PRKACA somatic mutations are rare findings in aldosterone-producing adenomas

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Introduction

Primary aldosteronism (PA) is the predominant endocrine cause of secondary hypertension, affecting 5-10% of hypertensive patients and up to 20% of patients with treatment-resistant hypertension. The two predominant causes of PA are aldosterone-producing adenomas (APA) and bilateral adrenal hyperplasia resulting in an elevated aldosterone to renin ratio (ARR) often associated with hypokalemia. So far, at least five candidate genes are implicated in PA: *KCNJ5, CACNA1D, ATP1A1, ATP2B3*, and *CACNA1H* (1-5), mutations result in electrophysiological alterations, consecutive increase in intracellular calcium levels and ultimately increase in the expression of *CYP11B2*, which encodes aldosterone synthase required for aldosterone biosynthesis. Another key activator for adrenocortical steroidogenesis and cell growth is cyclic AMP (cAMP), a second messenger, which regulates the activation of protein kinase A (PKA). Recently, somatic mutations of *PRKACA*, which codes for the α isoform of the C subunit (Cα), have been reported in adenomas of the adrenal cortex (6-10). In particular, the most frequent mutation (p.Leu206Arg) was found to be restricted to cortisol producing adenomas (CPA) associated with overt Cushing´s syndrome. Although aldosterone- and cortisol co-secreting adenoma and subclinical Cushing's syndrome can occur in PA patients (11, 12), the molecular causes for steroid co-secretion have remained uncertain. We report on in depth investigation of two cases of PA presenting with somatic mutations of *PRKACA* identified by exome sequencing and evaluated for their clinical and molecular phenotypes.

Materials and Methods

Patients were diagnosed with PA according to institutional and Endocrine Society Clinical Practice Guidelines and were included in the German Conn's Registry. Baseline clinical characterization included multi-steroid analysis of peripheral blood samples. Subtype differentiation was done by cross-sectional imaging (MRI) and adrenal venous sampling in PA patients.
APA tissues were collected from 122 patients who underwent unilateral adrenalectomy for PA between 2005 and 2015 at the Klinikum der Universität München. Surgically resected adrenocortical tissues were examined by a clinical pathologist. Identification of *PRKACA* somatic variants in APA was performed in the 122 APA by whole-exome sequencing (58/122) or direct bidirectional Sanger sequencing (64/122), followed by *in vitro* analysis of the enzymatic activity of *PRKACA* variants using the PepTag non-radioactive cAMP-dependent protein kinase assay and functional characterization by double immunofluorescence of CYP11B2 and CYP11B1 expression in the corresponding tumor tissues. All patients provided written informed consent and the study was approved by the ethics committee of the Ludwig-Maximilian University of Munich. Biochemical and clinical data were prospectively collected.

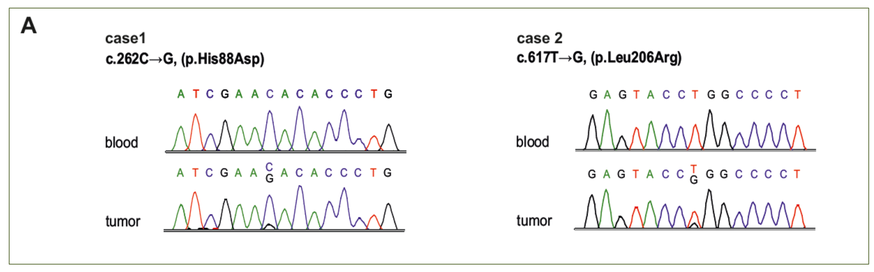
Results

Somatic *PRKACA* mutations were identified by exome sequencing in 2/122 (1.6%) cases described in Table 1. Case 1 presented a c.262C>G (p.His88Asp) mutation in exon 4 while a c.617A>C (p.Leu206Arg) mutation in exon 7 was identified in case 2 (Fig.1A). His88 is the only residue in the small lobe of the conserved catalytic core of the C subunit of PKA to interact with the phosphate on residue Thr198, the essential phosphorylation site on the surface of the large lobe (Fig.1B). Situated at the cleft interface, His88 was found to complement Ser100 at the auto-inhibitor sequence P+2 in the type I regulatory subunit and therefore His88 is also involved in the interaction with the RIα subunit of PKA. Measurement of PKA catalytic activity demonstrated that mutated His88Asp Cα subunit of PKA resulted in a significantly lower enzymatic PKA activity in comparison to the wild-type enzyme when co-transfected with either RIα or with RIIβ in HEK293 cells, both in presence and in absence of cAMP (Fig.1C). On the contrary, mutated Leu206Arg resulted in a constitutive elevated activity not suppressed by any of the regulatory subunits, as previously described (6). The APA resected from the two cases were of similar size (0.9 *vs* 1.2 cm) and both were composed of *zona fasciculata* and *zona glomerulosa*-like cells. Double immunofluorescence analysis (Fig. 2A) showed CYP11B2 expression and, to a lesser extent, CYP11B1 in case 1; conversely, the adenoma cells in case 2 were mostly positive for CYP11B1. Multi-steroid analysis of peripheral plasma from case 2 found hybrid serum steroids 18-oxocortisol and 18-hydroxycortisol to be elevated as observed with the multi-steroid fingerprint of patients carrying *KCNJ5* somatic mutations (Fig.2B).

Table1: Clinical and biochemical parameters of the PA-patients with *PRKACA* somatic mutations.

	Case 1		Case 2		Reference Intervals/Cut-off
Age, years	32		51		
BMI, kg/m²	26.5		32.3		
Dyslipidemia	No		Yes		
Type 2 Diabetes	No		Yes		
Maximal adenoma size, mm	9.0		12.0		
	Preoperative	Postoperative	Preoperative	Postoperative	
Systolic blood pressure, mm Hg	>210	125	159	127	
Diastolic blood pressure, mm Hg	114	100	90	77	
Aldosterone-to-renin ratio, pg/mL·mU/L	131.4	63.5	16.4	9.2	<10
PAC, pg/mL	552	216.0	154	645	
PRA, mU/L	4.2	3.4	9.4	70	
Serum Potasium, mmol/L	2.3	4.1	3.1	4.4	3.5-5.0
Basal plasma ACTH, pg/mL	22.9	nd	6	19	4 - 50
Basal serum cortisol, µg/dL	9.3	10.4	10.1	11,8	4.5-24
Late-night salivary cortisol, ng/mL	2.2	nd	2.1	0.9	<1.8
Urinary free cortisol, µg/24h	184	69	285	183	50-150
Serum cortisol after 1 mg dexamethasone, µg/dL	1.8	nd	5.1	2.2	<1.8

Figure 1: Identification and functional characterization of *PRKACA* variants

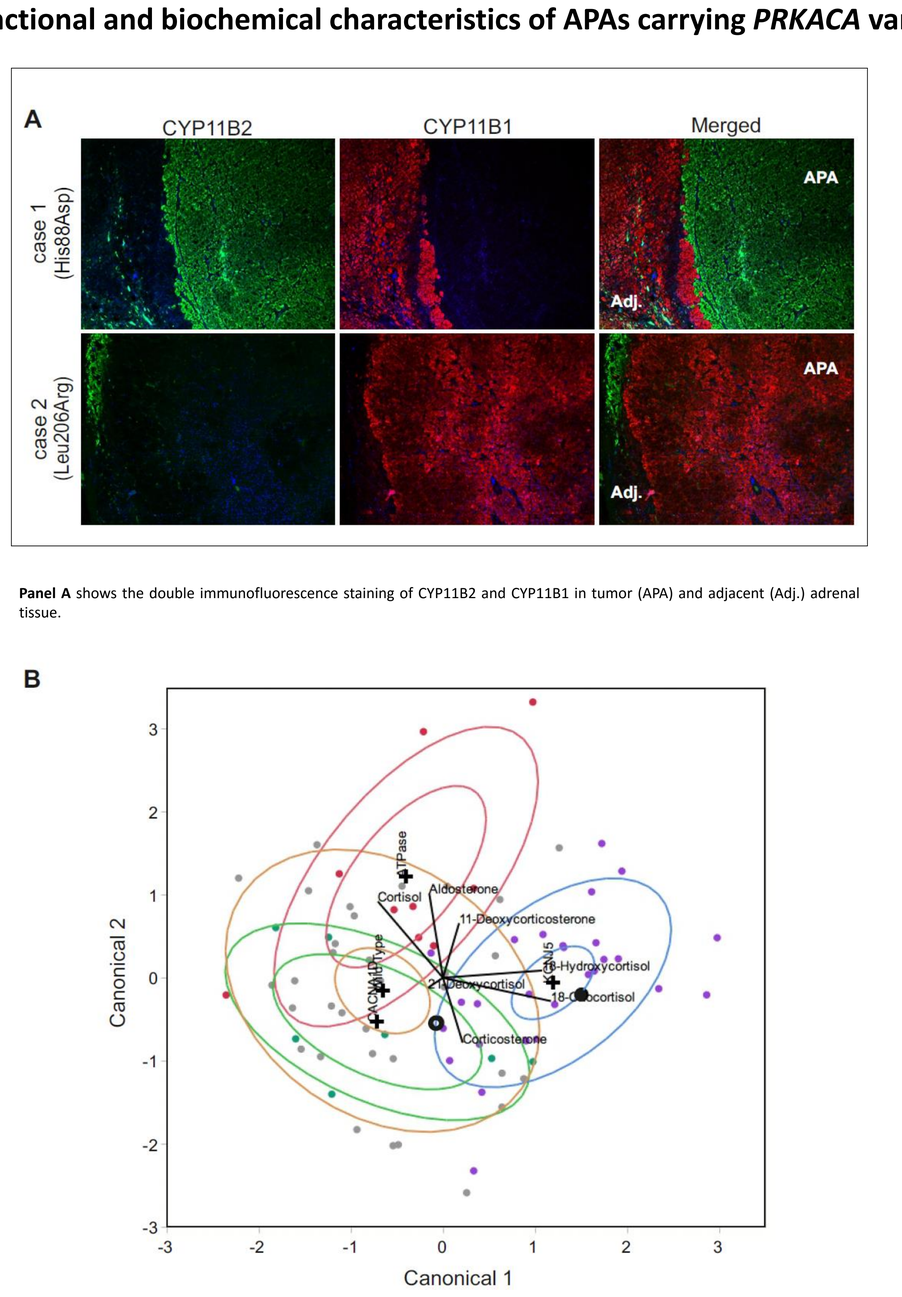


Panel A shows two paired sequence chromatograms of tumor and peripheral blood from case 1 and case 2. In case 1 (left) a somatic variant in *PRKACA* (c.262C→G) was identified in the aldosterone-producing adenoma, resulting in a His88Asp substitution. In case 2 (right) a somatic mutation in *PRKACA* (c.617A→C) was found, resulting in a Leu206Arg substitution.

Panel B shows the location of His88 within the tridimensional structure of the Cα subunit of PKA. This residue, together with Arg166 and Lys 190, binds and stabilizes phospho-Thr198 (left). Conversely, the substitution Asp88 (right, in red) disrupt this interaction.

Panel C shows the functional characterization of *PRKACA* mutations. Enzymatic PKA activity was quantified on lysates of human embryonic kidney 293 cells co-expressing Cα (mutant or non-mutant) and RIα (left) or RIIβ (right) in a molar ratio of R:C equal to 1:8. Activity was measured in presence or absence of cyclic AMP (cAMP) using a specific peptide substrate and measured by a fluorescent, in-gel migration assay. The His88Asp variant exhibits a lack of response to cAMP stimulation while the Leu206Arg mutant is constitutively active. "*" indicates *P*<0.05 for the comparison with the wild-type PKA activity in absence of cAMP; "#" indicates *P*<0.05 for the comparison with the wild-type PKA activity in presence of cAMP. Representative input blots are shown below the graphs.

Figure 2: Functional and biochemical characteristics of APAs carrying *PRKACA* variants



Panel A shows the double immunofluorescence staining of CYP11B2 and CYP11B1 in tumor (APA) and adjacent (Adj.) adrenal tissue.

Panel B shows the 2D-canonical plot derived from discriminant analysis for plasma concentrations of seven adrenal steroids (aldosterone, 18-oxocortisol, 18-hydroxycortisol, corticosterone, 11-deoxycorticosterone, 21-deoxycortisol and cortisol) used for 79 aldosterone-producing adenomas APAs with and without (wild-type, grey) somatic mutations of KCNJ5 (blue), CACNA1D (green) and ATP1A1 or ATP2B3 (red) genes. The crosses represent the centroids for each group. The empty and bold circles indicate the location within canonical plots of the two adenomas with the PRKACA somatic variant p.His88Asp (case 1) and the PRKACA somatic mutation p.Leu206Arg (case 2) respectively.

Conclusion

We describe for the first time *PRKACA* mutations in two cases of PA patients: a novel *PRKACA* variant (p.His88Asp) occurring in a case of sudden onset of PA and a *PRKACA* mutation (p.Leu206Arg) in context of hypokalemic aggravation of long term hypertension. These genetic alterations were not found in a subsequent series of 120 APA and thereby appear to be infrequent events. The molecular basis for co-secretion of aldosterone and cortisol as observed in a subgroup of PA patients remains to be elucidated.

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