Reviewer's report

Title: A case of autonomous cortisol secretion in a patient with subclinical Cushing's syndrome, GNAS mutation, and paradoxical cortisol response to dexamethasone

Version: 0 Date: 14 Oct 2018

Reviewer: Stefan Bilz

Reviewer's report:

The authors describe an interesting case of a 65 years old patient with (1) bilateral macronodular adrenal disease (2) primary aldosteronism, (3) ACTH independent subclinical cushing's syndrome (4) spotty skin pigmentation (5) a variety of tumors in extraadrenal tissues (meningioma, pituitary adenoma, thyroid, colon, heart, pancreas) and (6) absence of a germline PRKAR1A mutation. The major novelty described is a paradoxical cortisol response during the Liddle test that has previously been found in patients with PPNAD and the Carney complex.

Although AVS was indicative of bilateral aldosterone secretion left adrenalectomy was performed and the workup of the adenoma removed showed a somatic GNAS mutation (p.Arg201His) usually encountered in MAS. IHC of the surgical specimen indicated expression of enzymes of adrenal cortisol and androgen but not aldosterone synthesis. Unilateral left adrenalectomy was followed by a subtle improvement in biochemical hypercortisolism while aldosterone oversecretion was not affected.

The authors conclude that a paradoxical cortisol response to dexamethasone may provide a diagnostic clue to cAMP-PKA-pathway dependent hypercortisolism including GNAS mutations.

General comments

It is certainly worthwhile to report this very intriguing case. However, several points should be clarified or discussed in more depth for a better understanding of the clinical and genetic results.

Specific comments:

Although primary aldosteronism is not part of CNC the PRKAR1A genetic analysis should be described in detail. Did the methods employed account for copy number variants?
If aldosterone synthase (CYP11B2) was immunohistochemically absent in the surgical specimen, how can the authors explain the clear biochemical evidence for bilateral aldosterone oversecretion?

Both, the co-occurrence of AIMAH and primary aldosteronism (Tokumoto et al. BMC Surgery (2017) 17:97) and of primary aldosteronism, subclinical cushing's syndrome and somatic GNAS mutations (Nakajima et al. Endocrine Journal (2016) 63: 199) have been previously described. It may be helpful to refer to this work in the discussion.

In the original publication by Stratakis et al. the paradoxical increase in the 24h FUC excretion was not obvious until day 5 & 6 of the Liddle test when the high dexamethasone dose (2 mg q 6 hours) was applied. In the current patient the increase in the 24h FUC was much more pronounced when the low dexamethasone dose (0.5 mg q 6 hours) was applied and decreased thereafter when the high dose was given. Can the authors offer an explanation for this discrepancy?

Cases of AIMAH, which may present with quite subtle cushingoid features, and somatic GNAS mutations but no apparent paradoxical reaction to dexamethasone have been reported (Hsiao et al., J Clin Endocrinol Metab (2009) 94: 2930). This should be discussed.

Does the pattern of the additional tumors observed in the patient follow a pattern that is compatible with enhanced cAMP- PKA- and ultimately Wnt signaling (Salpea & Stratakis, Mol Cell Endocrinol. (2014) 386: 85)? Please discuss.

Based on the authors observations it seems justified to conclude that in patients with primary aldosteronism and subclinical CS/bilateral macronodular disease a paradoxcial increase in 24h FUC may point to enhanced cAMP- and PKA signalling and/or an underlying somatic GNAS mutation, respectively.
Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Unable to assess

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No

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