Published in final edited form as:

J Hypertens. 2015 December ; 33(12): 2546–2549. doi:10.1097/HJH.0000000000000748.

Normoaldosteronemic aldosterone-producing adenoma: immunochemical characterization and diagnostic implications

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Abstract

Background—A high aldosterone–renin ratio (ARR) is commonly used to identify primary aldosteronism, but the ARR is high when renin is low, even if plasma aldosterone concentration values are normal, suggesting the existence of ‘normoaldosteronemic’ primary aldosteronism. However, most such cases did not undergo adrenalectomy; moreover, because of the lack of antibody for the human CYP11B2 (aldosterone synthase), conclusive demonstration of a normoaldosteronemic aldosterone-producing adenoma was not possible thus far.

Method—In 2003, a lady presented with severe hypertension a right adrenal nodule, low renin, high ARR, but normal plasma aldosterone concentration. As adrenal vein sampling showed lateralized aldosterone secretion, she underwent left adrenalectomy, which consistently normalized blood pressure (BP) and renin during 11-year follow-up.

Result and conclusion—The development of a novel monoclonal antibody for the human CYP11B2 in 2014 allowed immunochemically identification of a CYP11B2-positive adenoma in the resected adrenal. Moreover, this case unequivocally demonstrates for the first time the existence of normoaldosteronemic aldosterone-producing adenoma, which suggests that many cases of ‘low renin-essential hypertension’ might instead have a surgically curable form of primary aldosteronism.

Keywords

aldosterone-producing adenoma; aldosterone synthase; immunohistochemistry; low renin-essential hypertension

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Conflicts of interest

There are no conflicts of interest.

INTRODUCTION

Primary aldosteronism is usually identified by a high aldosterone–renin ratio (ARR), alongside elevated plasma aldosterone concentration (PAC) values. However, available guidelines for the case detection of primary aldosteronism do not recommend use of a certain minimum threshold for PAC when calculating the ARR, mainly because of the variability of available PAC assays [1,2]. This approach might lead to diagnosis as normoaldosteronemic primary aldosteronism in many cases, wherein PAC is normal and the ARR is overinflated by low plasma renin.

Accordingly, the boundary between low-renin primary (essential) hypertension and normoaldosteronemic primary aldosteronism remains somewhat vague and unclear, because most such latter cases did not undergo surgical confirmation and even when adrenalectomy was performed, demonstration of an aldosterone-producing adenoma (APA) could not be obtained, because of the lack of specific antibodies for the human CYP11B2 (aldosterone synthase).

The development of such antibodies has been a challenge for decades because of the high degree of homology between CYP11B1 (11 β -hydroxylase) and CYP11B2 [3]. Therefore, even when an adenoma was found at pathology, whether it was the source of the aldosterone excess remained uncertain. Moreover, the criterion of correction of the hyperaldosteronism could obviously not be applied to these cases.

We herein report on a unique case with normoaldoster-onemic primary aldosteronism that was long-term cured with left adrenalectomy. Using novel monoclonal antibodies for CYP11B1 and CYP11B2 developed inhouse, we could unequivocally prove the presence of an APA in this case. This is the first conclusive demonstration of the existence of a normoaldosteronemic APA. Moreover, it suggests that many cases that are labelled as low-renin essential hypertension could instead harbour a small surgically curable APA.

METHODS

Clinical case

A 32-year-old lady presented in 2003 with severe hypertension (190/120 mmHg) and mild hypokalemia (3.4 mEq/l) while she was taking ramipril 5 mg/day and felodipine 5 mg/day. She was prescribed K chloride supplementation and switched to verapamil 180 mg/day and doxazosine 2 mg/day to allow undertaking a workup for secondary forms of arterial hypertension. This showed low plasma renin activity (PRA) both at baseline (0.40 μ g/l per h) and after captopril challenge (0.64 μ g/l per h), but repeatedly normal plasma aldosterone (5.4 and 4.3 ng/dl) (149.6 and 119.1 pmol/l, respectively) and cortisol concentrations (100 ng/ml). The ARR was 13.3 ng/dl/ μ g/l/h (NV <26). An abdominal ultrasound examination showed a node in the right adrenal, which was confirmed to be 20 mm in diameter at MR; the left adrenal gland was apparently normal.

Based on the low PRA, the hypokalaemia, and the resistance of BP to treatment, she underwent adrenal vein sampling without cosyntropin stimulation [4], which was bilaterally

selective and showed left lateralization of aldosterone secretion (left lateralization index = 3.2). Long PCR testing for familial hyperaldosteronism type 1 showed no chimeric gene [5]. As her BP fell with spironolactone 100 mg/d she consented to undergo left laparoscopic adrenalectomy, which was performed uneventfully in 2004. Pathology examination showed an 8-mm adenoma made of zona glomerulosa-like cells. Sequencing of the tumour DNA showed no KCNJ5 mutations [6].

Normalization of BP values and PRA and PAC levels at repeated testing during long-term (12 years) follow-up was observed. In 2006 and 2012, two 24-h ambulatory BP monitoring, while she was off of any treatment, showed values of $112 \pm 12/67 \pm 13$ and $130 \pm 11/74 \pm 13$ mmHg, respectively, with a preserved circadian rhythm. These findings indicated complete cure of the hypertension and a diagnosis of APA by the 'four corners criteria' [7].

In 2014, using novel monoclonal antibodies for human CYP11B2 and CYP11B1 [8], immunochemical characterization of the adrenal gland removed in 2004 was performed. This showed a predominant expression of CYP11B1 throughout the excised adrenal gland, but CYP11B2 staining of a small adenoma, thus unequivocally demonstrating an APA [Fig. 1, panel (a)–(d)]. Double and triple immuno-fluorescence for CYP11B1, CYP11B2, and 17 α -hydroxylase did not evidence coexpression of CYP11B1 and CYP11B2 enzymes in the same adrenocortical cells [Fig. 1, panel (g)]. However, they demonstrated coexpression of 11 β -hydroxylase (CYP11B1) with the zona fasciculata marker 17 α -hydroxylase [Fig. 1, panel (h)].

Immunohistochemistry and immunofluorescence

Double immunohistochemistry for CYP11B2 and CYP11B1 [8] and triple immunofluorescence for CYP11B1, CYP11B2, and 17 α -hydroxylase [9] of the paraffin-embedded resected adrenal was performed as previously described [10].

DISCUSSION

The diagnosis of APA was confirmed in this case beyond any reasonable doubt at pathology, at long-term follow-up by the cure of the primary aldosteronism and hypertension [7], and also by immunochemical characterization of the excised adrenal. The PAC values were consistently normal within the range and thus led to a diagnosis of normoaldosteronemic APA.

This case illustrates, in our view, several interesting points. First, the small APA produced enough aldosterone to cause severe hypertension and blunt renin, but not to raise PAC to abnormal values: only the finding of mild spontaneous hypokalaemia with a low renin suggested the presence of primary aldosteronism at the baseline evaluation. Second, the immunochemical characterization revealed quite interesting features: even though the APA was composed of an admixture of CYP11B2 and CYP11B1-positive cells, for example of aldosterone and cortisol-producing cells, the CYP11B1 and CYP11B2 were never coexpressed in the same cells. Instead, the CYP11B1-positive cells evidenced a coexpression of 17 α -hydroxylase, indicating that they make cortisol. Furthermore, the multiple islets of CYP11B2-expressing cells in the subcapsular region of the adrenal

indicated that the dominant aldosterone-producing node was unable to suppress aldosterone production in the surrounding adrenal cortex.

Moreover, these findings indicate that the disease is often multifocal from the pathological standpoint [11], which casts doubts about the appropriateness of adrenal-sparing surgery for primary aldosteronism.

The severity of the syndrome of primary aldosteronism does not always correlate with the degree of abnormality in the production of aldosterone by the adrenal [12–15]. This led to the search for new mineralocorticoids that might be secreted by adrenal adenomas and to the isolation of other mineralocorticoids, including 18-oxocortisol [15]. Their biological activity is, however, much lower than that of aldosterone.

Hence, overall, these findings suggest that currently many cases that we currently dismiss as ‘low renin-essential hypertension’ might instead have small undetected APA. The latter can be diagnosed only with a high degree of alertness, and use of AVS in selected cases.

Acknowledgments

The authors would like to thank the Foundation for Advanced Research in Hypertension and Cardiovascular Diseases (www.forica.it), the FP7-funded COST ADMIRE network (BM1301), and Horizon2020 Grant SEP-210176891. This work was supported by The Foundation for Advanced Research in Hypertension and Cardiovascular Diseases (www.forica.it), the Società Italiana dell’Ipertensione Arteriosa, and the University of Padua.

Abbreviations

APA	aldosterone-producing adenoma
ARR	aldosterone–renin ratio
CYP11B1	11 β -hydroxylase
CYP11B2	aldosterone synthase
CYP17α	17 α -hydroxylase
PAC	plasma aldosterone concentration
PRA	plasma renin activity

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FIGURE 1. CYP11B1 and CYP11B2 adrenal immunohistochemistry, and triple immunofluorescence for CYP11B1, CYP11B2, and 17 α -hydroxylase: panels (a)–(d) are different areas at different magnification of the same adrenal double stained for CYP11B1-80-7-5 and CYP11B2 antibodies. Panels (e)–(h) are images of triple immuno-fluorescence for CYP11B1, CYP11B2, and 17 α -hydroxylase at the same magnification [10]. Panels (e) and (f) show CYP11B1 and CYP11B2, respectively, evidencing the different cell localization of the two enzymes. Panels (g) and (h) are the merged image of CYP11B2 and CYP11B1 staining, CYP11B1, and 17 α -hydroxylase, respectively. For a colour rendition of this figure, the reader is advised to see supplemental data, <http://links.lww.com/HJH/A563>.