PRKACA somatic mutations are rare findings in aldosterone-producing adenomas

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Context: Somatic mutations have been found causative for endocrine autonomy in aldosterone-producing adenomas (APAs). While mutations of *PRKACA* (catalytic subunit of protein kinase A) have been identified in cortisol-producing adenomas (CPAs), the presence of *PRKACA* variants in APAs is unknown, especially in those that display co-secretion of cortisol.

Objective: To investigate *PRKACA* somatic variants identified in APA cases.

Design: Identification of *PRKACA* somatic variants in APAs by whole-exome sequencing followed by *in vitro* analysis of the enzymatic activity of *PRKACA* variants and functional characterization by double immunofluorescence of CYP11B2 and CYP11B1 expression in the corresponding tumor tissues.

Setting and Patients: APA tissues were collected from 122 patients who underwent unilateral adrenalectomy for PA between 2005 and 2015 at a single institution.

Results: *PRKACA* somatic mutations were identified in two APA cases (1.6%). One APA carried a newly identified p.His88Asp variant while in a second case a p.Leu206Arg mutation was found, previously described only in CPA with overt Cushing´s syndrome. Functional analysis showed that the p.His88Asp variant was not associated with gain of function. While CYP11B2 was strongly expressed in the p.His88Asp mutated APA, the p.Leu206Arg carrying APA predominantly expressed CYP11B1. Accordingly, biochemical Cushing's syndrome was present only in the patient with the p.Leu206Arg mutation. Following adrenalectomy, both patients improved with a reduced number of antihypertensive medications and normalized potassium levels.

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Abbreviations: ACE angiotensin-converting-enzyme; ACTH adrenocorticotropin; APA aldosterone-producing adenoma; ARR aldosterone-to-renin-ratio; *ATP1A1* ATPase Na^+/K^+ transporting subunit alpha 1; *ATP2B3* ATPase plasma membrane Ca^{2+} transporting 3; BMI body mass index; *CACNA1D* calcium voltage-gated channel subunit alpha1 D; *CACNA1H* calcium voltage-gated channel subunit alpha1 H; cAMP 3',5'-cyclic AMP; CPA cortisol-producing adenoma; CYP11B1 cytochrome P450, family 11, subfamily B, polypeptide 1; CYP11B2 cytochrome P450, family 11, subfamily B, polypeptide 2; FFPE formalin-fixed, paraffin-embedded; *KCNJ5* potassium voltage-gated channel subfamily J member 5; MRI Magnetic resonance imaging; PA primary aldosteronism; PAC plasma aldosterone concentration; PKA cAMP-dependent protein kinase A; PRA plasma renin activity; *PRKACA* protein kinase cAMP-activated catalytic subunit alpha; SBP systolic blood pressure

Conclusions: We describe for the first time *PRKACA* mutations as rare findings associated with unilateral PA. As cortisol co-secretion occurs in a sub-group of APAs, other molecular mechanisms are likely to exist.

Primary aldosteronism (PA) is the predominant endocrine cause of secondary hypertension (1), 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 (2, 3). PA constitutes an independent risk factor for increased cardiovascular morbidity (4). Since 2011, next generation sequencing approaches have identified acquired somatic mutations in around 50% of APA cases. So far, at least five candidate genes are implicated in PA: *KCNJ5, CACNA1D, ATP1A1, ATP2B3*, and *CACNA1H* (5–9). In all instances, mutations result in electrophysiological alterations, consecutive increase in intracellular calcium levels and ultimately in an increase in the expression of *CYP11B2*, which encodes aldosterone synthase required for aldosterone biosynthesis. While the functional consequences of these somatic mutations towards calcium signaling and hormonal autonomy have been studied in great detail over the recent years, direct effects on adenoma formation remain uncertain and might require additional yet unresolved environmental or genetic factors.

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). In its inactive form, the PKA holoenzyme exists as a tetrameric complex of two catalytic (C) and two regulatory (R) subunits. Binding of cAMP to the R subunits induces the dissociation and activation of the C subunits (10). Recently, somatic mutations of *PRKACA* (NM_002730), which codes for the α isoform of the C subunit (Cα), have been reported in adenomas of the adrenal cortex (11–15). 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 cosecreting adenoma and subclinical Cushing's syndrome can occur in PA patients (16, 17), the molecular causes for steroid cosecretion have remained uncertain. Herein, 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

Patient samples and clinical data

Patients were diagnosed with PA according to institutional and Endocrine Society Clinical Practice Guidelines (18, 19) and were included in the German Conn's Registry. Baseline clinical characterization included multisteroid analysis of peripheral blood samples as described recently (20). Subtype differentiation was done by imaging and adrenal venous sampling in PA patients who underwent subsequent unilateral adrenalectomy. Surgically resected adrenocortical tissues were examined by a clinical pathologist and molecular and functional characterization of APA tissues were performed subsequently. 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.

DNA extraction and sequencing

In 122 surgically resected unilateral adrenal tissues from PA-patients, genomic DNA was extracted from 113/122 frozen adrenal tissues using the Maxwell Tissue DNA Kit (Promega, Mannheim, Germany) and from 9/122 formalin-fixed, paraffin-embedded (FFPE) adrenal tissues using the FFPE DNA kit (Qiagen, Hilden, Germany) according to each manufacturer's recommendations. DNA quality and concentration were assessed by spectrophotometry.

Whole exome sequencing was performed on gDNA from 58/122 samples as previously described (11). Additionally, in 64/122 samples, exons 4, 6 and 7 of *PRKACA* were amplified by PCR and Sanger sequenced using intron-spanning primers (Exon 4: 5′-ACTGGGACCCCACCTTG-3′ and 5′-AGGCATT-AGGGGAAATGAGG-3′; exons 6 and 7: 5′-GTTTCTGACG-GCTGGACTG-3′ and 5′-AGTCCACGGCCTTGTTGTAG-3′). The PCR program was as follows: denaturation at 95°C for two minutes, eight amplification cycles (15 seconds at 95°C, 15 seconds at 65°C with - 1°C at each cycle, and 30 seconds at 72°C) followed by 30 amplification cycles (15 seconds at 95 C, 15 seconds at 58°C, and 30 seconds at 72°C) and 5 minutes at 72°C. The results of the automated bidirectional Sanger sequencing of the amplicons were analyzed with the Mutation Surveyor software (Soft Genetics).

In silico analysis

Structural images were generated as described previously (11) using the PyMOL software (www.pymol.org). The structure of the mouse holoenzyme (PDB entry 3TNP) was used to display the mutation affecting the Ca subunit of PKA.

Plasmids, cell culture and transfection

Plasmids encoding wild-type human *PRKAR1A, PRKAR2B*, and the mutant *PRKACA*-Leu206Arg have been described previously (11, 21). A plasmid coding for human *PRKACA* (pCMV6-XL4-PRKACA) was purchased from OriGene Technologies (Rockville, MD, USA). The substitution c.262C>G (p.His88Asp) was introduced in the plasmid pCMV6-XL4-

PRKACA with the use of the QuikChange II Site-Directed Mutagenesis Kit (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's protocol and the primers 5′-CTGAAACAGATCGAAGACACCCTGAATGAAAAGC-3′ and 5′-GCTTTTCATTCAGGGTGTCTTCGATCT-GTTTCAG-3′. The complete open reading frame was sequenced to confirm the presence of the specific mutation by direct Sanger sequencing. HEK293AD cells were obtained from ATCC®. Cells were cultured in Dulbecco´s modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, 0.1 mg/ml streptomycin, and 100 U/ml penicillin at 37°C, 5% CO_2. Cell culture reagents were from PAN-Biotech (Pan-Biotech GmbH, Aidenbach, Germany). HEK293AD cells were seeded 24h prior to transfection with the Effectene transfection kit (Qiagen, Hilden, Germany) and the corresponding plasmids according to the manufacturer´s protocol. The molar ratio of C and R subunit DNA was 1:8. All experiments were performed 48h after transfection.

PKA enzymatic activity assay

Cells were washed twice with phosphate-buffered saline (PBS) at room temperature, scraped from the plate, and resuspended in 300μl buffer containing 5 mM Tris-HCl, 2 mM EDTA, pH 7.4. Samples were homogenized using an Ultraturrax device for 20 seconds on ice and centrifuged at 50 000 x g for 30 minutes at 4°C to remove membranes. Expression of Cα, RIα and RIIβ PKA subunits in cell lysates were determined by Western blot analysis with specific antibodies (anti-PKA Cα 1:7000, #4782, Cell Signaling Technology; anti-PKA RIα 1:1000, #610609, BD Transduction Laboratories; anti-PKA RIIβ 1:1000, #610625, BD Transduction Laboratories). PKA catalytic activity was measured with or without the addition of cAMP using the PepTag nonradioactive cAMP-dependent protein kinase assay (Promega, Mannheim, Germany), following the manufacturer´s instructions. Images of the gels were analyzed using the ImageJ software (http://rsbweb.nih.gov/ij). Quantified enzymatic activities were adjusted to the immunoreactivities of the corresponding PKA subunits in the immunoblots in order to compensate for variable transfection efficiencies (22).

CYP11B2 and CYP11B1 immunofluorescence and immunohistochemistry

The antibodies directed against human CYP11B2 and CYP11B1 have been previously described (23). Double immunofluorescence was performed as follows. Adrenal tissue sections were deparaffinised and heat-induced epitope-retrieval was performed in a steamer with Tris-EDTA buffer at pH 9.0 for 45 minutes. Nonspecific staining was blocked with 20% human AB serum in PBS solution for 1h. The slides were incubated overnight at 4°C with a mixture of the mouse monoclonal anti-hCYP11B2 clone 41–17B (1:500) and the rat monoclonal anti-hCYP11B1 clone 80–2–2 (1:50) in 20% human AB serum in PBS. Slides were then incubated with the secondary antibodies donkey antimouse IgG Alexa Fluor 488 (Life technologies #A21202, 1:500) and goat antirat IgG Alexa Fluor 647 (Abcam # ab150167, 1:200) for 1h at room temperature. Coverslips were mounted using Vectashield mounting media with DAPI (Vector Labs, Burlingame, CA, USA).

Additionally, CYP11B2 immunohistochemistry was performed with the previously described anti-hCYP11B2 monoclonal antibody (23).

Steroid metabolome measurement

Determination of multisteroid measurement and grouping for the presence of somatic mutations was done as described before (20). Discriminant analysis was performed based on plasma concentrations of seven adrenal steroids (aldosterone, 18-oxocortisol, 18-hydroxycortisol, corticosterone, 11-deoxycorticosterone, 21-deoxycortisol and cortisol).

Statistical analysis

Results were analyzed using a two-way ANOVA corrected for multiple comparisons by means of Tukey's honest significance test. Statistical significance was considered for those comparisons with P values < 0.05. Statistical analysis was performed using Prism3 (GraphPad Software Inc.).

Results

Prevalence of *PRKACA* variants in APAs

Within a series of 122 APAs cases described in the Supplemental Table 1, 58/122 cases were processed with whole exome sequencing leading to the identification of somatic *PRKACA* mutations in two cases which both were found negative for mutations in *KCNJ5, CACNA1D, ATP1A1, ATP2B3 and CACNA1H*. 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 (Figure 1A). Both mutations were heterozygous and absent in peripheral DNA samples. Therefore, 64 additional cases were processed by direct bidirectional Sanger sequencing for mutations in *KCNJ5, CACNA1D, ATP1A1, ATP2B3, CACNA1H* and *PRKACA*. In total, none of the two identified *PRKACA* mutations were found in the remaining 120/122 APA cases. Thereby, the overall prevalence of *PRKACA* variants was 2/122 (1.6%) in our examined series.

case 1

A 32-year-old female patient of African origin with a BMI of 26.5 kg/m^2 and no personal or familiar medical history of hypertension presented with a hypertensive episode (SBP > 210 mm/Hg) while hospitalized for a planned myomectomy. Following antihypertensive medication with an ACE inhibitor, her blood pressure (BP) remained elevated in association with severe hypokalemia at 2.3 mmol/L. She was diagnosed with PA based on an aldosterone-to-renin ratio (ARR) of 131 ng/mU (upper cut-off: 10) with plasma aldosterone concentration (PAC) at 552 ng/L and plasma renin activity (PRA) at 4.2 mU/L and postsodium infusion PAC at 329 ng/L (upper cut-off: 50). Serum cortisol and plasma ACTH were measured at 8 AM and found to be in the normal range with 9.3 μg/dL and 22.9 pg/mL respectively. Serum cortisol was suppressed at 1.8 μg/dL following administration of 1 mg of

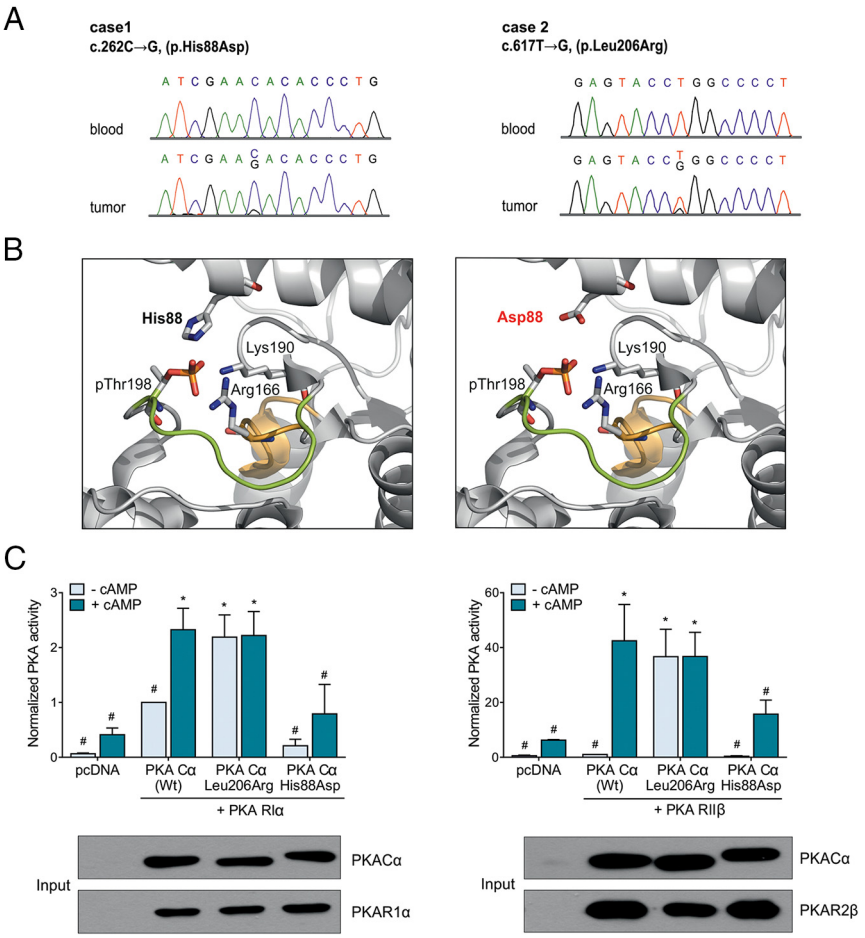

Figure 1. Identification and functional characterization of *PRKACA* variants **Panel A** shows two paired sequence chromatograms of tumor and peripheral blood from case 1 and case 2. In case 1 (left) a somatic variant in *PRKACA* (c.262C→G) was identified in the aldosterone-producing adenoma, resulting 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 Ca subunit. 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 coexpressing Ca (mutant or nonmutant) and RIa (left) or RIIß (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 $P < .05$ for the comparison with the wild-type PKA activity in absence of cAMP; "#" indicates $P < .05$ for the comparison with the wild-type PKA activity in presence of cAMP. Representative input blots are shown below the graphs.

dexamethasone overnight. A lateralization index of 24.2 during adrenal venous sampling indicated unilateral autonomous secretion of aldosterone by the left adrenal gland. Accordingly, the patient was referred for unilateral adrenalectomy. The surgically resected specimen presented a 9 mm adrenocortical adenoma. Postsurgical serum potassium levels normalized and BP was measured at 125/100 mm/Hg under methyldopa monotherapy at one-year follow-up.

case 2

A 51-year-old Caucasian female patient presented with a medical history of 17 years of hypertension. She was diagnosed with PA at age 34 and medically treated until presentation in our hospital for persistent hypokalemic hypertension. Her BMI of 32.3 kg/m^2 was associated with dyslipidemia and type 2 diabetes. Baseline ARR was elevated with 16.4 ng/mU (upper cut-off: 10) and a confirmatory captopril test was performed, resulting in a pre and post-test PAC at respectively 154 and 164 ng/L and PRC of respectively 9.4 and 12.2 mU/L. Late-night salivary cortisol and urinary free cortisol were elevated with 2.1 ng/mL (upper cut-off: 1.8) and 285 μg/24h (reference interval 50–150), respectively, with low baseline plasma ACTH of 6 pg/ml. 1 mg dexamethasone suppression testing resulted in an elevated serum cortisol level of 5.1 μg/dL (upper cut-off: 1.8). MRI indicated the presence of a single nodule in the right adrenal gland and adrenal vein sampling demonstrated a lateralization index of 7.3 indicating a unilateral autonomous secretion of aldosterone by the right adrenal gland. The patient underwent unilateral adrenalectomy and pathological examination revealed a 12 mm adenoma of the adrenal cortex. Postsurgical serum potassium levels normalized and sodium loading test revealed good suppression of aldosterone from baseline 50.9 ng/L to levels < 35 ng/L. At the first yearly follow-up, circadian cortisol normalized with a late-night salivary cortisol at 0.9 ng/mL and baseline ARR was found normal at 9.2 ng/mU.

For both case 1 and case 2, the AVS results and cross-sectional imaging by MRI are detailed in Supplemental Table 2 and Supplemental Figure 1 respectively.

Structural analysis of identified *PRKACA mutations*

The newly identified c.262C>G (p.His88Asp) mutation in exon 4 of *PRKACA* found in the adenoma of case 1 affects the residue His88 situated at the beginning of the Ca helix. 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 (24) (Figure 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 RIa subunit of PKA (25). In case 2, the c.617A>C (p.Leu206Arg) mutation in exon 7 of *PRKACA* is identical to that previously described by our group and others in cortisol-producing adenomas (11–13). Leu206 is part of the hydrophobic $P + 1$ motif of the C subunit, important for the structure of the enzyme and involved in substrate recognition (26, 27). Mutation of this residue impairs the interaction between C and R subunits and, thus, leads PKA to a state of constitutive activation (3).

Functional characterization of *PRKACA mutations*

Measurement of PKA catalytic activity by means of enzymatic experiments demonstrated that mutated His88Asp Cα subunit of PKA resulted in a significantly lower enzymatic PKA activity in comparison to the wild-type enzyme when cotransfected with either RIα or with RIIβ in HEK293 cells, both in presence and in absence of cAMP (Figure 1C). On the contrary, mutated Leu206Arg resulted in a constitutive elevated activity not suppressed by any of the regulatory subunits, as previously described (11).

Histology, immunohistochemistry and CYP11B2/ CYP11B1 double immunofluorescence

The APAs 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 (Figure 2A) showed expression of CYP11B2 and, to a lesser extent, CYP11B1 in case 1 (carrying the His88Asp variant); conversely, the adenoma cells in case 2 (with the Leu206Arg mutation) were predominantly positive for CYP11B1, although CYP11B2 expression was detected in few adenoma cells (Figure 2B).

Plasma steroid metabolome

Multisteroid analysis of peripheral plasma from case 2, who had biochemical and clinical evidence of subclinical Cushing's syndrome, found hybrid serum steroids 18-oxocortisol and 18-hydroxycortisol to be elevated (data not shown). Thereby, the multisteroid fingerprint (20) was grouped into patients carrying *KCNJ5* somatic mutations (Figure 2C). In contrast, multisteroid analysis from case 1 resulted in grouping into the patient subgroup with adenomas devoid of somatic mutations in any of the candidate genes.

Discussion

This is the first report of somatic mutations in the *PRKACA* gene in adrenocortical adenomas of patients with PA. Furthermore, to our knowledge, the missense substitution in *PRKACA* resulting in p.His88Asp has not been identified previously in any exome variant database and was not present in any of the in-house 7000 exomes. Analysis of the crystal structure of the PKA Cα subunit has shown that the interaction between His88 and phospho-Thr198 is the only direct electrostatic interaction between the two lobes of the PKA catalytic subunit (24, 28). Interestingly, this interaction is present in the closed conformation (ie, in the presence of ATP) and is lost during the opening of the active site cleft between the two lobes (28). Previous structural and kinetic analysis performed with purified *E.coli* PKA Ca point to an important role of His88 in the interaction with substrate peptides, physiological inhibitors and the RI subunit, ultimately leading to a change in enzyme kinetics (25, 28). We investigated the enzymatic activity of p.His88Asp in cell lysates and found reduced basal and maximal (ie, cAMP-stimulated) activities compared to the wild-type enzyme. Overall, our results are in favor of a partial loss-of-function mutation. Accordingly, the pathological and histological examination of the adenoma in case 1 did not show strong CYP11B1 expression in line with a lack of increased cAMP signaling. Cross-signaling of the p.His88Asp with modulation of CYP11B2 expression cannot be excluded but is less likely to be causative for the overt functional, biochemical and clinical phenotype of aldosterone hypersecretion in this patient. In fact, exome sequencing also indicated the presence of an *ARMC5* germline genetic alteration in this patient, which was predicted to be benign by *in silico* analysis (c.125C>G; p.Thr42Arg; known SNP: rs28451331). A previous report had indicated that *ARMC5* germline variants may be associated with PA in 6/56 cases examined, with all predicted damaging mutations occurring in African American patients (29). While the association of these two rare genetic alterations (germline *ARMC5* and somatic *PRKACA* variants) might have contributed to the onset of PA in this patient, we demonstrate that the His88Asp variant was not associated with clinically overt hypercortisolism.

Conversely, the p.Leu206Arg variant is the most frequent somatic mutation to be found in cortisol-producing adenomas and has been associated with severe forms of adrenal Cushing's syndrome (11). As previously described, *ex vivo* analysis indicates that the substitution of leucine by a positively charged arginine in the catalytic subunit impairs the interaction with the regulatory subunit, leading to a constitutive activation of PKA (11, 21).

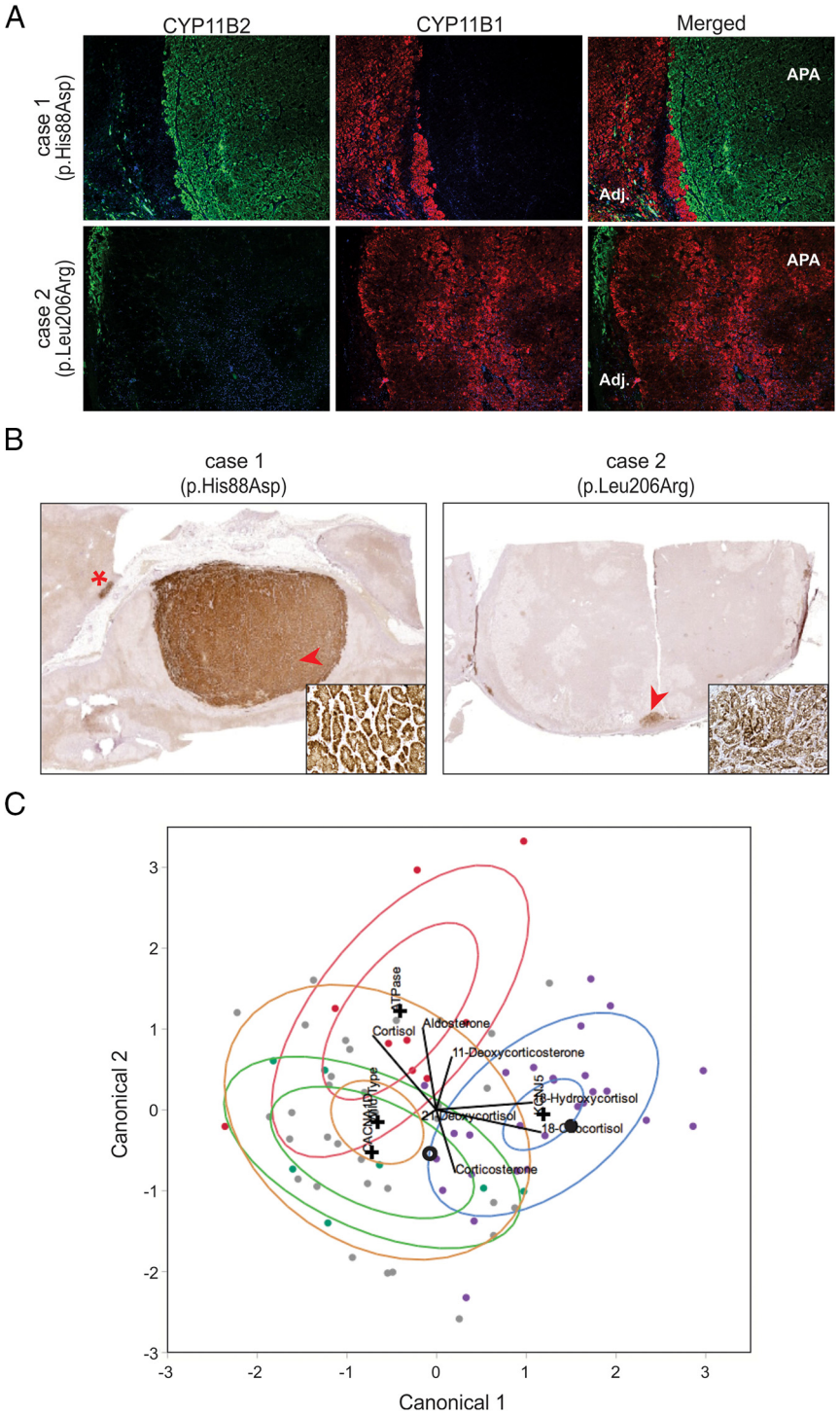

Figure 2. Functional and biochemical characteristics of APAs carrying *PRKACA* variants **Panel A** shows the double immunofluorescence staining of CYP11B2 and CYP11B1 in tumor (APA) and adjacent (Adj.) adrenal tissue. The APA in case1 (upper row) is positive for CYP11B2 and in case 2 (lower row) is positive for CYP11B1. **Panel B** highlights immunohistochemical staining of CYP11B2 in the adenoma (arrows) and adjacent (*) adrenal tissue. Pictures are presented with both magnifications of x20 (larger frame) and x200 (smaller frame). **Panel C** 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, gray) 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.

In contrast to adrenal Cushing's syndrome, *PRKACA* p.Leu206Arg somatic mutations in patients with unilateral hyperaldosteronism has not yet been reported. Although subclinical Cushing´s syndrome has been observed in up to 21% of PA cases in some series (30), no *PRKACA* mutations have been identified in two recent reports (31, 32). In fact, considering the current series of 122 APAs, *PRKACA* mutations seem to occur only in a very small fraction of PA cases. CYP11B1 expression was positive in adenoma cells composed of both small compact cells and large clear cells whereas CYP11B2 was only positive in few adenoma cells and adjacent tissue. These morphological findings corresponded to a clinical phenotype of hypokalemia and hyperaldosteronism associated with biochemical hypercortisolism. Overall, the two presented cases highlight the spectrum of functional and genetic findings associated with unilateral PA: APAs with known driver mutations or unexpected somatic variants, adenomas with functional and genetic heterogeneity, unilateral double adenomas with distinct genetic features and coexistence of functionally active or inactive adenomas with zona glomerulosa hyperplasia.

While cortisol excess might have contributed to elevated BP and hypokalemia, in variance to earlier reports, in the current case overt Cushing's syndrome was not detected on a clinical basis. Biochemically, autonomous cortisol secretion was evident. Furthermore, serum hybrid steroids in case 2 were elevated in accordance with previously published observations by Späth and colleagues (17) on PA patients presenting with aldosterone and cortisol cosecretion. Accordingly, the hybrid steroids measurements in case 2 segregated with the pattern otherwise

found in patients carrying *KCNJ5* somatic mutations.

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 very rare events. The molecular basis for cosecretion of aldosterone and cortisol as observed in a subgroup of PA patients remains to be elucidated.

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