Mitotane Treatment Combined With Unilateral Adrenalectomy in a Patient With Primary Pigmented Nodular Adrenocortical Disease Caused by a Start Codon Mutation of PRKAR1A Gene

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Research

Keywords: primary pigmented nodular adrenocortical disease (PPNAD), Carney complex (CNC), PRKAR1A (Protein Kinase CAMP-Dependent Type I Regulatory Subunit Alpha) mutation, Cushing's syndrome (CS), low-dose Mitotane

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Abstract

Background: Primary pigmented nodular adrenocortical disease (PPNAD) is a rare cause of Cushing's syndrome (CS). This study aimed to explore molecular basis and treatment strategy in a patient with PPNAD.

Methods: Whole-exome sequencing (WES) was used to reveal the mutation of PRKAR1A gene, with immunohistochemistry (IHC) to observe the expression of mutant PRKAR1A. Low-dose Mitotane followed by unilateral adrenalectomy were performed to control hypercortisolism.

Results: A case of 45-year-old female present with classical hypercortisolism as excessive weight gain, central obesity, and intractable hypertension. She experienced adrenal adenoma surgery 10 years ago with no improvement. Low dosage of Mitotane was used for 7 months to control the severe hypercortisolism. Then laparoscopic unilateral adrenalectomy was performed and pathological features supporting PPNAD. The germline mutation (c.1A>G) in the start codon of PRKAR1A (Protein Kinase cAMP-Dependent Type I Regulatory Subunit Alpha) gene was identified. Notably, the body weight and hypertension were improved obviously one year later even if she discontinued with the Mitotane treatment.

Conclusion: Low-dose Mitotane followed by unilateral adrenalectomy showed satisfied treatment effect in this patient, which may be an alternative treatment for PPNAD patient instead of bilateral adrenalectomy.

Introduction

PPNAD, a rare cause of hypercortisolism, accounting for only 0.6%-1.9% in Cushing's syndrome (CS) [1, 2], is resulted from spontaneous overproduction of cortisol by bilateral adrenal [3]. Pathologically, PPNAD is characterized by multiple black or brown, limited micronodules containing lipofuscin in the adrenal cortex, usually involving bilateral adrenal glands [4]. The Carney complex (CNC) is a rare hereditary endocrine disorder, characterized by myxomas (mainly in the heart, skin and breast), abnormal cutaneous and mucosal pigmentation, blue nevus, multiple endocrine neoplasias including primary pigmented nodular adrenocortical disease (PPNAD). Approximately, 70% of CNC cases were familial and follow autosomal dominant inheritance pattern [5]. For CNC patients, hypercortisolism caused by PPNAD is the most common clinical manifestation, which can be detected in 90% of patients [1]. Inactivating mutations of PRKAR1A gene have been demonstrated to be related with the disorder. PRKAR1A is a tumor suppressor gene that encodes type I- α regulatory subunit of protein kinase A (PKA), playing an important role in cAMP signal transduction. The mutation of PRKAR1A gene was reported to be related with multiple endocrine neoplasias [5].

Herein, we report a patient presenting with typical symptoms of Cushing's syndrome (CS) due to prolonged exposure to excessive secretion of cortisol, was revealed to be afflicted with PPNAD. Low dose

of Mitotane therapy followed by unilateral adrenalectomy showed satisfied treatment effect in this patient, who was identified a start codon mutation of PRKAR1A gene.

Patient And Methods

Patient

This was an observational study. Phenotype and genotyping analysis were described in a patient with PPNAD. The low-dose Mitotane and unilateral adrenalectomy protocol were used for the improvement of CS. The patient gave informed consent for this study.

Whole-exome sequencing and data analysis

Genomic DNA of the patient and family members was automatically isolated from peripheral blood lymphocytes using the QuickGene DNA Whole Blood Kit L (Kurabo, Osaka, Japan) according to the manufacturer's instructions. We accomplished the targeted exome design and capture on the Access Array system and Illumina Sequencing System (Fluidigm, San Francisco, CA, USA). The entire coding regions and exon–intron boundaries of the genes were amplified by multiplex PCR using the Access Array™ microfluidics platform (Fluidigm, South San Francisco, CA, USA). PRKAR1A gene mutation was confirmed by direct sequencing polymerase chain reaction (PCR) (primers sequences will be provided upon requested). The PCR products were directly sequenced by Sanger sequencing.

Immunohistochemistry (IHC) for PRKAR1A

The adrenal tissues were fixed in 4% formalin and then embedded in paraffin wax. Tissue sections were made at 4µm and stained with Hematoxylin-eosin (HE). Before immunohistochemistry was performed, the tissue sections were dewaxed and antigen was repaired for complete exposure. Then the tissue sections were processed to inactivate endogenous peroxidase with 3% hydrogen peroxide and incubated with the primary antibody for 12 hours at 4°C. Rabbit polyclonal antibody against PRKAR1A (Cat. no. PA5-62112, Invitrogen, California, USA, working dilutions 1:250) was used as the primary antibody. After washing with Tris buffered saline Tween (TBST) for 10 minutes, the tissue sections were incubated with the secondary antibody conjugated with horseradish Peroxidase (HRP), which can catalyze 3,3'-diaminobenzidine tetrahydrochloride (DAB) to produce brown precipitation. And positive reactions were visualized with DAB. The tissue sections were lightly counterstained with hematoxylin.

Results

Clinical Data

A 45-year-old female with classical presentation of hypercortisolism, was transferred from the Department of Urology in Shanghai 9th hospital. The patient was diagnosed with right adrenal adenoma ten years ago owing to weight gain, hypertension and right lumbar pain. She accepted the surgery of right adrenal adenoma resection. But the cushingoid feature, such as excessive weight gain, central obesity

and hypertension, etc., did not improve after the surgery. She experienced progressive weight gain from 45Kg to 95Kg in the past decade, with progressing edema in the face and lower limbs. Moreover, she took anti-hypertensive drugs with poorly control of blood pressure by Nifedipine Controlled-release Tablets (60mg/day), Olmesartan Medoxomil (20mg/day) and Metoprolol Tartrate Tablets (25mg/day). Hirsutism and acne also developed gradually with menstrual cycle disturbances. Her family history was negative for similar presentations.

Upon admission, she showed the typical hypercortisolism appearance, including moonlike face, abdominal fat deposits, dorsal and supraclavicular fat pads, facial acne, slight hirsutism (Fig. 1A). She weighed 95Kg and her height was 159cm with body mass index (BMI) 37.6kg/m². High blood pressure (165/101mmHg) was noticed with the heart rate of 78 bpm under three or four types of anti-hypertension drugs. Black and brown moles were observed on the patient's face and pigmented skin was notice in the hypochondria region (Fig. 1C, 1D). Wide purple striae was observed on the skin of abdomen, waist and thighs (Fig. 1E). Ecchymoses and pitting edema were also found with the bilateral lower limbs.

The laboratory tests revealed that morning cortisol level was close to the upper limit of the reference range (23.2ug/dl, ref: 5-25ug/dl) with disturbed circadian rhythm of cortisol secretion (4PM 18.8ug/dl, 0AM 21.7ug/dl, ref: 2.5-12.5ug/dl), elevated urinary free cortisol (UFC) level (171.7ug/24h, ref: 21-111ug/24h). But morning serum ACTH levels showed significantly reduced or undetectable (8AM 11.7pg/ml, 4PM 34.7pg/ml, 0AM 19.3pg/ml, ref: 0-46pg/ml). High-dose dexamethasone suppression test (HDDST) (8mg dexamethasone orally overnight) failed to suppress serum cortisol level (23.2→20.7ug/dl) and UFC level (171.7→147.84ug/24h), indicating the high level of cortisol autonomously secreted by bilateral adrenal glands. Serum androgen levels including testosterone (T-2, 0.35ng/ml, ref: 0.11-0.57ng/ml), androstenedione (AD, 2.95ng/ml, ref: 0.3-5.5ng/ml) seemed to be in the reference range. Normal aldosterone level 143.85pg/ml (ref: 65.2-295.7pg/ml) is helpful to exclude primary hyperaldosteronism (PHA). The detailed laboratory tests are listed in Table 1. Meanwhile, multiple micronodules in bilateral adrenal glands were discovered by computed tomography (CT) scan of adrenal with the largest size of 8mm on the right side (Fig. 2A, 2B). Echocardiography showed mild left ventricular hypertrophy without cardiac myxoma. Pituitary MRI showed partial empty sella. Ultrasound of thyroid was found to be normal with no signs of the nodule.

Sequencing of the PRKAR1A gene

The patient underwent genetic diagnosis by Whole-exome sequencing (WES). The heterozygous missense mutation NM_212472.2: c.1A>G, p.(Met1Val) in the start codon of PRKAR1A was identified, which is validated by the Sanger sequencing of PCR products (Fig. 2E). This variant was not present in dbSNP135, the 1000 genomes project nor ExAC database. Moreover, mutation analysis of PRKAR1A gene was negative in both her parents and daughter, who have no similar presentations (Fig. 2F).

Treatment progress

Based on her typical manifestations, the most likely diagnosis was considered as hypercortisolism caused by PPAD. However, severe obesity and poorly controlled hypertension tended to be the barrier of surgical anesthesia. Then the patient accepted Mitotane therapy to suppress hypercortisolism provisionally. After seven months of low-dose Mitotane intake (The initial dose was 250 mg/day for one week, then gradually increased to 500 mg/day), her body weight reduced to 80kg and hypertension improved significantly. Serum cortisol (8AM 23.2→16.0ug/dl, 4PM 18.8→14.4ug/dl, 0AM 21.7→15.3ug/dl) and UFC levels (171.7→25.68ug/24h) showed decreasing (Table 1 and Fig. 3). Her blood pressure could be controlled with Nifedipine, Olmesartan and Metoprolol. And the size of adrenal nodules was observed to be reduced significantly by CT scan (Fig. 2C). Subsequently, the patient accepted laparoscopic right adrenalectomy. Considering the existence of autonomous secretion left adrenal, serum cortisol level reduced to 9.0ug/dl on the next day after surgery (Table 1 and Fig. 3). Mitotane was suggested to maintain 250mg/day. But the patient discontinued this medicine one month later.

Pathological analysis and immunohistochemistry (IHC)

Pathological analysis of the resected adrenal gland was consistent with the features of PPAD. Multiple pigmented cortical nodules composed of fine granular pigment cells filled with lipofuscin and atrophic inter-nodular cortex were observed by Hematoxylin-eosin staining (Fig. 4A, 4B). By using immunohistochemistry (IHC), the expression of PRKAR1A was found to be remarkably reduced, compared with the peri-tumorous normal tissues from a patient with adrenal adenoma, indicating that the mutation located in the start codon impairs the translation of the PRKAR1A (Fig. 4C, 4D).

Follow-up

Three months after laparoscopic right adrenalectomy, the patient's blood pressure was well controlled with the anti-hypertensive drugs. Her ACTH levels were still less than 10pg/ml, whereas the cortisol level showed significantly reduced but disturbed circadian rhythm of cortisol (8AM 6.65 ug/dl, 4PM 6.87 ug/dl, 0AM 6.15ug/dl) (Table 1 and Fig. 3). Meanwhile, the remarkable decrease of androgen levels including testosterone (T-2, 0.35→0.15ng/ml, ref: 0.11-0.57ng/ml), androstenedione (AD, 2.95→0.96ng/ml, ref: 0.3-5.5ng/ml) and dehydroepiandrosterone sulfate (DHEA-S, 75.6→<15ug/dl, ref: 35-430ug/dl) were noticed, suggesting the suppressed hormone secretion of the reticular zone of the left adrenal gland.

One year later, the patient went to our hospital to seek the comprehensive assessment of her condition again. Her body weight reduced to 70Kg continuously in this year, with 25kg weight loss compared with that of the first visit (Fig. 1B). Her blood pressure could be well controlled by Valsartan, Nifedipine and Metoprolol. Wide purple striae was obviously lighter than before (Fig. 1F). The cortisol level of 8 AM was found to be in the reference range, but the circadian rhythm of cortisol was mildly abnormal (8AM 14.5 ug/dl, 4PM 9.48 ug/dl, 0AM 10.6ug/dl) (Table 1 and Fig. 3). And ACTH level showed less than 10pg/ml. The cortisol level was found to be 16.4 ug/dl after low-dose dexamethasone suppression test (LDDST). CT scan showed the left adrenal nodule with the size of 5mm (Fig. 2D). Considering the patient was reluctant to accept the left adrenalectomy, low dosage intake of Mitotane (250mg/day) was suggested.

Discussion

ACTH-dependent hypercortisolism is responsible for 80% of CS cases, which are caused either by pituitary ACTH hypersecretion or ectopic ACTH secretion, mainly by lung cancer and bronchial carcinoid. Adrenocortical adenoma, adrenocortical carcinoma, primary bilateral macronodular adrenal hyperplasia (PBMAH) and primary pigmented nodular adrenocortical disease (PPNAD) could explain the remaining cases, with autonomous secretion of cortisol by the adrenal glands. PPNAD is extremely rare, accounting for only 1% of patients with ACTH-independent CS [1, 4, 6].

PPNAD, one reason for ACTH-independent CS, is characterized by decreased or undetectable serum ACTH levels and elevated serum cortisol levels with abnormal circadian rhythm of cortisol secretion [1]. In most cases, radiology findings of the adrenal gland with PPNAD could be completely normal or showed macronodular hyperplasia, rather than the typical features with multiple micronodules in bilateral adrenal glands [2]. Moreover, the clinical manifestations of PPNAD may not be atypical or present cyclic CS characterized by excessive and normal cortisol secretion alternately [7]. Moreover, it was reported that only few cases of sporadic PPNAD occurred during childhood present no other signs of CNC [1]. All these factors can increase the difficulty for clinicians to identify PPNAD. In 1999, Stratakis et al. [8, 9] observed paradoxical response to dexamethasone in some patients with CS after performing Liddle test. Only patients with PPNAD were identified by 100% increase of UFC compared with baseline levels, suggesting paradoxical increase of glucocorticoid excretion in response to dexamethasone. It was reported that increased expression of glucocorticoid receptor (GR) may be responsible for the contradictory phenomenon [10].

Our patient was managed as CS caused by adrenocortical adenoma ten years ago, with gradually worsened clinical manifestations. Her severe hypercortisolemia features of elevated blood pressure, central obesity, purple striae and small ecchymoses were typical when she came to our hospital. She was attributed to PPNAD, due to multiple micronodules in bilateral adrenal glands reflected by adrenal enhanced CT. The characteristics of cortisol secretion after HDDST also proved that hypercortisolism was caused by autonomous secretion of adrenal nodules. It was also confirmed by histopathological analysis, characterized by numerous multiple pigmented micronodules of adrenal cortex. However, our patients only specifically presented with PPNAD, but not with cardiac myxoma, blue nevus and other classical CNC manifestations. The unclassical pigmented skin was noticed in the hypochondria region. Pereira et al. [11] reported cases with the same mutation in the start codon of PRKAR1A. Related functional studies showed that the expression level of mutant protein was extremely low. But a shorter protein of approximately 43 kDa was detected, which was translated by the second ATG codon located 141 bp downstream from the original start codon. Consistently, IHC analysis of the right adrenal of our patient showed reduced signal of PRKAR1A protein, compared with normal adrenal tissue adjacent to adenoma (Fig. 4C, 4D).

Similar to c.1A>G,exon 7 IVS del (-7→-2) of the PRKAR1A gene was also confirmed to be associated with PPNAD only [12]. Guo et al. [13] reported c.491_492delTG mutation of PRKAR1A gene in two Chinese

siblings, who present with CNC, including multiple cardiac myxomas, PPNAD and spotty skin pigmentation. A novel frameshift mutation in PRKAR1A (c.4_13del10) was identified in a 14-year-old Japanese boy who suffered from cardiac myxoma and spotty skin pigmentation on the face and lip [14]. It has been reported that 80% of cases with PRKAR1A mutations could present with PPNAD and CNC. Moreover, most of them carried inactivating mutations, leading to a premature stop codon and truncated translated product [15]. Cases identified with frameshift mutations and a premature termination codon were assumed to have more severe phenotype.

The patient with the same mutation in the start codon of PRKAR1A reported by Pereira et al. [11] was arranged for bilateral laparoscopic adrenalectomy, which is the frequent management for CS secondary to PPNAD. But this type of surgery leads to permanent adrenal insufficiency and requires life-long replacement with glucocorticoid and mineralocorticoid hormone, which may reduce the quality of life and increase the risk of adrenal crisis in severe stress events [16]. However, Mitotane has been widely used in the treatment of adrenocortical carcinoma as an orally administered adrenocorticolytic agent [17]. And some reports suggest that its satisfactory efficacy and safety in ACTH-dependent CS when using as a steroidogenesis inhibitor [18]. Our patient took low-dose Mitotane to obtain the improvement of hypercortisolism, which create opportunities for the surgery. Then she received unilateral adrenalectomy and low-dose Mitotane after surgery. Although the patient discontinued the medicine one month after the surgery, her body weight reduced to 70Kg and blood pressure did not rebound one year later. The clinical manifestations related to hypercortisolism improved remarkably, compared with those before treatment (Fig. 1B,1F). Moreover, we found that the cortisol level was slightly lower three months after surgery compared with one year after surgery (6.65→11.4ug/dl) (Table 1 and Fig. 3), which may be explained by a long half-life (18-159 days) of Mitotane stored in adipose tissue [19]. Even though, we assumed that the right adrenal gland may have priority of cortisol secretion, while the secretion of the left adrenal remained relatively in low level. Therefore, low-dose Mitotane and unilateral adrenalectomy may be another treatment for PPNAD as an alternative way instead of bilateral laparoscopic adrenalectomy in some patients who are unwilling to accept bilateral adrenalectomy in view of its severe adverse reactions [18]. Moreover, Mitotane may be beneficial for the preoperative control of severe hypercortisolemia [20]. Considering both therapeutic strategy require the long-term medicine intake and the mineralocorticoid is difficult to obtain in some country, low-dose Mitotane treatment appeared to be accepted easily in this patient.

Conclusions

In summary, we report a case of PPNAD in a middle-aged Chinese woman with c.1A>G mutation in the start codon of PRKAR1A gene. The expression of PPNAD protein was undetectable by IHC analysis. For the severe patient with PPNAD, the low dose of Mitotane followed by unilateral adrenalectomy showed significant improvement of the presentation of hypercortisolism.

Abbreviations

CS: Cushing's syndrome

PPNAD: primary pigmented nodular adrenocortical disease

CNC: Carney complex

WES: Whole-exome sequencing

IHC: immunohistochemistry

PKA: protein kinase A

UFC: urinary free cortisol

HDDST: High-dose dexamethasone suppression test

T-2: testosterone

AD: androstenedione

PHA: primary hyperaldosteronism

CT: computed tomography

PBMAH: primary bilateral macronodular adrenal hyperplasia

GR: glucocorticoid receptor

DXM: dexamethasone

ACTH: adrenocorticotrophic hormone

Ang II: angiotension

ALD: androstenedion

Declarations

Ethics approval and consent to participate

The Ethics Committee (Institutional Review Board) of the Ninth People's Hospital of Shanghai approved all protocols. Patients provided written informed consent.

Consent for publication

The consent for publication has been obtained from the patient.

Availability of data and materials

All data generated during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

J. Qiao and H. D. Song conceived and revised this paper. X. M. Liu and M. H. Guo collected the clinical data and did follow-up. Y. Xu and B. Han collected blood samples and performed IHC. S. X. Zhao analyzed the data of WES and Sanger sequencing. B. Xu performed the surgery of unilateral adrenalectomy. X. M. Liu wrote the paper. All authors read and approved the final manuscript.

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Table

Table 1. Laboratory tests results including serum cortisol, ACTH, T-2, AD, DHEA-S, UFC and other hormones

Hormonal data (reference ranges)	Before surgery	After 7 months of intermittent low-dose Mitotane intake	3 months after surgery	A year after surgery
Serum cortisol 8AM (5-25 ug/dl)	23.2	16.0	6.65	14.5
Serum cortisol 4PM (2.5-12.5 ug/dl)	18.8	14.4	6.87	9.48
Serum cortisol 0AM (2.5-12.5 ug/dl)	21.7	15.3	6.16	10.6
Serum ACTH 8AM (0-46 pg/ml)	11.7	18.7	<10	<10
Serum ACTH 4PM (0-46 pg/ml)	34.7	17.4	<10	<10
Serum ACTH 0AM (0-46 pg/ml)	19.3	19.9	<10	<10
Serum cortisol after 8 mg DXM administration (high-dose suppression test) (ug/dl)	20.7			
Serum ACTH after 8 mg DXM administration (high-dose suppression test) (pg/ml)	<10			
Serum cortisol after 2 mg DXM administration (low-dose suppression test) (ug/dl)			7.93	16.4
Serum ACTH after 2 mg DXM administration (low-dose suppression test) (pg/ml)			<10	<10
UFC (21-111ug/24h)	171.7	25.68	20.02	20.28
UFC after 8 mg DXM administration (high-dose suppression test) (ug/24h)	147.84			
Serum T-2 (0.11-0.57 ng/ml)	0.35		0.15	0.16
Serum AD (0.3-3.5 ng/ml)	2.95		0.96	1.34
Serum DHEA-S (35-430 ug/dl)	75.6		<15	16.1
Serum renin (0.93-6.56 ng/ml/h)	0.95			
Serum Ang II (85.3±30 pg/ml)	104.77			
Serum ALD (65.2-295.7 pg/ml)	143.85			

Abbreviations: ACTH, adrenocorticotrophic hormone; DHEA-S, dehydroepiandrosterone sulfate; AD, androstenedione; UFC, urinary free cortisol;XM, dexamethasone; T-2, testosterone; Ang II, angiotension; ALD, androstenedion.

Figures

Figure 1

(A) The patient's clinical image on initial visit. Moonlike face, abdominal fat deposits, dorsal and supraclavicular fat supported the existence of hypercortisolemia. (B) The patient's presentation one year after laparoscopic right adrenalectomy. The clinical manifestations related to hypercortisolemia remarkably improved. (C–D) Black and brown moles were observed on the patient's face and pigmented skin in the hypochondria region. (E) Wide purple striae was observed on the skin of abdomen, waist and thighs. (F) Wide purple striae was obviously lighter after treatment.

Figure 2

Computed tomography (CT) images of bilateral adrenal glands before and after treatment. Multiple adrenocortical micronodules in the right (A) and left (B) were observed. After seven months of low-dose Mitotane intake, the size of adrenal nodules was observed to be reduced significantly by CT scan (C). The patient underwent laparoscopic right adrenalectomy (D). Sanger sequencing verified the missense mutations (c.1A>G) of the PRKAR1A identified from the patient (E). Mutations at the same site were not found in her family members (F).

Figure 3

Elevated serum cortisol levels, urinary free cortisol (UFC) level and disturbed circadian rhythm of cortisol of the patient were detected since the first admission. After seven months of low-dose Mitotane intake, serum cortisol levels decreased significantly. And serum cortisol level of 8AM reduced to 9.0ug/dl on the next day after laparoscopic right adrenalectomy. Even if the patient discontinued Mitotane one month later, her serum cortisol levels did not rebound three months and one year after surgery.

Figure 4

Hematoxylin-eosin staining and Immunohistochemistry (IHC) analysis of the resected adrenal gland. (A) Adrenocortical micronodules (arrows) characterized by multiple pigmented cortical nodules and atrophy of the inter-nodular cortex were observed (10×magnification). (B) Adrenocortical micronodules are composed of fine granular pigment cells filled with lipofuscin (arrow). (C) The expression of PRKAR1A of our patient was found to be remarkably reduced by IHC when compared with the normal expression of PRKAR1A of the tissue surrounding adrenal adenoma (D).