An unusual presentation of Carney complex with diffuse primary pigmented nodular adrenocortical disease on one adrenal gland and a nonpigmented adrenocortical adenoma and focal primary pigmented nodular adrenocortical disease on the other

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Abstract. A 24-year-old female patient with cushingoid appearance was admitted in May 2000. The endocrine studies showed ACTH-independent Cushing’s syndrome. A 2-day high-dose dexamethasone suppression test (HDDST) revealed paradoxical increase of 24 h urinary free cortisol (UFC). Abdominal computed tomography demonstrated a left adrenal nodule (3 x 2 cm in diameter). An adrenal scintigram with ¹³¹I-6β-iodomethyl-19-norcholesterol showed uptake of the isotope in the left adrenal gland and non-visualization in the right adrenal gland throughout the examination course. A retroperitoneoscopic left total adrenalectomy was performed in July 2000. The cut surface of the left adrenal was yellow-tan grossly. Microscopically, the left adrenal nodule contained a nonpigmented adrenocortical adenoma (NP) and another focal primary pigmented nodular adrenocortical disease (PPNAD, FP) mixed lesion. The immunohistochemical studies of CYP17 demonstrate positive in NP and FP of the left adrenal gland. Very low baseline morning plasma cortisol (0.97 µg/dL) and subnormal ACTH (8.16 pg/mL) levels were measured 1.5 months after left adrenalectomy. Right adrenal gland recovered its function 6 months after left adrenalectomy. Plasma cortisol could be suppressed to 3.47 µg/dL by overnight low-dose dexamethasone suppression test 65 months after left adrenalectomy. Cushingoid features still did not appear 122 months after left adrenalectomy. In May 2011, this patient was readmitted due to cushingoid characteristics. Paradoxical rise of 24-h UFC to 2-day HDDST was demonstrated. Ultrasonography of thyroid showed bilateral thyroid cysts. Subtotal right adrenalectomy about 80% of right adrenal was performed. Diffuse PPNAD of the right adrenal was proved pathologically. Immunohischemical stain for CYP17 is positive in the right adrenal gland but weaker positive than that in the left adrenal gland. The genetic study of the peripheral blood, left adrenocortical nodule, and right PPNAD all showed p.R16X (c.46C>T) mutation of the PRKARIA gene.

Key words: Carney complex, Diffuse primary pigmented nodular adrenocortical disease (PPNAD), Focal PPNAD

CARNEY COMPLEX (CNC) was first described in 1985 as the complex of myxoma, spotty pigmentation, and endocrine overactivity. It is an autosomal dominantly inherited multisystem tumor syndrome in which the tumors are multicentric (heart and skin) in affected organs and bilateral in paired organs (adrenal, breast, and testis) [1-4]. The most frequent endocrine manifestation of CNC is adrenocorticotrophic hormone (ACTH) independent Cushing’s syndrome (CS) caused by primary pigmented nodular adrenocortical disease (PPNAD) [5]. In the disease process of PPNAD, both
adrenal glands are involved and feature small brown-black nodules separated by atrophic adrenal cortex. Microscopically, the nodules consist of large cortical cells with eosinophilic cytoplasm and lipofuscin pigment, and internodular cortical atrophy [3, 4, 6].

Genetic linkage analysis has revealed two distinct loci for CNC, one on chromosome 17q22-24 (CNC1) and the other on chromosome 2p16 (CNC2) [3, 7]. The CNC1 gene has been identified as the regulatory subunit R1A of protein kinases A (PRKAR1A). Heterozygous inactivating mutations of PRKAR1A gene have been reported in about 45% of CNC families. In CNC patients with CS, the frequency of PRKAR1A mutations is about 80%, suggesting that families with PPNAD are more likely to be associated with 17q22-24 defect [3].

Herein, we report an unusual clinical manifestation of a non-pigmented adrenocortical adenoma and focal PPNAD in one side of adrenal and diffuse PPNAD in another side with PRKAR1A mutation in a case of CNC.

Materials and Methods

Plasma and urine cortisol levels were measured by commercially available radioimmunoassay kits. Plasma ACTH levels were measured by immunoradiometric assay (Nichols Institute Diagnostics, San Juan Capistrano, California, USA).

Genomic DNA was extracted from the peripheral blood leukocytes of the patient and from the paraffin-embedded blocks of the excised adrenal glands by using QuickExtract FFPE DNA Extraction Kit (Epicentre Biotechnologies, Madison, WI) according to the manufacture’s protocol. All 10 exons in the PRKAR1A gene and their intron-exon boundaries were amplified by polymerase chain reaction (PCR) using primers described previously [2]. Semi-nested PCR analysis of exon 2 in the DNA obtained from the paraffin-embedded block was performed by using the reverse primer 5’-CAACCTCTCAAAGTATTCCCTGA-3’ for the second PCR cycle. PCR products were separated by electrophoresis on a 1.5% agarose gel and sequenced by ABI 3730XL DNA Analyzer (Applied Biosystems, Foster City, CA). The study protocol was approved by the institutional review board and informed consent was obtained from the patient.

Case Report and Results

A 24-year-old woman was admitted in May 2000 due to cushingoid appearance for half a year and weight increase of 10 kg in the proceeding 9 months. This patient’s father was a reported case of CNC [8]. His right and left adrenal glands were excised in October and December 1991, respectively and bilateral diffuse PPNADs were confirmed pathologically. Bilateral total thyroidectomies were performed in March 2002 and microscopic findings revealed papillary thyroid carcinomas. The patient’s height and weight were 156 cm and 59 kg, respectively and her blood pressure was 150/90 mm Hg. Physical examination showed moon face, buffalo hump, central obesity, striae over the abdomen and thighs, and hirsutism over the upper limbs and back. Her renal function and electrolytes were normal and fasting plasma glucose was 109 mg/dL. The endocrine studies (Table 1) before left adrenalectomy revealed adrenal CS. Paradoxical increase of 24 h urinary free cortisol (UFC, 1489 µg/day) during the 2-day high-dose dexamethasone suppression test (HDDST) was measured (Table 1). Her plasma growth hormone concentration was very low (< 0.1 ng/mL). Abdominal computed tomography (CT) revealed a left adrenal nodule (diameter, 3 x 2 cm) (Fig. 1A) and a normal-sized right adrenal gland. Adrenal scintigraphy with 131I-6β-iodomethyl-19-norcholesterol (NP-59) showed tracer uptake in the left adrenal gland and non-visualization of the right adrenal gland throughout the examination course (Fig. 1B). Ultrasonography of the heart did not demonstrate any cardiac myxoma.

A retroperitoneoscopic left total adrenalectomy was performed in July 2000. On the pathologic examination, the cut surface of the left adrenal nodule was yellow-tan (Fig. 2A) grossly with no black pigmented nodule. Microscopically, the adrenocortical nodule primarily contained clear and oxyphilic cells with encapsulation and a smaller focal area of large cortical cells with eosinophilic cytoplasm and lipofuscin pigment (Fig. 2B and 2C). A mixed lesion of a nonpigmented adrenocortical adenoma (NP) and focal PPNAD (FP) in the left adrenal gland was confirmed pathologically (Fig. 2B). After left adrenalectomy, cortisone acetate 25 mg once daily was prescribed for one month then 12.5 mg once daily for another month. The cushingoid features of the patient gradually disappeared thereafter. A very low baseline morning plasma cortisol (0.97 µg/dL) was measured 1.5 months after left adrenalectomy (Table 1). The baseline plasma cortisol and ACTH levels at 0800 h were normal 6 months later (Table 1). The patient’s weight was 55 kg and her blood pres-
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*Baseline plasma cortisol level (µg/dL)
  0800 h (normal range, 9-23)
  2200 h (normal range, 3-13)

Baseline 24 h urinary free cortisol level (µg/24 h)
(normal range, 34-122)

Baseline plasma ACTH level (pg/mL)
0800 h (normal range, 9-52)

Overnight low-dose dexamethasone suppression test*
  Plasma cortisol level at 0800 h

Two day low-dose dexamethasone suppression test**
  Plasma cortisol level at 0800 h
  24 h urinary free cortisol level

Two day high-dose dexamethasone suppression test†
  Plasma cortisol level at 0800 h
  24 h urinary free cortisol level

*Overnight low-dose dexamethasone suppression test: dexamethasone 1 mg orally at 2300 h and blood sampling for cortisol level the following morning 0800 h. **Two-day low-dose dexamethasone suppression test: dexamethasone 0.5 mg orally every 6 h for 2 days and blood sampling for cortisol level at 0800 h on the third day of testing. Collecting 24 h urine for cortisol level from 0800 h on the second day to 0800 h on the third day after dexamethasone administration. †Two-day high-dose dexamethasone suppression test: dexamethasone 2 mg orally every 6 h for 2 days and blood sampling for cortisol level at 0800 h on the third day of testing. Collecting 24 h urine for cortisol level from 0800 h on the second day to 0800 h on the third day after dexamethasone administration. §Normal range: 75-270 µg/24 h  §May 2011

Fig. 1 A. Abdominal computed tomography revealing a nodule (white arrow) on the left adrenal gland. B. Adrenal scintigram with 131I-6β-iodomethyl-19-norcholesterol on the 7th day after the tracer was injected showing tracer uptake (white arrow) in the left adrenal gland.

Table 1  Endocrinological Studies of the Case

<table>
<thead>
<tr>
<th>Tests</th>
<th>Before left adrenalectomy</th>
<th>After left adrenalectomy</th>
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<tbody>
<tr>
<td></td>
<td>May 2000</td>
<td>1.5 months 6 months 65 months 122 months 132 months*</td>
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<tr>
<td>Baseline plasma cortisol level (µg/dL)</td>
<td></td>
<td></td>
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<tr>
<td>0800 h (normal range, 9-23)</td>
<td>17.5</td>
<td>0.97</td>
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<tr>
<td>2200 h (normal range, 3-13)</td>
<td>19.0</td>
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<tr>
<td>Baseline 24 h urinary free cortisol level (µg/24 h) (normal range, 34-122)</td>
<td>952</td>
<td>145†</td>
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<tr>
<td>Baseline plasma ACTH level (pg/mL)</td>
<td></td>
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</tr>
<tr>
<td>0800 h (normal range, 9-52)</td>
<td>2.05</td>
<td>8.16</td>
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<tr>
<td>Overnight low-dose dexamethasone suppression test*</td>
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<td></td>
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<tr>
<td>Plasma cortisol level at 0800 h</td>
<td>20.9</td>
<td>3.47</td>
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<tr>
<td>Two day low-dose dexamethasone suppression test**</td>
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<tr>
<td>Plasma cortisol level at 0800 h</td>
<td>17.3</td>
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<td>24 h urinary free cortisol level</td>
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<td>Two day high-dose dexamethasone suppression test†</td>
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<tr>
<td>Plasma cortisol level at 0800 h</td>
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</tr>
<tr>
<td>24 h urinary free cortisol level</td>
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In May 2011, the patient was readmitted because of body weight gain of 13 kg within the past 6 months. We observed recurrence of the cushingoid appearance and a paradoxical rise of 24 h UFC during the 2-day HDDST (Table 1). Thyroid ultrasonography demonstrated bilateral cysts. Both abdominal CT and magnetic resonance imaging showed a normal-sized right adrenal gland. Subtotal right adrenalectomy (about 80% of the right adrenal gland) via the traditional thoracoabdominal approach was performed on May 25, 2011. The resected tissue fragment of the right adrenal gland measured 5.0 x 2.0 x 0.6 cm and weighed 6.5 gram. The cut surface of the right adrenal gland was brown-black grossly. Microscopically, the right adrenal gland showed diffuse cortical involvement with multiple pigmented nodules. The cortical cells contained eosinophilic cytoplasm with lipofuscin pigment (Fig. 3A and 3B). A diffuse PPNAD of right adrenal gland...
Fig. 2  A. The cut surface of the nonpigmented left adrenal nodule showing yellow-tan.  B. Microscopically, the left adrenal gland containing a major part of nonpigmented adrenocortical adenoma (NP), which is composed of clear (lipid-rich) and oxyphilic cells, and an area of focal primary pigmented nodular adrenocortical disease (FP) (hematoxylin and eosin; x 20).  C. The FP area in the left adrenal gland contains cortical cells with eosinophilic compact (lipid-poor) cytoplasm and lipofuscin pigment (hematoxylin and eosin; x 100).  D. Immunohistochemical stain of CYP17 in left adrenal gland is observed in focal PPNAD (FP) and nonpigmented adrenocortical adenoma (NP) (x 40).

Fig. 3  A. Microscopically, the pigmented nodules in the right adrenal gland are circumscribed but not encapsulated with considerable variation in sizes (black arrows; hematoxylin and eosin; x 20).  B. The cortical cells of the right pigmented nodules containing eosinophilic compact (lipid-poor) cytoplasm with lipofuscin pigment (white arrows) (hematoxylin and eosin; x 400).  C. Immunohistochemical stain of CYP17 in the right adrenal gland is positive (x 20) but weaker positive than that in the left adrenal (Fig. 2D).
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ible plasma cortisol and 24 h UFC during the 2-day HDDST, and very low plasma ACTH concentration. A paradoxical increase of 24 h UFC during the 2-day HDDST (Table 1) was observed in May 2000 and 2011. Analysis of the genomic DNA obtained from the peripheral blood and tissue sample of both adrenal glands showed a mutation in the PRKAR1A gene. Thyroid ultrasonography demonstrated bilateral cysts. Our patient’s father was a reported case of CNC due to PPNAD and bilateral papillary thyroid carcinomas occurring 11 years apart [8]. No spotty skin pigmentation or lentigines were found on the patient’s face or oral lips. However, she had PPNAD, thyroid gland abnormalities, first degree relative of CNC, and mutation of the PRKAR1A gene. Those clinical features meet the criteria for CNC presented by Stratakis et al. [10]. In this case, gross observation of the nodule on the left adrenal gland showed no pigmentation because the nodule revealed only a small part of the focal PPNAD. Travis et al. [15] reported an unusual pathological feature that had one nonpigmented (yellow) nodule and multiple pigmented nodules (focal PPNAD) on one side of adrenal glands in a PPNAD patient. However, they did not mention the pathological features of the contralateral adrenal gland. This is the first case report of CNC caused by a nonpigmented adrenocortical adenoma and focal PPNAD in one adrenal gland and a diffuse PPNAD in the other with the same mutation of the PRKAR1A gene in both glands.

Was there any possible that the focal PPNAD developed into diffuse PPNAD in right adrenal gland? The possibility was very low. In our case, there was a capsule between focal PPNAD and nonpigmented adenoma in the left adrenal gland. The diffuse PPNAD in the right adrenal gland did not contain any nonpigmented nodule. In the Travis et al. [15] report, pigmented nodules are not adenomatous or premalignant. We suppose that the existence of nonpigmented adenoma and focal PPNAD in the left adrenal gland is coincident.

PPNAD can be overt (CS present clinically and biochemically), subclinical (no clinical features of CS, but adrenocortical hyperfunction, or autonomy, or both on testing), or latent (no clinical or laboratory evidence of the CS, but presence of the genetic trait) [6, 16]. At 1.5 months after undergoing left adrenalectomy, our patient showed very low baseline morning plasma cortisol (0.97 µg/dL) and subnormal ACTH (8.16 pg/mL) levels (Table 1), which indicated suppressed functioning of the contralateral (i.e. right) adrenal gland or a latent

Discussion

CNC includes myxomatous masses (cardiac myxoma, cutaneous myxoma, and mammary myxoid fibroadenoma), spotty pigmented lesions of the skin (lentigines and blue nevi), endocrine disorders (PPNAD, various testicular tumors (large-cell calcifying Sertoli cell tumor, Leydig cell tumor, and adrenocortical rest tumor), and growth hormone-producing pituitary adenoma) [1]. In 1997, Stratakis et al. added thyroid gland abnormalities to the components of CNC [9]. They set the diagnostic criteria for CNC in 2001 [10]. A predisposition for CNC patients to develop a variety of carcinomas, including thyroid and ovarian cancer, was noted [11].

In a large study by Bertherat et al. [12], 258(73%) of the 353 CNC patients carried 80 different PRKAR1A mutations. The detection rate of PRKAR1A mutation in 185 CNC families was 62 % (114 families). This increased to 80% among CNC patients presenting with CS due to PPNAD [13]. In 2010, Horvath et al. [13] reported that 117 different PRKAR1A mutations were identified in 387 unrelated families of various ethnic origins. Some studies have reported the heterogeneity in genetic and clinical manifestations in CNC patients [10, 12-14].

The endocrinological studies (Table 1) in our patient revealed ACTH-independent CS (unsuppress-
PPNAD state in the right adrenal gland. The adrenal scintigraphy of NP-59 in May 2000 also demonstrated non-visualization of the right adrenal gland. At 6 months after left adrenalectomy, normal baseline plasma cortisol (8.70 µg/dL) and ACTH (38.8 pg/mL) levels (Table 1) were measured which indicated normal cortisol-secreting function of the right adrenal gland. Overnight LDDST suppressed the plasma cortisol level to 3.47 µg/dL at 65 months after left adrenalectomy. Although the overnight LDDST failed to suppress the plasma cortisol level (7.12 µg/dL) at 122 months after left adrenalectomy, the CS did not appear clinically (subclinical PPNAD). In May 2011, overt CS was observed and 2-day LDDST did not suppress plasma cortisol level (overt PPNAD). Our case clearly demonstrated that the period of complete 3 stages from the suppressed or latent to the clinically overt stage in a diffuse PPNAD was at least 11 years. Some of PPNADs are difficult to detect because they may not only present as subclinical or cyclical CS, but also radiologic imaging can be normal or show only subtle nodularity [7, 11].

The treatment of choice for PPNAD patients is bilateral adrenalectomy [16]. Several studies have reported the mortality and morbidity caused by adrenal insufficiency after bilateral total adrenalectomy in patients with PPNAD [17-19]. Carney et al. [16] reported that less than total adrenalectomy might be considered for some PPNAD patients who were asymptomatic or only minimally symptomatic. They found that some cases of PPNAD underwent unilateral adrenalectomy with remission of the clinical features of the CS. We also reported a case of PPNAD with remission of the CS after unilateral adrenalectomy in 2009 [20]. The mechanism that the involved bilateral adrenal glands in PPNAD including the present case displayed the features of the CS in different time is unclear. Bourdeau et al. [21] reported that glucocorticoid receptor (GR) immunoreactivity was detected in PPNAD nodular cells, not seen in surrounding cortical cells, and weak in adrenal adenomatous tissue. The GR explains paradoxical dexamethasone responses and can’t explain the clinical presentations of the CS in our cases and the reported cases by Carney et al. [16]. We suppose that there may have unknown trigger factors in these PPNAD patients. Onoda et al. [22] reported a case of CYP17A1 strongly expressed in the right adrenocortical adenoma secreting cortisol and weaker expressed in the left adrenocortical adenoma secreting aldosterone. Our present case showed that CYP17 immuno-reactivity in the left adrenal gland (Fig. 2D) was stronger than that in the right adrenal gland (Fig. 3C). The adrenal scintigraphy of NP-59 in May 2000 revealed non visible right adrenal gland (Fig. 1B). Those two clinical examinations demonstrated lesser cortisol production in the right adrenal gland than that in the left adrenal. Therefore, the onset of clinical features of CS delayed on the right adrenal gland. The causes of discrepancy in cortisol secretion of left and right adrenal glands with the same mutation of PRKAR1A gene are unknown. In our patient, about 80% of the right adrenal gland was excised because she did not wish to receive lifelong glucocorticoid treatment which is necessary after bilateral total adrenalectomy.

In summary, we present the unusual different pathological features of PPNAD in both adrenal glands with the same mutation of the PRKAR1A gene (c.46C>T). We also report the serial plasma cortisol concentrations and clinical states of CS in a patient with diffuse PPNAD. In this case of PPNAD in the right adrenal gland, an interval of at least 11 years is observed from latent to overt CS.

Conflicts of Interest

All authors declare no conflicts of interest.

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Ethics Committee Approval

All human studies were approved by the Kaohsiung Chang Gung Memorial Hospital Institutional Review Board.

Author Contributions

SC Tung performed the data collection, analysis, and interpretation, and wrote the relevant sections of the manuscript. DY Hwang performed the genetic analysis and literature search, provided the figures, and wrote the relevant sections of the manuscript. CT Lee performed the genetic analysis and literature search, and wrote the relevant sections of the manuscript. WJ Chen performed the adrenal pathology analysis and provided the figures. JW Yang performed the adrenal surgery.
References


