adrenal gland; IHC, immunohistochemistry; NFA, non functioning adenoma; APCC, aldosterone producing cell cluster.

Figure 6.

a. n=58 Clinical cure

Fisher exact p value <0.001

KCNJ5 v Other
Univariate Proportional Odds Model
Odds Ratio of going up 1 level (95% CI)
10.37 (2.50, 42.99) p=0.001

CACNA1D v Other
Univariate Proportional Odds Model
Odds Ratio of going up 1 level (95% CI)
0.38 (0.11, 1.36) p=0.014

Brant p value = 0.44

b. Logistic regression analysis (complete clinical cure)

Variable	OR (95% CI) P-value
Explanatory Variables at Baseline	
Gene: <i>KCNJ5</i> (c.w. Other Mutations)	11.12 (2.11 , 75.68) ; p=0.007
Gene: <i>CACNA1D</i> (c.w. Other Mutations)	0.79 (0.12 , 5.05) ; p=0.798
Age: Decades from mean	0.94 (0.86 , 1.02) ; p=0.145

c. n=37

Figure 6

a. Number of participants achieving each definition of clinical cure by genotype. b. Results from logistic regression analysis of variables predicting complete clinical cure. Note the two participants with double GNAQ/CTNNB1 mutations were excluded from this analysis since 16/16 patients with this double mutation were previously reported to be completely cured.³⁰ c.w., compared with. c. Urine steroid profile (18 hydroxycortisol cortisol ratio) at baseline by genotype.

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