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CLINICAL STUDY

Lack of influence of somatic mutations on steroid gradients during adrenal vein sampling in aldosterone-producing adenoma patients

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Abstract

Objective: Adrenal vein sampling (AVS) is a technically demanding procedure required for the identification of suitable candidates for unilateral adrenalectomy in primary aldosteronism. Recently, somatic *KCNJ5* K^+-channel mutations in aldosterone-producing adenoma (APA) patients have been shown to influence steroid gradients during AVS. These and other recently identified genetic modifiers (*ATP1A1* and *ATP2B3*) might affect the final diagnosis and treatment of the affected patients.

Design: Fifty-nine patients with APAs who had undergone successful AVS (adrenal vein cortisol:peripheral cortisol ratio ≥ 2) and had undergone a mutation analysis of their tumor tissue were studied. The mutation status of the APAs was as follows: 19 *KCNJ5* mutations, eight ATPase mutations (five *ATP1A1* and three *ATP2B3*), and 32 patients with none of these mutations.

Methods: The lateralization index (ratio of aldosterone:cortisol on the side of the adenoma to aldosterone to cortisol on the contralateral side) and the contralateral suppression index (ratio of aldosterone:cortisol on the contralateral side to aldosterone to cortisol in the periphery) were calculated for the *KCNJ5*-mutated, ATPase-mutated, and the *KCNJ5*/ATPase mutation-negative APA patients.

Results: The lateralization indices of the ATPase mutation carriers had a median of 19.9 compared with a median of 16.0 in the *KCNJ5* mutation carriers and that of 20.5 in the *KCNJ5*/ATPase mutation-negative patients. The contralateral suppression indices of the ATPase-mutated patients had a median of 0.1 compared with a median of 0.4 in the *KCNJ5* mutation carriers and that of 0.2 in the *KCNJ5*/ATPase mutation-negative patients. The differences between the genetic groups were not statistically significant.

Conclusions: We did not find evidence for a clinically important impact of mutation status on steroid gradients during AVS.

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Introduction

Primary aldosteronism (PA) is the most common cause of secondary arterial hypertension. It has been reported to occur in ~7% of hypertensive patients in population-based studies and in up to 20% of those with resistant hypertension in specialized centers (1, 2). Cardiovascular and renal morbidities are increased in patients with PA compared with patients with essential hypertension (3, 4, 5). Therefore, early diagnosis and specific therapy are of crucial importance (6). The two main causes of PA are aldosterone-producing adenomas (APAs) and bilateral idiopathic adrenal hyperplasia. While APAs can effectively be cured with unilateral adrenalectomy, bilateral idiopathic adrenal hyperplasia is treated with lifelong therapy with mineralocorticoid receptor antagonists. Subtype differentiation between unilateral adrenal disease and bilateral adrenal disease necessitates adrenal vein sampling (AVS) (7).

Recently, somatic mutations in APA patients have become a focus of research. In 2011, Choi *et al.* (8) discovered mutations in the *KCNJ5* gene, coding for the potassium channel KIR3.4, in 36% of the sporadic APA cases. The detected mutations were localized near the selectivity filter of the channel. Thus, *KCNJ5*-mutated potassium channels lose their ion selectivity and permit sodium influx, leading to the depolarization of the cell (9). This leads to the opening of voltage-dependent calcium channels and influx of calcium. The enhanced calcium concentration induces aldosterone production

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via the calcium signaling cascade. In many consecutive studies including our own series, affected patients were demonstrated to be predominantly females (10). We have recently discovered somatic mutations in the Na^+, K^+-ATPase (*ATP1A1*) and Ca^{2+}-ATPase (*ATP2B3*) genes in 5.2 and 1.6% of the APA patients respectively (11). These mutations were localized in the ion-binding pocket. Functional studies of *ATP1A1* mutations have demonstrated the loss of function of the pump and depolarization of the cells. Patients affected by these mutations are predominantly males and have a more severe endocrine and cardiovascular phenotype.

AVS is a technically demanding procedure that is required for the identification of suitable candidates for unilateral adrenalectomy in PA. A recent study has shown that steroid gradients during AVS are influenced by the *KCNJ5* mutation status of the APA (12). However, the impact of ATPase mutations has not been studied yet. This might have an impact on final diagnosis and treatment.

Subjects and methods

Patient cohort

Patients with APAs were recruited consecutively in three different German centers (Munich, $n=50$; Berlin, $n=8$; and Würzburg, $n=1$) of the German Conn's Registry – Else Kröner-Fresenius Hyperaldosteronismus Registry (www.conn-register.de). Of the 59 patients recruited, 46 were part of the study carried out by Beuschlein *et al.* (11). Case detection and subtype identification of PA were carried out according to institutional guidelines and in accordance with the Endocrine Society Guidelines (7, 13). The final diagnosis of APAs was based on the following criteria: biochemical diagnosis of hyperaldosteronism, lateralization of aldosterone production during AVS, histological confirmation of adrenocortical adenomas and normalization of hypokalemia, hypertension, and aldosterone-to-renin ratio after adrenalectomy (14, 15). The Ethics Committee of the participating centers approved the protocol of the German Conn's Registry, and all patients provided written informed consent for genetic and clinical investigations.

Adrenal vein sampling

Before AVS, the use of interfering medications such as mineralocorticoid receptor antagonists, diuretics, and β-blockers was stopped. Instead, verapamil (maximum dose of 240 mg twice daily) and doxazosin (maximum dose of 16 mg daily) were used and hypokalemia was corrected. AVS was carried out between 0800 and 1200 h by experienced radiologists. Blood samples were sequentially collected from both adrenal veins without adrenocorticotrophic hormone (ACTH) stimulation. Hydrophilic 4 French catheters with different configurations were used, depending on the anatomy of the adrenal veins. The samples were collected by gravity or gentle suction. Corresponding peripheral samples were collected at the time of AVS of each adrenal vein ($n=48$) or only once during AVS ($n=11$). The mean selectivity index of the two groups was similar.

A selectivity index (adrenal vein cortisol to peripheral cortisol) of at least 2 on both sides and a lateralization index (ratio of aldosterone:cortisol on the side of the adenoma to aldosterone to cortisol on the contralateral side) of 4 or above were set for the diagnosis of unilateral aldosterone excess (16). The contralateral suppression index was calculated as the ratio of the cortisol-corrected aldosterone ratio (AC) of the nondominant adrenal gland to the peripheral AC ($AC_{nondominant\ adrenal}/AC_{periphery}$). The decision for adrenalectomy was not based on the contralateral suppression of aldosterone secretion.

Biochemical measurements

Plasma aldosterone concentration was measured using Coat-a-Count RIA (Biermann DPC, Bad Nauheim, Germany). Active renin concentration was measured using the Diasorin assay (Liaison, Saluggia, Italy) in Munich and Würzburg and using the Cisbio assay (Berlin, Germany) in Berlin. In this study, the respective within-assay and between-assay coefficients of variation were below 9 and 12% for aldosterone and below 5.6 and 12.2% for renin respectively. All other biochemical variables were assayed using plasma or serum in our central laboratory using standard methods. Serum potassium concentration was measured using flame photometry (ISE Indirect, Cobas Integra, the Roche platform; Roche).

KCNJ5, ATP1A1, and ATP2B3 sequencing

DNA was extracted from APA tissue using the RNeasy DNA extraction kit (Qiagen) and amplified using intron-spanning primers as described previously (8, 11). Bi-directional Sanger sequencing was carried out using the ABI 3730xl Analyzer.

Statistical analysis

Data were extracted from the German Conn's Registry – Else Kröner-Fresenius Hyperaldosteronismus Registry. If not stated otherwise, group results are reported as medians and interquartile ranges (IQRs). Data of the groups were compared using the Kruskal–Wallis test followed by a two-sided test for pairwise comparison of two groups. Power calculation of the study was based on the data of the study carried out by Seccia *et al.* (12) and on a conservative assumption of mean lateralization indices of 30 and 15 (S.D. $\pm$15) in mutated vs nonmutated APA patients. This estimation required

Table 1 Clinical characteristics of the patient cohort.

	***KCNJ5*/ATPase**			***KCNJ5***			**ATPase**			**All**	***KCNJ5* vs *KCNJ5*/ATPase**	**ATPase vs *KCNJ5*/ATPase**	***KCNJ5* vs ATPase**
	n	Median	IQR	*n*	Median	IQR	*n*	Median	IQR	*P*[a]	*P*[b]	*P*[b]	*P*[b]
Age at diagnosis (years)	32	54	16	19	40	12	8	56	5	0.001	0.002	NS	0.015
Adenoma size (mm)	32	15	8	19	16	3	8	15	7	0.248			
Serum potassium concentration (mmol/l)	32	3.2	0.6	19	3.4	1.0	8	2.8	0.6	0.069			
Plasma aldosterone concentration (ng/l)	31	285	301	19	228	191	8	611	628	0.106			
Plasma renin concentration (mU/l)	30	4.5	5.7	19	2.2	8.2	8	3.5	16.2	0.270			
Aldosterone-to-renin ratio (ng/mU)	30	63.0	109.1	19	75.3	227.3	8	127.2	617.8	0.395			
Systolic blood pressure (mmHg)	32	146	20	19	142	26	8	168	51	0.045	NS	NS	0.038
Diastolic blood pressure (mmHg)	32	91	20	19	89	16	8	96	16	0.788			
No. of antihypertensive agents	17	3.0	3.0	11	2.0	2.0	8	2.0	2.0	0.074			
Serum potassium concentration (mmol/l) postoperative	31	4.3	0.4	18	4.2	0.5	7	4.7	0.8	0.124			
Plasma aldosterone concentration (ng/l) postoperative	28	44	35	18	56	57	7	35	1	0.464			
Plasma rennin concentration (mU/l) postoperative	28	11.3	22.9	18	16.5	27.0	7	23.8	54.5	0.478			
Aldosterone-to-rennin ratio (ng/mU) postoperative	28	3.7	6.0	18	4.3	5.1	7	1.5	2.6	0.185			
Systolic blood pressure (mmHg) postoperative	25	142	18	18	131	33	7	141	12	0.187			
Diastolic blood pressure (mmHg) postoperative	25	86	14	18	82	16	7	90	24	0.473			
No. of antihypertensive agents post-operative	24	2	2	16	0.5	2	7	2	3	0.069			

n, number of subjects for each group; IQR, interquartile range; NS, not significant.
[a]Data of the groups were compared using the Kruskal–Wallis test followed by a two-sided test.
[b]For pairwise comparison of the two groups. Conversion of aldosterone (ng/l) to SI unit (pmol/l) by multiplication by 2.77.

Table 2 AVS values of the patient cohort.

	KCNJ5/ATPase			*KCNJ5*			ATPase			All	*KCNJ5* vs *KCNJ5*/ATPase	ATPase vs *KCNJ5*/ATPase	*KCNJ5* vs ATPase
	n	Median	IQR	*n*	Median	IQR	*n*	Median	IQR	*P*[a]	*P*[b]	*P*[b]	*P*[b]
Aldosterone adenoma (ng/l)	32	8328.5	25 953	19	17 980.0	31 200	8	15 707.5	60 389.0	0.204			
Aldosterone contralateral (ng/l)	32	428.0	875	19	466.0	879	8	1184.5	2379	0.196			
Cortisol adenoma (μg/dl)	32	55.0	111.0	19	174.8	320.7	8	207.3	544.3	0.162			
Cortisol contralateral (μg/dl)	32	112.5	275.1	19	45.4	216.9	8	261.7	1548.9	0.081			
AC adenoma	32	123.8	216	19	146.7	469.0	8	120.7	127.4	0.645			
AC contralateral	32	4.7	8.1	19	5.0	6.7	8	2.0	8.0	0.291			
Aldosterone peripheral (AVS ipsilateral; ng/l)	32	149.6	138.0	19	214.0	264.0	8	402.5	697.0	0.075			
Aldosterone peripheral (AVS contralateral; ng/l)	32	125.5	109.0	19	214.0	132.0	8	256.5	432.0	0.037	NS	NS	NS
Cortisol peripheral (AVS ipsilateral; μg/dl)	32	8.8	7.9	19	12.0	8.1	8	14.1	12.4	0.382			
Cortisol peripheral (AVS contralateral; μg/dl)	32	8.6	10.3	19	12.7	9.0	8	13.9	12.1	0.430			
AC peripheral (AVS ipsilateral)	32	13.8	16.3	19	19.2	20.2	8	29.8	37.7	0.230			
AC peripheral (AVS contralateral)	32	12.5	23.5	19	14.7	17.9	8	19.1	35.2	0.266			
Selectivity index (minimal)	32	4.7	7.5	19	3.3	6.9	8	11.7	24.0	0.250			

n, number of subjects for each group; IQR, interquartile range; NS, not significant; AC, aldosterone:cortisol ratio; AVS ipsilateral and AVS contralateral, time points during AVS when the peripheral sample was withdrawn.

[a]Data of the groups were compared using the Kruskal–Wallis test followed by a two-sided test.

[b]For pairwise comparison of two groups. Conversion of aldosterone (ng/l) to SI unit (pmol/l) by multiplication by 2.77. Conversion of cortisol (μg/dl) to SI unit (nmol/l) by multiplication by 27.59.

17 patients in each group to detect a significant difference ($P<0.05$) with a power of 80%. Statistical analysis was carried out using the standard statistical software (SPSS 21).

Results

To analyze the potential influence of somatic mutations on steroid gradients during AVS, 59 consecutive patients with APAs in three German centers were prospectively studied. Among the patients, 37 were men and 22 were women. All the subjects were diagnosed according to the German Conn's Registry standard. Following genetic analysis of the adenoma tissue, it was found that 19 patients had *KCNJ5* mutations (11 *G151R* and eight *L168R*) and eight had ATPase mutations (five *ATP1A1* and three *ATP2B3*), while 32 APA patients did not harbor any of these mutations.

The baseline and follow-up characteristics of the cohort are summarized in Table 1. As has been reported previously, patients with *KCNJ5* mutations were predominantly females and younger at the time of surgery, whereas patients with ATPase mutations displayed a male predominance and were older.

AVS was carried out without ACTH stimulation. The AVS values are summarized in Table 2, and individual values are given in Supplementary Table 1, see section on supplementary data given at the end of this article.

To analyze the lateralization of aldosterone production during AVS, the lateralization index was calculated. The lateralization index of the nonmutated and *KCNJ5*-mutated patients had a median of 20.5 (IQR 30.3) and 16.0 (IQR 41.9) respectively (Fig. 1A). The ATPase mutation carriers had a median lateralization index of 19.9 (IQR 122.3). These differences between the genetic groups were not significant ($P=0.959$).

Contralateral suppression was most distinct in the ATPase-mutated patients with a median of 0.1 (IQR 0.3) compared with a median of 0.4 (IQR 0.5) in the *KCNJ5*-mutated patients and that of 0.2 (IQR 0.6) in the nonmutated patients (Fig. 1B), but these differences were not significant ($P=0.060$).

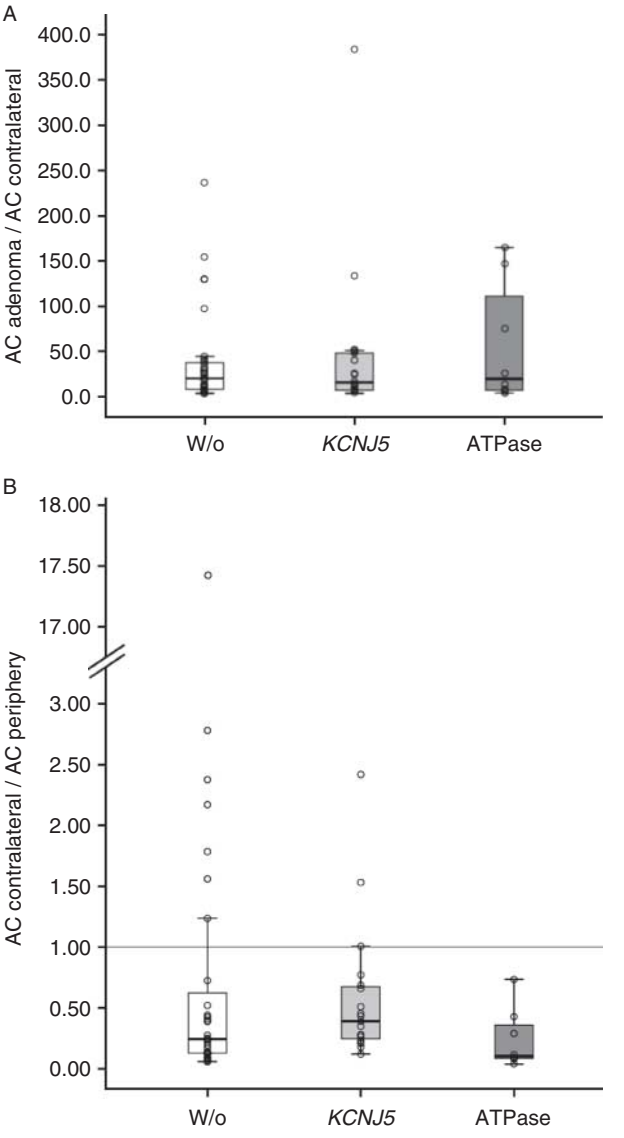

Figure 1 (A) Lateralization index. The box and whisker plot (median and 25–75th percentiles) shows the ratio of cortisol-normalized aldosterone on the APA side:cortisol-normalized aldosterone on the contralateral side for *KCNJ5*/ATPase-nonmutated (w/o) patients and for the *KCNJ5*- and ATPase-mutated patients. (B) Suppression index. The box and whisker plot (median and 25–75th percentiles) shows the ratio of cortisol-normalized aldosterone on the contralateral side:cortisol-normalized aldosterone in the periphery for the *KCNJ5*/ATPase-nonmutated (w/o) patients and for the *KCNJ5*- and ATPase-mutated patients.

Discussion

AVS is the recommended procedure for the identification of patients with APAs that can be cured with unilateral adrenalectomy. However, it is unclear whether the mutation status of APAs affects steroid gradients during AVS. Seccia *et al.* (12) have reported on the impact of *KCNJ5* mutations on aldosterone gradients during AVS and have found a more profound lateralization index in their cohort. These data have not been confirmed in independent cohorts, and the newly identified ATPase mutations have not been appreciated in this context yet.

Only patients who had undergone technically successful AVS (selectivity index ≥ 2.0) exhibiting a lateralization of aldosterone production (lateralization index ≥ 4.0) were included in this study as these were set as the criteria for unilateral adrenalectomy within the German Conn's Registry. The patient cohort

displayed a composition similar to the ones reported previously (17). Among the subjects, 32% had *KCNJ5* mutations and 14% had ATPase mutations. Gender distribution and age at diagnosis also resembled published data (10).

In this study, the lateralization indices of the nonmutated and ATPase-mutated patients were nearly equal and were higher than those of the *KCNJ5* mutation carriers. This finding is in contrast to the findings of the study of Seccia *et al.*, who demonstrated that *KCNJ5*-mutated patients have a significantly higher lateralization index than the *KCNJ5* mutation-negative patients. This discrepancy might be explained by different inclusion criteria for their patient cohort based on a selectivity index of 2 and a lateralization index of 2, impeding the direct comparison of the two studies (12, 18). A lateralization index of 4, as used in the present study, would have placed 68% of the *KCNJ5* mutation-negative patients and 24% of the *KCNJ5* mutation-positive cases of the Seccia study in the category of bilateral adrenal hyperplasia. These patients probably would not have been adrenalectomized in our clinical setting. Notably, the *KCNJ5*-mutated patients in the study carried out by Seccia *et al.* exhibited a more severe phenotype with higher aldosterone levels and lower potassium levels than our patients. One speculation might be that this cohort was diagnosed later and suffered longer from hyperaldosteronism than our patients. On the other hand, the Italian *KCNJ5* mutation-negative patients seemed to exhibit a less severe phenotype with higher potassium levels and lower blood pressure in comparison with our nonmutated patients, despite the fact that within this group an unknown proportion of yet undiagnosed ATPase mutation carriers might have been included. In this context, it is not surprising if this group has high lateralization indices similar to the *KCNJ5*-mutated patients.

We found contralateral suppression to be most distinct in the ATPase-mutated patients. In general, a low contralateral suppression index indicates a more pronounced inhibition of aldosterone production from the contralateral adrenal gland. In our series, all the ATPase-mutated patients displayed full contralateral suppression (100%), whereas only 84% of the *KCNJ5*-mutated patients and 78% of the nonmutated patients had suppression indices below 1. In the patient cohort of Seccia *et al.*, 74% of the *KCNJ5*-mutated APA patients exhibited contralateral suppression compared with 54% of the *KCNJ5* mutation-negative APA patients. These findings again support the hypothesis that the nonmutated patients in our cohort are different from the ones of the study of Seccia *et al.*

In summary, AVS is a clinically accurate method in the presence of APAs with or without *KCNJ5* and ATPase mutations. We did not find convincing evidence for a clinically important impact of *KCNJ5* and ATPase mutations on steroid gradients during AVS.

Supplementary data

This is linked to the online version of the paper at http://dx.doi.org/10.1530/EJE-13-0551.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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