Original Article

KCNJ5 gene somatic mutations affect cardiac remodelling but do not preclude cure of high blood pressure and regression of left ventricular hypertrophy in primary aldosteronism

Gian Paolo Rossi[a], Maurizio Cesari[a], Claudio Letizia[b], Teresa M. Seccia[a], Maria Verena Cicala[c], Laura Zinnamosca[b], Maniselvan Kuppusamy[a], Sara Mareso[a], Susanna Sciomer[d], Maurizio Iacobone[e], Franco Mantero[c], and Achille C. Pessina[a]

Objective: Aldosterone exerts detrimental cardiovascular effects, and patients with an aldosterone-producing adenoma (APA) carrying somatic mutations in the KCNJ5 K^+ channel (mutAPA) have higher plasma aldosterone concentration than wild-type APA (wtAPA) patients. We therefore investigated whether mutAPA patients develop a more prominent cardiovascular damage than wtAPA patients.

Methods and findings: From 257 consecutive primary aldosteronism patients, we identified 176 who had both a diagnosis of APA by the 'four corners' criteria and high-quality echocardiographic data. Of them, 129 with KCNJ5 sequencing information and long-term follow-up data were compared for echocardiographic changes according to presence (mutAPA, 26%) or absence (wtAPA, 74%) of the KCNJ5 mutations. At baseline, the mutAPA were similar to the wtAPA for blood pressure (BP) and need for antihypertensive medications. However, they had higher left ventricular mass index (59 ± 19 vs. 51 ± 13 g/h$^{2.7}$; $P < 0.05$) and plasma aldosterone concentration [49 (32–68) vs. 36 (25–52) ng/dl); $P = 0.048$] than the wtAPA patients. In spite of their more prominent cardiac involvement, the mutAPA patients exhibited a fall of BP and plasma aldosterone similar to wtAPA, and a regression of left ventricular mass index.

Conclusions: Compared to the wild-type APA patients those with KCNJ5 mutations showed more prominent cardiovascular damage. Notwithstanding this, their chances of being cured from the hyperaldosteronism and the high BP, and of regression of left ventricular hypertrophy after adrenalectomy, were not compromised by the presence of these mutations.

Keywords: adrenal, aldosteronism, cardiac hypertrophy, gene mutations, potassium channel KCNJ5

Abbreviations: APA, aldosterone-producing adenoma; KCNJ5, Kir 3.1 potassium channel; LV, left ventricle; LVH, left ventricular hypertrophy; LVM, left ventricular mass; LVMI, left ventricular mass index; mutAPA, APA patients with KCNJ5 mutations; preLVM, predicted value of left ventricular mass; RWT, relative wall thickness; wtAPA, wild-type APA patients

INTRODUCTION

The molecular mechanisms of primary aldosteronism, a common curable cause of high blood pressure (BP) [1], were unknown until Choi *et al.* [1] identified three mutations (G151R, L168R and T158A) in the selectivity filter of the KCNJ5 (Kir 3.4) potassium channel, which caused enhanced Na^+ influx and cell membrane depolarization. At least one of such mutations, the T158A, was thereafter shown to enhance aldosterone production *in vitro* [2]. In large surveys of aldosterone-producing adenoma (APA), these mutations were detected at the somatic level in about one-third of the cases [3–5], indicating that they are not uncommon. They even seem to be more common in Asia where they can involve the majority of APA patients, as shown by a prevalence of 59% in China (Professor Zeng, personal communication) and of 65% in Japan [5]. The germ-line mutations (T158A, G151R and G470T) initially reported to cause severe drug-resistant hypertension requiring bilateral adrenalectomy are, by contrast, exceedingly rare [1,6]. Moreover, they can exhibit a milder adrenal phenotype featuring drug-responsive hypertension notwithstanding a similarly increased Na^+ conductance, as shown for the G151E mutation found in two

Journal of Hypertension 2014, 32:1514–1522

[a]Department of Medicine-DIMED, Internal Medicine 4, University of Padua, Padua, [b]Internal Medicine and Medical Specialities, 'La Sapienza' University, Rome, [c]Department of Medicine-DIMED, Endocrinology, University of Padua, Padua, [d]Department of Cardiovascular and Respiratory Sciences, 'La Sapienza' University, Rome and [e]Minimally Invasive Endocrine Surgery Unit, Department of Surgery, Oncology and Gastroenterology, University of Padua, Padua, Italy

Correspondence to Professor Gian Paolo Rossi, MD, FACC, FAHA, DIMED – Internal Medicine 4, University Hospital, Via Giustiniani, 2, 35128 Padua, Italy. Tel: +39 049 821 2279, or 7821; fax: +39 049 821 7873; e-mail: gianpaolo.rossi@unipd.it

Received 19 October 2013 **Revised** 25 February 2014 **Accepted** 25 February 2014

J Hypertens 32:1514–1522

DOI:10.1097/HJH.0000000000000186

kindred [7,8]. By far, the vast majority of the KCNJ5 mutations operate within the tumour tissue [3,4] (for review see ref. [9]) where they sustain hyperaldosteronism, as documented with measurement of both CYP11B2 mRNA in the APA and the hormone in the venous effluent from the APA side [10]. Through this mechanism, they can maintain excess constitutive aldosterone production in spite of the suppression of the rennin–angiotensin system, the high BP, and the hypokalemia – all factors that by themselves should blunt aldosterone secretion.

When coupled with a high salt intake, hyperaldosteronism detrimentally affects the cardiovascular system [11,12], and causes an excess rate of cardiovascular events [13–16]. We therefore sought to determine if APA patients with KCNJ5 mutations develop more cardiovascular damage than APA patients without such mutations.

METHODS

Study design and patients

We recruited consecutive primary aldosteronism patients among those referred to the Specialized Centres for Hypertension of the University of Padua and Rome, who were submitted to adrenalectomy, according to the current guidelines recommendations [17], and had high-quality echocardiography data at baseline and follow-up. The patients' refusal to undergo adrenalectomy and/or contraindications to the general anaesthesia required for laparoscopic adrenalectomy was the only exclusion criterion. The protocol followed the principles of the Declaration of Helsinki and the institutional guidelines. An informed written consent was obtained from each participant.

Diagnostic criteria

The diagnosis of APA was based on the following 'four corners' criteria [18], as follows: biochemical diagnosis of primary aldosteronism, lateralization of aldosterone secretion at bilaterally selective AVS or NP59 scintigraphy, evidence of adrenocortical nodule at histopathology, and cure or improvement of hypertension, and correction of the biochemical picture of primary aldosteronism at follow-up after adrenalectomy.

KCNJ5 mutations

DNA was extracted from APA tissue using QIAquick DNA purification kit (Qiagen, Courtaboeuf Cedex, France) and quantified with Nanodrop 2000c spectrophotometer (Thermoscientific, Wilmington, Delaware, USA). PCR was performed on 250 ng DNA in a final volume of 50 μl containing 300 nmol/l $MgCl_2$, 400 nmol/l of each primer, 200 μmol/l deoxynucleotide triphosphate and 2.6 U expand high-fidelity enzyme mix (Roche Applied Science, Monza, Italy). Direct sequencing of PCR products spanning amino acids 122–199 was performed using the ABI Prism Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California, USA) on an ABI Prism 3700 DNA Analyser (Applied Biosystems, Carlsbad, California, USA).

Phenotypic assessment

Blood pressure was measured according to guidelines [19]; the mean of three measurements taken in the supine position at least 3 min apart from one another was used. Plasma renin activity (PRA) and plasma aldosterone concentration (PAC) were measured after 1 h in the supine position and again 45 min after administration of 50 mg captopril p.o. Serum K^+ levels, PRA, and PAC were measured as described [20]. The cross-reactivity of the antibody against aldosterone for the other steroids was below 0.001%; normal ranges, and intra-assay and inter-assay coefficients of variation were for PRA 0.65–2.64 ng/ml per h, and 8 and 10%; for PAC 1–15 ng/dl, and both less than 5.6% [20].

Echocardiography

M-mode and two-dimensional (2D)-echocardiography was performed in all patients by experienced cardiologists (M.C. and S.S.), blinded to the cause of high BP and on-going medical therapy, as described [16]. The left ventricular mass index (LVMI), estimated according to Devereux *et al.* [21], was indexed to height$^{2.7}$. Relative wall thickness (RWT) was calculated at end-diastole left ventricular geometry, as RWT = (inter-ventricular septum thickness + posterior wall thickness)/left ventricular diameter. At both the Padua and Rome laboratory, the between-assessment coefficient of variation was below 4% for both LVMI and RWT.

The criteria for left ventricular hypertrophy (LVH) were LVMI above 50 g/m$^{2.7}$ for men and 47 g/m$^{2.7}$ for women [21,22]; in both sexes, a RWT at least 0.42 and below 0.42, respectively, identified concentric and eccentric LVH [23,24]. Left ventricular end-diastolic and end-systolic volumes were calculated with the Teicholz's correction of the cube formula [25]. Left ventricular ejection fraction was calculated by standard methods [23]. Stroke work was estimated as SBP (measured after the echocardiographic study) times stroke volume and converted into gram-meters by multiplying by 0.0144 as described [16].

The predicted value of left ventricular mass (preLVM), which provides an estimate of the left ventricular mass expected for cardiac workload and height$^{2.7}$ (used as surrogate for genetically programmed lean body mass for that height) and sex, was calculated using an indicator variable for sex, height$^{2.7}$, and stroke work (as a measure of cardiac workload), as follows:

PreLVM = 55.37 + 6.64 × height (m$^{2.7}$) + 0.64 × stroke work − 18.07 × sex (where sex was coded as male = 1 and female = 2) [23,24].

The observed LVM divided by the preLVM expressed as a percentage (observed LVM/predicted LVM × 100) was categorized as inappropriate as reported [16,23,24].

Statistical analysis

Results are expressed as mean and SD, or median and interquartile range, as appropriate. Due to skewed distribution, PRA and PAC values were examined after log transformation. Paired and unpaired *t* test was used to compare the patients' values before and after adrenalectomy, and between mutAPA and wtAPA, respectively.

An exploratory analysis showed differences of sex, PAC, and other variables between groups; thus, a GLM univariate procedure with a backward (Wald) stepwise linear regression and analysis of variance (with age, PAC at baseline, and sex as co-variables, and KCNJ5 mutation status as factor) was used to search for predictors of LVMI and for

their interactions. Unadjusted and predictors-adjusted LVMI values were compared between mutAPA and wtAPA. When an overall *F* test showed significance, differences between specific means were assessed by Bonferroni's post-hoc test. Estimated marginal means of predicted mean values in the GLM model were calculated; an interaction plot of these means was determined to visualize the relationships. Statistical analysis was performed using SPSS 20.0 for Mac (SPSS Inc., Bologna, Italy); significance was defined as *P* less than 0.05 (two-sided).

RESULTS

From 257 consecutive primary aldosteronism patients, 10 were excluded for poor-quality echocardiography data, and 71 because they did not undergo adrenalectomy as primary aldosteronism could not be unequivocally attributed to a clinically recognizable APA. In the 176 adrenalectomized APA patients, the tumour tissue was available for KCNJ5 genotyping in 129 patients, in whom the presence or absence of the KCNJ5 somatic mutations could be conclusively determined (Fig. 1). One novel KCNJ5 mutation consisting of a 149Tins, which was found to be similar to those already known from the electrophysiological standpoint, was also found (data submitted). The biochemical and echocardiographic data of the APA patients without KCNJ5 genotypes were also examined as a reference group to determine what might have occurred. In three patients, adrenalectomy did not result in correction of the hyperaldosteronism; therefore they were judged to have bilateral adrenal hyperplasia (BAH). Two of them were genotyped and showed no KCNJ5 mutations; one could not be genotyped. All three were excluded from the analysis per protocol, because BAH was not found to be associated with somatic KCNJ5 mutations (Fig. 1) [3].

Most of the patients recruited between 2006 and 2013 were part of the multicentre survey on KCNJ5 mutations prevalence carried out within the European Network for the Study of Adrenal Tumours (ENS@T) [3]; those of the Padua cohort were also examined in a study on the impact of these mutations on adrenal vein sampling results [10]. For the present study, 67 patients were recruited in Rome and 60 in Padua.

Features of aldosterone-producing adenoma with KCNJ5 mutations

The G151R and L168R mutations were found in 14.9 and 7.9% of APA, respectively; no G151E or T158A mutations were found. The overall prevalence of the KCNJ5 mutations was 24.4% (20.9% in Rome, 28.3% in Padua); it was higher in women than in men (33.3 vs. 15.6%; $\chi^2 = 5.39$, $P = 0.024$).

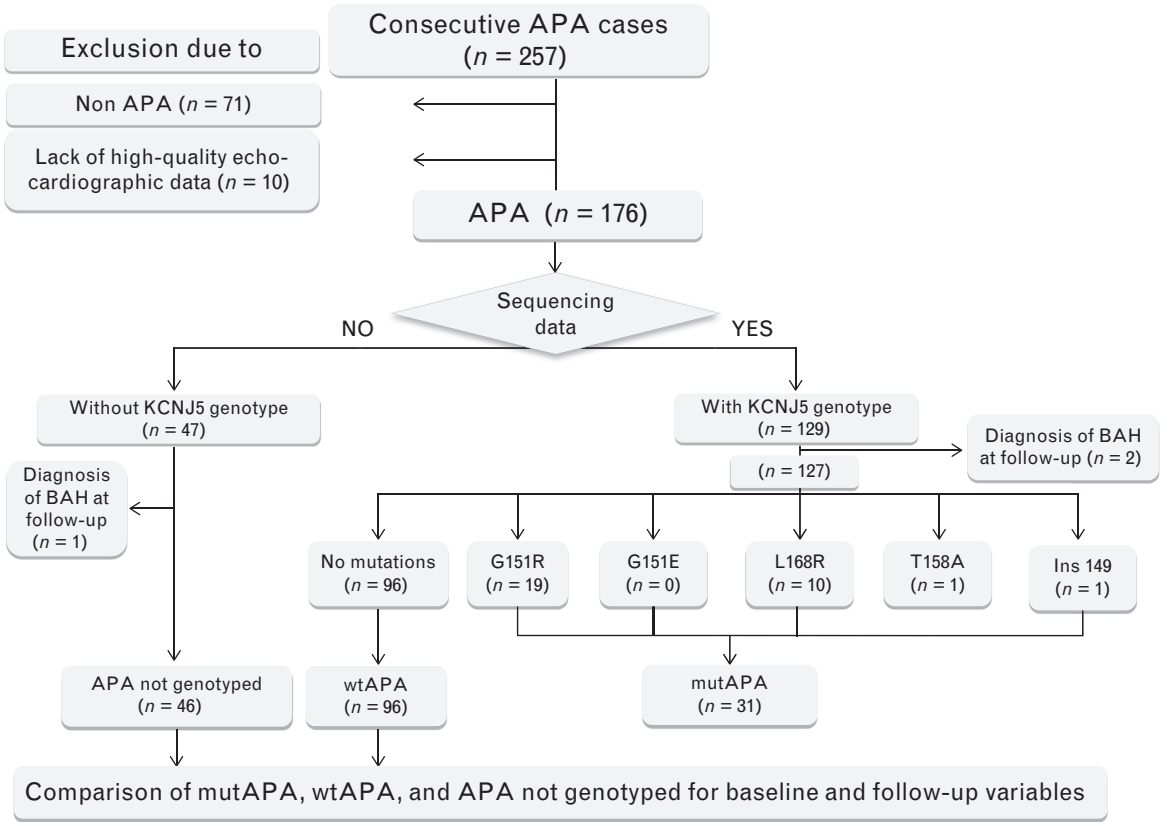

FIGURE 1 Flowchart of the study. Consecutive patients with primary aldosteronism were initially evaluated. Patients without conclusive evidence for an APA were not eligible for search of somatic KCNJ5 mutations as they did not undergo adrenalectomy. Those without unequivocal information on the presence or absence of the KCNJ5 mutations, and those without high-quality echocardiographic data, were excluded from the downstream analysis. Likewise three patients without correction of the hyperaldosteronism after adrenalectomy, who were judged to have bilateral adrenal hyperplasia (BAH), were excluded from further analysis because BAH was not found to be associated with somatic KCNJ5 mutations. Therefore, 127 APA patients with full APA DNA sequencing and echocardiographic data were recruited. Comparison between mutAPA and wtAPA KCNJ5 mutations, and between mutAPA and APA without KCNJ5 genotype data, as a large reference group, was also performed for all the variables of interest to rule out a selection bias. APA, aldosterone-producing adenoma; mutAPA, APA carrying somatic KCNJ5 mutations; wtAPA, wild-type APA.

TABLE 1. Clinical and biochemical features at baseline and follow-up in adrenalectomized aldosterone-producing adenoma (APA) patients with (mutAPA) or without (wild-type, wtAPA) somatic KCNJ5 mutations, and in APA patients not genotyped

Variable	mutAPA ($n=31$)			wtAPA ($n=96$)			APA not genotyped ($n=46$)		
	Baseline	Follow-up	P (two-tailed)	Baseline	Follow-up	P (two-tailed)	Baseline	Follow-up	P (two-tailed)
Sex (m/f, %)	32/68	–	–	56/44**	–	–	67/33**	–	–
BMI (kg/m^2)	25.5 ± 4.7	25.8 ± 4.6	0.581	26.9 ± 4.8	27.1 ± 4.8	0.337	26.6 ± 4.4	27.1 ± 4.8	0.185
DBP (mmHg)	95 ± 11	80 ± 8	<0.0001	95 ± 10	83 ± 8	<0.0001	101 ± 12*	86 ± 9*	<0.0001
Antihypertensive treatment (number of drugs)	2.5 ± 1.3	1.6 ± 1.2	0.008	2.7 ± 1.6	1.8 ± 1.7	<0.0001	2.5 ± 1.1	1.8 ± 1.2	<0.0001
Serum K$^+$ (mmol/l)	3.1 ± 0.5	4.2 ± 0.5	<0.0001	3.3 ± 0.6	4.2 ± 0.3	<0.0001	3.3 ± 0.5	3.9 ± 0.4	<0.0001
PRA (ng/ml/h)	0.36 (0.21–0.48)	2.65 (0.80–4.20)	0.002	0.20 (0.20–0.57)	1.30 (0.66–2.26)	0.001	0.35 (0.20–0.70)	2.05 (0.86–4.05)	0.001
PAC (ng/dl)	49 (32–68)	8 (5–17)	<0.0001	36 (25–52)*	11 (6–15)	<0.0001	31 (21–56)	12 (10–21)	0.001
ARR (ng/dl)/(ng/ml per h)	158 (70–219)	5 (2–9)	0.025	142 (65–277)	8 (7–11)	<0.0001	93 (40–250)	6 (4–12)	0.035

APA, aldosterone-producing adenoma; ARR, aldosterone-to-renin ratio; PAC, plasma aldosterone concentration; PRA, plasma renin activity.
The APA not genotyped differed from the mutAPA and the wtAPA only for higher DBP at baseline ($P<0.05$).
Mean ± SD, or median (25th–75th percentile), except than for known duration of hypertension for which the 95% confidence interval is reported.* $P<0.05$, **$P<0.01$ vs. mutAPA.

Table 1 and Fig. 2 show the anthropometric and BP data of the mutAPA and wtAPA patients at baseline and postadrenalectomy at last available follow-up. As expected by definition, all groups had low PRA and raised PAC with ensuing marked elevation of the aldosterone-to-renin ratio (ARR) [26].

Compared to wtAPA patients, the mutAPA patients had higher PAC and ARR values, albeit the latter did not attain statistical significance. They were similar for serum K$^+$, PRA, and known duration of hypertension (all $P=$NS). The recruited APA patients were similar to a group of APA patients who could not be genotyped for demographic and biochemical variables (all $P=$NS), with the exception of a slightly lower DBP. Therefore, they likely are a representative sample of the Caucasian patients with APA.

Effects of KCNJ5 mutations on cardiac remodelling

All APA patients had a normal systolic function, as shown by the normal values of left ventricular ejection fraction (Table 2). At baseline, the mutAPA patients showed an increase of LVMI compared to the wtAPA and the APA not genotyped (Table 2 and Fig. 3). This was due to both an outward type of remodelling, for example, to left ventricular cavity dilatation, and to thickening of the left ventricular walls (Table 2). Accordingly, the mutAPA patients had a higher prevalence of LVH than the wtAPA patients (74 vs. 52%; $\chi^2=4.84$, $P=0.002$). Among those with LVH, the concentric type predominated in both the mutAPA (91.3%) and in the wtAPA (70.2%) ($P<0.05$). The mutAPA tended to have a higher rate of inappropriate LVM, for example, a LVM disproportionally increased for sex and for the prevailing cardiac workload than the wtAPA patients (56.5 vs. 44.7%; NS), albeit this difference did not reach significance. Thus, even though the overall prevalence of LVH was high (55.3%) in the entire cohort, there were major differences between APA patients with and without KCNJ5 mutations. In spite of these left ventricular differences, left atrium and aortic root diameter, their ratio, and all indices of left ventricular diastolic filling, including the ratio of early (E wave) over presystolic filling peak flow velocity (A wave), atrial contribution to left ventricular filling, and tissue Doppler peak early diastolic mitral inflow/annular velocity (E/E') ratio showed only minimal differences between mutAPA and wtAPA patients (Table 2).

Predictors of left ventricular mass index

A regression model with sex, baseline age, KCNJ5 status, and PAC predicted LVMI ($F=6.41$, $P<0.0001$), the strongest predictors being sex and KCNJ5 status. The latter two variables significantly interacted ($F=4.41$, $P=0.038$) in determining LVMI (Table 3 and Fig. 4). Even after adjustment for these variables, the differences of LVMI between mutAPA and wtAPA remained significant.

Clinical and echocardiographic data at follow-up

At follow-up (median 38 months; range 3–225 months) in both the mutAPA and the wtAPA patients, serum K$^+$ and PRA increased, whereas PAC and the ARR normalized, indicating a 100% hormonal cure rate (Table 1). These changes were seen also in the APA patients not genotyped.

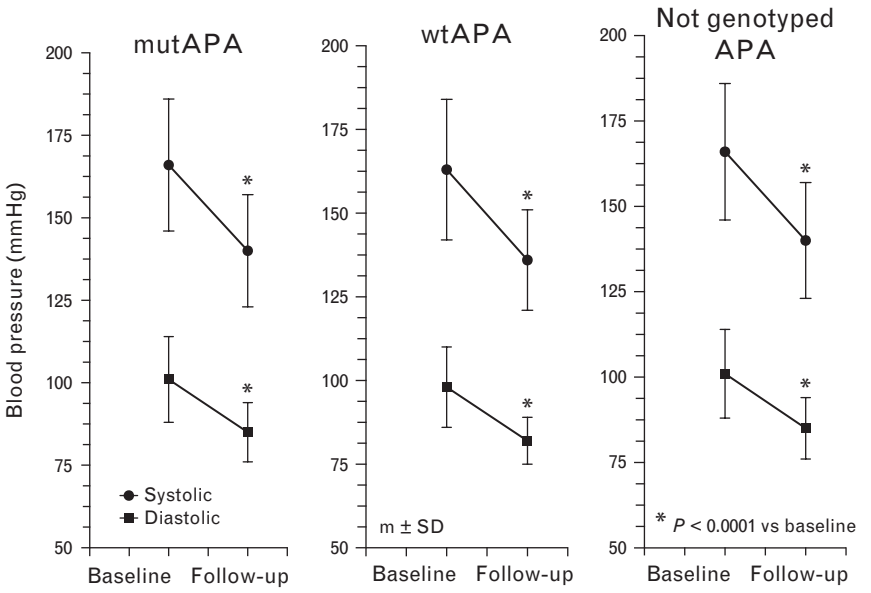

FIGURE 2 The plot shows the mean (± SD) values of SBP and DBP at baseline, and at long-term follow-up in the APA with (mutAPA) and without (wtAPA) somatic KCNJ5 mutations. A large group of APA patients in whom the KCNJ5 mutations could not be determined is shown for comparison purposes. Please note that the fall in both SBP and DBP, of the adrenalectomy, albeit highly significant, was similar in all groups.

Both the mutAPA and the wtAPA patients exhibited a similar marked decrease of SBP and DBP (Table 1 and Fig. 2) despite a tapering of number and doses of the antihypertensive agents. High BP was long-term cured, as defined by normotension with no antihypertensive drugs, in 57% of the mutAPA and in 52% of the wtAPA ($\chi^2 = 0.67$, NS), respectively; in the rest, it was markedly improved. All changes did not differ between the follow-up evaluation within 1 year and at long term (not shown).

The nadir of LVMI fall was seen after 1 year follow-up in the mutAPA patients: at the last available follow-up the fall in LVMI was more marked in the mutAPA than in the wtAPA patients (delta LVMI: -8.0 ± 2.5 vs. -1.8 ± 1.0 g/m^2; $P = 0.007$), which accounted for the fall in the prevalence of LVH (Fig. 5). In the mutAPA, the regression of LVH occurred mostly through an inward left ventricular remodelling, since the inter-ventricular septum changed slightly and the posterior wall thickness did not decrease significantly. The left atrium and aortic root diameter and their ratio did not change significantly in any groups, but the atrial contribution to left ventricular filling fell significantly in the mutAPA group only (Table 2). The fall of LVMI at follow-up was predicted by the KCNJ5 status ($F = 4.50$, $P = 0.036$), but not by sex, age, baseline PAC, and SBP.

DISCUSSION

The left ventricle (LV) adapts to the increased afterload of patients with high BP by developing hypertrophy (LVH), which predicts cardiovascular events [27] and should be regressed to improve prognosis [24]. Aldosterone is key in LVH development: in the setting of a high sodium intake apart from affecting pre-load and after-load, it augments the effects of angiotensin II on AT-1 receptors [28,29], and also causes inflammation and fibrosis by promoting oxidative stress and enhancing collagen type I and III gene transcription and fibroblast proliferation [28]. Altogether, these actions can explain both the worse prognosis of patients with hyperaldosteronism compared to essential hypertensive patients [15,30], and the survival benefit conferred by MR antagonists on patients with left ventricular dysfunction [31–33].

In a proportion of the APA patients, the KCNJ5 mutations imply opening of voltage-activated Ca^{2+} channels [1,2] and Ca^{2+} influx leading to enhanced CYP11B2 expression and a constitutive over-secretion of aldosterone from the tumour, as shown using adrenal vein sampling and ex-vivo gene expression studies [10]. Given that KCNJ5 mutations cause more marked hyperaldosteronism, we hypothesized that APA patients carrying the mutations could develop more cardiac damage, including LVH, and exhibit a lower chance of cure after adrenalectomy than wtAPA patients. To test this hypothesis, we used echocardiography and follow-up observation in a relatively large cohort of APA patients, who were searched for mutations in the KCNJ5 potassium channel. The vast majority of them had a normal left ventricular systolic function at baseline. They were submitted to long-term follow-up to determine the impact of the mutation and its removal on regression of LVH and rate of cure of high BP.

The first important finding of this study is that the mutAPA patients had a greater LVM and a higher rate of LVH than their wtAPA counterpart (Fig. 5, Table 2), indicating a more marked cardiac damage [11]. This would imply *per se* a higher likelihood of persistent high BP after surgical correction of the hyperaldosteronism [11], which did not seem to be the case. In fact, a second important finding that contradicts this expectation was the observation that the KCNJ5 somatic mutations did not extend their detrimental effect after adrenalectomy: a similar consistent correction of the hyperaldosteronism, along with a prominent fall of both SBP and DBP, and a lowered need for antihypertensive drugs, was found in the mutAPA and in the wtAPA patients (Table 1). In all patients of this cohort, the cure rate of hypertension was very high, likely because

TABLE 2. Echocardiography and Doppler variables at baseline and follow-up in adrenalectomized aldosterone-producing adenoma (APA) patients with (mutAPA) or without (wild-type, wtAPA) somatic KCNJ5 mutations, and in APA patients not genotyped

Variable	mutAPA ($n = 31$)			wtAPA ($n = 96$)			APA not genotyped ($n = 46$)		
	Baseline	Follow-up	*P* (two-tailed)	Baseline	Follow-up	*P* (two-tailed)	Baseline	Follow-up	*P* (two-tailed)
LVEDD (mm)	50.6 ± 6.4	48.6 ± 4.1	0.039	49.6 ± 4.9	48.9 ± 4.3	0.015	49.4 ± 4.6	47.9 ± 4.3	0.001
IVSd (mm)	12.2 ± 2.1	11.4 ± 1.8	0.025	11.4 ± 1.4*	11.5 ± 1.6	0.855	12.1 ± 2.3	11.6 ± 1.6	0.151
PWd (mm)	11.5 ± 2.1	10.9 ± 1.7	0.046	11.1 ± 1.9	10.9 ± 1.4	0.171	11.4 ± 1.9	11.1 ± 1.4	0.755
RWT	0.47 ± 0.08	0.46 ± 0.07	0.408	0.46 ± 0.07	0.45 ± 0.06	0.575	0.48 ± 0.09	0.47 ± 0.06	0.565
LVMI ($g/h^{2.7}$)	59 ± 19	51 ± 14	0.003	51 ± 13*	49 ± 10	0.099	51 ± 12*	46 ± 9	0.001
LVED volume index (ml/m^2)	68 ± 16	61 ± 10	0.064	62 ± 13	60 ± 10	0.041	60 ± 12*	56 ± 10	0.008
LV stroke work (g/beat)	197 ± 74	142 ± 27	0.003	178 ± 46	141 ± 39	<0.0001	186 ± 48	145 ± 28	<0.0001
E/A	1.0 ± 0.2	1.0 ± 0.3	0.546	1.0 ± 0.4	1.0 ± 0.4	0.562	1.0 ± 0.3	1.0 ± 0.3	0.682
LV ejection fraction (%)	70 ± 4	69 ± 10	0.526	68 ± 8	65 ± 11	0.067	68 ± 6	70 ± 6	0.056
E/E′	10.8 ± 4.9	9.9 ± 5.5	0.646	11.6 ± 6.7	11.2 ± 9.3	0.499	9.7 ± 2.8	8.8 ± 2.7	0.727
DT/E wave ($s^2/cm \times 10^{-3}$)	3.2 ± 1.0	3.6 ± 1.7	<0.0001	3.1 ± 1.2	3.5 ± 1.8	<0.0001	3.2 ± 0.9	3.7 ± 1.6	<0.0001
LAD (mm)	37.6 ± 4.5	36.8 ± 3.9	0.237	38.8 ± 4.6	39.2 ± 4.3	0.700	39.0 ± 4.1	38.8 ± 4.6	0.541
Aortic dimension (mm)	33.9 ± 2.9	33.7 ± 3.4	0.387	34.4 ± 3.9	35.2 ± 3.9	0.090	34.3 ± 3.7	35.3 ± 3.8	0.039
LAD/AoD	1.1 ± 0.1	1.1 ± 0.1	0.777	1.1 ± 0.1	1.1 ± 0.2	0.128	1.1 ± 0.1	1.1 ± 0.1	0.683
ACLVF (%)	42 ± 6	38 ± 7	0.030	40 ± 12	41 ± 10	0.761	42 ± 11	39 ± 7	0.683

ACLF, atrial contribution to left ventricular filling; AoD, aortic root diameter; DT/E wave, mitral E wave deceleration time/E wave duration; E/A, early/late diastolic left ventricular filling wave ratio; E/E′, peak early diastolic mitral inflow/annular velocity ratio; IVSd, end-diastolic inter-ventricular septum thickness; LAD, left atrial dimension; LV, left ventricle; LVED, LV end-diastolic; LVEDD, LVED dimension; LVMI, left ventricular mass index; PWd, end-diastolic left ventricular posterior wall thickness; RWT, relative wall thickness.
Mean ± SD. *$P < 0.05$; **$P < 0.01$ vs. mutAPA.

FIGURE 3 The box plot and whisker (median, interquartile interval and range) shows the values of left ventricular mass index (LVMI) at baseline, and at long-term follow-up in the APA with somatic KCNJ5 mutations (mutAPA), in the APA without mutation (wtAPA) and in a group of APA patients in whom the KCNJ5 mutations could not be determined (shown for comparison). The dots show the individual values. Please note that at baseline LVMI was significantly higher in the mutAPA than in the wtAPA. Such differences disappeared at long-term follow-up (all P = NS) because of the significantly fall of LVMI in the mutAPA group. APA, aldosterone-producing adenoma.

they were identified early on in the course of their primary aldosteronism. Of interest, the mitral E wave deceleration time/E wave duration, which is an index of decreased left ventricular afterload, increased significantly and similarly in all groups (Table 2) in keeping with findings in a larger dataset [16].

The third major outcome of this study was the demonstration of the feasibility of achieving regression of LVH with adrenalectomy in the mutAPA to an even greater extent than in the wtAPA, likely because they started from a higher baseline LVMI (Fig. 5). The more prominent fall of LVMI in the mutAPA group was associated with a significant decrease of the atrial contribution to left ventricular filling, an index of left ventricular diastolic filling (Table 2).

At regression analysis, we found that the presence of the KCNJ5 mutations affected both baseline LVMI and the reduction of LVMI. Of note, the effect of KCNJ5 status on baseline LVMI persisted after adjustment for PAC, suggesting that in APA patients, a single measurement of plasma aldosterone at baseline does not capture the detrimental effect of long-term exposure to the hyperaldosteronism. The constitutive over-secretion of aldosterone associated with the KCNJ5 mutations probably implied a different 24-h BP profile, a contention that deserves further investigation. Of interest, we also identified an interaction of sex and KCNJ5 mutation status on both LVMI (Fig. 4) and its fall after adrenalectomy, the nature of which remains to be mechanistically identified.

In summary, we herein provided the following clinically relevant novel results: a KCNJ5 mutation in the APA determine a primary aldosteronism phenotype featuring increased cardiac damage, presumably reflecting long-term exposure to higher aldosterone levels; and the presence of

TABLE 3. Results of a GLM univariate regression on LVMI

Source	Type III sum of squares	Degree of freedom	Mean square	*F*	Sig.
Corrected model	5673.9	5	1134.79	6.41	0.000
Intercept	9924.5	1	9924.5	56.08	0.000
PAC	30.01	1	30.01	0.170	0.681
Sex	3502.2	1	3502.2	19.79	0.000
Sex × KCNJ5 status	780.5	1	780.5	4.41	0.038
Error	18758.3	106	176.9		
Total	333321.4	112			
Corrected total	24432.3	111			

LVMI, left ventricular mass index; PAC, plasma aldosterone concentration; Sig., significance.

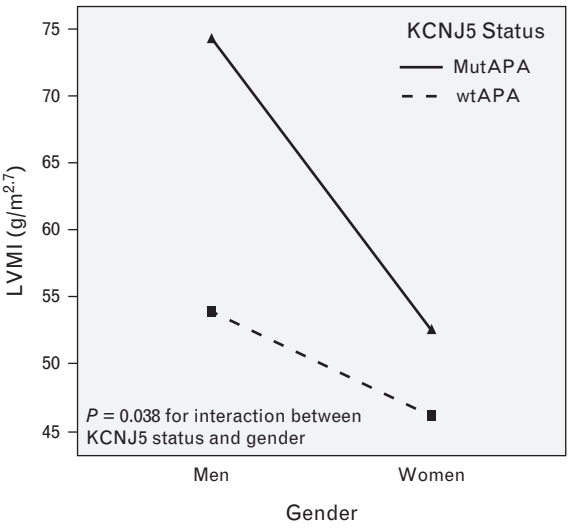

FIGURE 4 The plot shows the values of left ventricular mass index (LVMI) at baseline adjusted for the effect of sex, age, KCNJ5 mutation status, and plasma aldosterone concentration. The GLM model evidenced a significant interaction between sex and KCNJ5 mutation status ($F = 4.41$, $P = 0.038$) in determining LVMI, which is evidenced by the different slope of the line between mutAPA and wtAPA. Please note that men had a higher adjusted LVMI than women, but mutAPA women had a LVMI practically identical to wtAPA men, thus indicating the impact of the KCNJ5 somatic mutation. APA, aldosterone-producing adenoma.

these mutations did not preclude the possibility of achieving cure of the high BP and regression of LVH.

Limitations and strengths

This study involved selected patients referred to specialized centres, which carried limitations and strengths. These APA patients might not be entirely representative of the APA population at large in that they showed a lower prevalence of KCN5 mutations than in the previous series [1,3,34].

This could be due to an early detection of primary aldosteronism also in older patients, who might have a lower prevalence of KCNJ5 mutations [8], at both the Padua and the Rome Centres, where a systematic screening for primary aldosteronism is undertaken with no age selection criterion. This possible limitation was balanced by several strengths, including the unequivocal diagnosis of APA, the sequencing-based technique used for KCNJ5 genotyping, the careful assessment of left ventricular changes with echocardiography, and the long-term follow-up with painstaking assessment of the cardiac and BP changes.

In conclusion, we herein showed that apart from being more commonly women and featuring a higher and more lateralized aldosterone secretion [10], the APA patients with KCNJ5 mutations develop more left ventricular remodelling as evidenced by a higher LVMI, and more LVH, than wtAPA patients. These findings contradict the contention that the

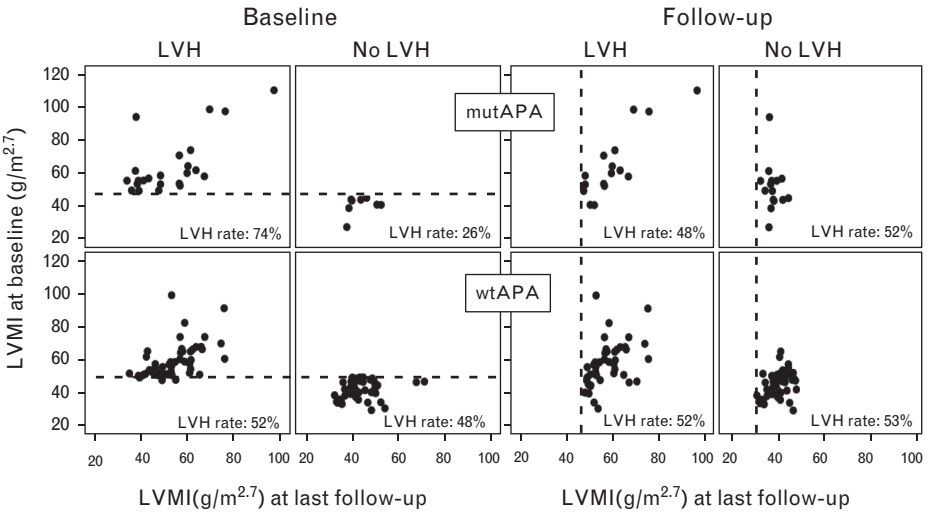

FIGURE 5 The scatter plots show the relationship between left ventricular mass index (LVMI) at baseline and at follow-up in the mutAPA (upper panels) and the wtAPA (lower panels) in the patients divided according to the presence or absence of LVH at baseline (left panels) or at follow-up (right panels). The horizontal and vertical dotted lines identify the cut-off for LVH at baseline and follow-up, respectively. Please note the more prominent regression of LVMI and, consequently, of rate of LVH, in the mutAPA than in the wtAPA patients. APA, aldosterone-producing adenoma; LVH, left ventricular hypertrophy; mutAPA, APA carrying somatic KCNJ5 mutations; wtAPA, wild-type APA.

KCNJ5 mutations do not translate into a peculiar phenotype [3]. Of clinical importance, both the hyperaldosteronism and the LVH regressed after adrenalectomy, thus proving a cause–effect relationship between the excess constitutive aldosterone secretion associated with the mutations and the development of LVH.

ACKNOWLEDGEMENTS

Funding sources: This study was supported by research grants from FORICA (The FOundation for advanced Research In Hypertension and CArdiovascular diseases, see at www.forica.it), the Società Italiana dell'Ipertensione Arteriosa and the University of Padua to G.P.R. and T.M.S., and EU FP7 to F.M.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Choi M, Scholl UI, Yue P, Bjorklund P, Zhao B, Nelson-Williams C, *et al.* K+ channel mutations in adrenal aldosterone-producing adenomas and hereditary hypertension. *Science* 2011; 331:768–772.
2. Oki K, Plonczynski MW, Luis Lam M, Gomez-Sanchez EP, Gomez-Sanchez CE. Potassium channel mutant KCNJ5 T158A expression in HAC-15 cells increases aldosterone synthesis. *Endocrinology* 2012; 153:1774–1782.
3. Boulkroun S, Beuschlein F, Rossi GP, Golib-Dzib JF, Fischer E, Amar L, *et al.* Prevalence, clinical, and molecular correlates of KCNJ5 mutations in primary aldosteronism. *Hypertension* 2012; 59:592–598.
4. Azizan EA, Murthy M, Stowasser M, Gordon R, Kowalski B, Xu S, *et al.* Somatic mutations affecting the selectivity filter of KCNJ5 are frequent in 2 large unselected collections of adrenal aldosteronomas. *Hypertension* 2012; 59:587–591.
5. Taguchi R, Yamada M, Nakajima Y, Satoh T, Hashimoto K, Shibusawa N, *et al.* Expression and mutations of KCNJ5 mRNA in Japanese patients with aldosterone-producing adenomas. *J Clin Endocrinol Metab* 2012; 97:1311–1319.
6. Charmandari E, Sertedaki A, Kino T, Merakou C, Hoffman DA, Hatch MM, *et al.* A novel point mutation in the KCNJ5 gene causing primary hyperaldosteronism and early-onset autosomal dominant hypertension. *J Clin Endocrinol Metab* 2012; 97:E1532–E1539.
7. Mulatero P, Tauber P, Zennaro MC, Monticone S, Lang K, Beuschlein F, *et al.* KCNJ5 mutations in European families with nonglucocorticoid remediable familial hyperaldosteronism. *Hypertension* 2012; 59:235–240.
8. Scholl UI, Nelson-Williams C, Yue P, Grekin R, Wyatt RJ, Dillon MJ, *et al.* Hypertension with or without adrenal hyperplasia due to different inherited mutations in the potassium channel KCNJ5. *Proc Natl Acad Sci U S A* 2012; 109:2533–2538.
9. Gomez-Sanchez CE, Oki K. Mini review: potassium channels and aldosterone dysregulation: is primary aldosteronism a potassium channelopathy? *Endocrinology* 2014; 155:47–55.
10. Seccia TM, Mantero F, Letizia C, Kuppusamy M, Caroccia B, Barisa M, *et al.* Somatic mutations in the KCNJ5 gene raise the lateralization index: implications for the diagnosis of primary aldosteronism by adrenal vein sampling. *J Clin Endocrinol Metab* 2012; 97:E2307–E2313.
11. Rossi GP, Bolognesi M, Rizzoni D, Seccia TM, Piva A, Porteri E, *et al.* Vascular remodeling and duration of hypertension predict outcome of adrenalectomy in primary aldosteronism patients. *Hypertension* 2008; 51:1366–1371.
12. Rossi GP, Sechi LA, Giacchetti G, Ronconi V, Strazzullo P, Funder JW. Primary aldosteronism: cardiovascular, renal and metabolic implications. *Trends Endocrinol Metab* 2008; 19:88–90.
13. Reincke M, Fischer E, Gerum S, Merkle K, Schulz S, Pallauf A, *et al.* Observational study mortality in treated primary aldosteronism: the German Conn's registry. *Hypertension* 2012; 60:618–624.
14. Savard S, Amar L, Plouin PF, Steichen O. Cardiovascular complications associated with primary aldosteronism: a controlled cross-sectional study. *Hypertension* 2013; 62:331–336.
15. Milliez P, Girerd X, Plouin PF, Blacher J, Safar ME, Mourad JJ. Evidence for an increased rate of cardiovascular events in patients with primary aldosteronism. *J Am Coll Cardiol* 2005; 45:1243–1248.
16. Rossi GP, Cesari M, Cuspidi C, Maiolino G, Cicala MV, Bisogni V, *et al.* Long-term control of arterial hypertension and regression of left ventricular hypertrophy with treatment of primary aldosteronism. *Hypertension* 2013; 62:62–69.
17. Funder JW, Carey RM, Fardella C, Gomez-Sanchez CE, Mantero F, Stowasser M, *et al.* Case detection, diagnosis, and treatment of patients with primary aldosteronism: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab* 2008; 93:3266–3281.
18. Rossi GP, Bernini G, Desideri G, Fabris B, Ferri C, Giacchetti G, *et al.* Renal damage in primary aldosteronism: results of the PAPY Study. *Hypertension* 2006; 48:232–238.
19. Mancia G, De Backer G, Dominiczak A, Cifkova R, Fagard R, Germano G, *et al.* 2007 ESH-ESC Practice Guidelines for the Management of Arterial Hypertension: ESH-ESC Task Force on the Management of Arterial Hypertension. *J Hypertens* 2007; 25:1751–1762.
20. Rossi GP, Bernini G, Caliumi C, Desideri G, Fabris B, Ferri C, *et al.* A prospective study of the prevalence of primary aldosteronism in 1,125 hypertensive patients. *J Am Coll Cardiol* 2006; 48:2293–2300.
21. Devereux RB, Alonso DR, Lutas EM, Gottlieb GJ, Campo E, Sachs I, Reichek N. Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. *Am J Cardiol* 1986; 57:450–458.
22. Wallerson DC, Ganau A, Roman MJ, Devereux RB. Measurement of cardiac output by M-mode and two-dimensional echocardiography: application to patients with hypertension. *Eur Heart J* 1990; 11 (Suppl I): 67–78.
23. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, *et al.* Recommendations for chamber quantification. *Eur J Echocardiogr* 2006; 7:79–108.
24. Muiesan ML, Salvetti M, Paini A, Monteduro C, Galbassini G, Bonzi B, *et al.* Inappropriate left ventricular mass changes during treatment adversely affects cardiovascular prognosis in hypertensive patients. *Hypertension* 2007; 49:1077–1083.
25. Teichholz LE, Kreulen T, Herman MV, Gorlin R. Problems in echocardiographic volume determinations: echocardiographic-angiographic correlations in the presence of absence of asynergy. *Am J Cardiol* 1976; 37:7–11.
26. Rossi GP. A comprehensive review of the clinical aspects of primary aldosteronism. *Nat Rev Endocrinol* 2011; 7:485–495.
27. Casale PN, Devereux RB, Milner M, Zullo G, Harshfield GA, Pickering TG, Laragh JH. Value of echocardiographic measurement of left ventricular mass in predicting cardiovascular morbid events in hypertensive men. *Ann Intern Med* 1986; 105:173–178.
28. Robert V, Van Thiem N, Cheav SL, Mouas C, Swynghedauw B, Delcayre C. Increased cardiac types I and III collagen mRNAs in aldosterone-salt hypertension. *Hypertension* 1994; 24:30–36.
29. Yamada M, Kushibiki M, Osanai T, Tomita H, Okumura K. Vasoconstrictor effect of aldosterone via angiotensin II type 1 (AT1) receptor: possible role of AT1 receptor dimerization. *Cardiovasc Res* 2008; 79:169–178.
30. Rossi GP, Sacchetto A, Pavan E, Palatini P, Graniero GR, Canali C, Pessina AC. Remodeling of the left ventricle in primary aldosteronism due to Conn's adenoma. *Circulation* 1997; 95:1471–1478.
31. Catena C, Colussi G, Nadalini E, Chiuch A, Baroselli S, Lapenna R, Sechi LA. Cardiovascular outcomes in patients with primary aldosteronism after treatment. *Arch Intern Med* 2008; 168:80–85.
32. Pitt B, Ahmed A, Love TE, Krum H, Nicolau J, Cardoso JS, *et al.* History of hypertension and eplerenone in patients with acute myocardial infarction complicated by heart failure. *Hypertension* 2008; 52:271–278.
33. Pitt B, Zannad F, Remme WJ, Cody R, Castaigne A, Perez A, *et al.* The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized Aldactone Evaluation Study Investigators. *N Engl J Med* 1999; 341:709–717.
34. Akerstrom T, Crona J, Delgado Verdugo A, Starker LF, Cupisti K, Willenberg HS, *et al.* Comprehensive re-sequencing of adrenal aldosterone producing lesions reveal three somatic mutations near the KCNJ5 potassium channel selectivity filter. *PLoS One* 2012; 7: e41926.

Reviewers' Summary Evaluations

Reviewer 2

The article provided some evidence to advance our understanding the effect of KCNJ5 gene somatic mutations on primary aldosteronism-induced hypertension and cardiac hypertrophy.

Reviewer 3

This is an interesting and well conducted study showing that among patients with an aldosterone-producing adenoma those carrying mutations in the KCNJ5 K^+ channel have higher left ventricular mass index and plasma aldosterone concentrations, but the excess left ventricular hypertrophy was corrected by adrenalectomy. The strength of the study is the careful phenotyping of the patients before and following adrenalectomy. A weakness, though inherent to the difficult availability of the patients being studied, is the lack of a sample size calculation with the results that some of the differences are of borderline significance.