**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 elevated aldosterone to renin ratio (ARR) often associated with hypokalemia. So far, at least 14 candidate genes are implicated in PA: \textit{PRKACA}, \textit{CAH1}, \textit{ATP1A2}, \textit{ATP2B1}, and \textit{CAH2} mutations result in electrophysiological abnormalities, consecutive increase in intracellular calcium levels and ultimately increase in the expression of \textit{CYP11B2}, which encodes aldosterone synthase required for aldosterone biosynthesis. Another key activator for adenocortical steroidogenesis and cell growth is cyclic AMP (cAMP), a second messenger, which regulates the activation of protein kinase A (PKA). Recently, somatic mutations of \textit{PRKACA}, which codes for the isoform of the C subunit of PKA in particular, the most frequent mutation (p.Leu206Arg) was found to be restricted to cortisol producing adenomas (CPA) associated with overt Cushing syndrome. Although aldosterone- and cortisol-secreting adenomas and subclival Cushing’s syndrome may occur in PA patients ([11, 12]), the molecular causes for steroid co-secretion have remained unclear. We report on in depth investigation of two cases of PA presenting with somatic mutations of \textit{PRKACA} identified by exon sequencing and evaluated for their clinical and molecular phenotypes.

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**Materials and Methods**

Patients were diagnosed with PA according to institutional and Endocrine Society Clinical Practice Guidelines and were included in the German Con’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 adrenal tissue specimens were examined by a clinical pathologist. Identification of \textit{PRKACA} somatic variants in APA was performed in the 122 APA by whole-exome sequencing [8/122] or direct bidirectional longer sequencing [50/122], followed by in situ analysis of the enzymatic activity of \textit{PRKACA} variants using the PepTag non-radioactive protein kinase assay and functional characterization by double immunofluorescence of \textit{CYP11B1} and \textit{CYP11B2} 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.

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**Results**

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**Table 1: Clinical and biochemical parameters of the PA-patients with \textit{PRKACA} somatic mutations.**

**Figure 1: Identification and functional characterization of \textit{PRKACA} variants**

**Figure 2: Functional and biochemical characteristics of APAs carrying \textit{PRKACA} variants**

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**Discussion**

We describe the first time \textit{PRKACA} mutations in two cases of PA-patients: a novel \textit{PRKACA} variant (p.His88Asp) occurring in a case of sudden onset of PA and a \textit{PRKACA} mutation (p.Leu206Arg) in context of hypokalemic aggravation of long term hypertension. These genetic alterations were not found in a subsequent series of 122 APAs and thereby appear to be of infrequent events. However, it remains unclear whether the molecular mechanisms of aldosterone and cortisol as observed in a subgroup of PA patients remains to be elucidated.