Heterogeneous Clinical Manifestations of Cushing’s Syndrome in a Family with Primary Pigmented Nodular Adrenocortical Disease

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Abstract

**Purpose:** To evaluate the clinical manifestations of Cushing’s Syndrome (CS) and associated genetic mutation in patients with Primary Pigmented Nodular Adrenocortical Disease (PPNAD).

**Methods:** Seven family members were screened for mutations of the PRKAR1A gene. Gene mutation screening used genomic DNA (from peripheral blood leukocytes and, in some cases, adrenal gland tissue) and subsequent DNA sequencing. The five patients showing genetic mutation were assessed clinically for baseline cortisol and adrenocorticotropic hormone and adrenal imaging studies (abdominal computed tomography and adrenal scintigraphy). Low-dose and high-low dexamethasone suppression tests were performed in these five patients.

**Results:** PRKAR1A gene mutation was detected in five of the seven family members. Four of the five gene mutation-positive patients presented with overt CS due to pathology-proven PPNAD. After unilateral adrenalectomy in these four patients, overt CS persisted for two patients and CS symptoms abated for the other two. The remission period of CS was >11 years in one instance (case III-2) and continues for >12 years in the other (case II-4), with no observable disease of the contralateral non-resected adrenal gland. For each of the two remission patients, one adrenal gland had been larger (by abdominal computed tomography) and had had stronger function (by 131I-6 β-iodomethyl-19-norcholesterol scintigraphy) than the other. In one (case III-2) of the two remission patients, one adrenal displayed focal PPNAD while the other displayed diffuse PPNAD. The clinical manifestations of CS did not resolve after unilateral adrenalectomy in case II-2 and III-7. The fifth gene mutation-positive patient (case III-4) is still in the latent stage of CS.

**Conclusions:** The clinical manifestations of CS and adrenal image features in patients with PPNAD are heterogeneous. Detailed adrenal imaging of these patients is necessary to assist with the decision to perform unilateral adrenalectomy.

**Keywords** Cushing’s syndrome; Primary pigmented nodular adrenocortical disease

Introduction

Primary Pigmented Nodular Adrenocortical Disease (PPNAD) is a rare cause of adrenal Cushing’s Syndrome (CS). It occurs sporadically or as part of a familial syndrome called Carney Complex (CNC) [1]. PPNAD was observed in about 26-32% of CNC patients [2,3]. However, histological evidence of PPNAD was found in almost every patient with CNC who underwent an autopsy [2]. In the disease process of PPNAD, both adrenal glands are involved and feature small brown black nodules separated by atrophic adrenal cortex. The adrenal sizes associated with PPNAD range from small to slightly enlarged. The cut surfaces of the adrenal nodules vary in color from yellow-tan to gray, brown, and black [4]. Microscopically, the nodules consist of large cortical cells with lipofuscin pigment and internodular cortical atrophy [1,5].

We present the clinical manifestations of CS and the adrenal imaging and pathological features of family members with PPNAD and a common, single mutation of the PRKAR1A gene.

Subjects and Methods

Subjects

Seven members of a family were screened for mutation of the PRKAR1A gene. The five patients with the mutation were aged 24-45 years and included one female and four males. Four of the five patients...
presented with clinical features of CS and eventually had an adrenalectomy.

Methods

Plasma and urine cortisol levels were measured by commercial radioimmunooassay kits. Plasma Adrenocorticotropic Hormone (ACTH) levels were measured by immunoradiometric assay. Low-dose and high-dose dexamethasone suppression tests were performed in these five gene mutation patients. Adrenal imaging studies included abdominal Computed Tomography (CT) and adrenal scintigraphy.

Genomic DNA was extracted from peripheral blood leukocytes from each of the seven family members using the QIAamp DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany) and from paraffin-embedded adrenal gland tissue available for four of the seven family members using the QuickExtract FFPE DNA Extraction Kit (Epigenet Biotechnologies, Madison, WI) according to the manufacturer’s protocol.

All 10 exons of the PRKAR1A gene and their intron-exon boundaries were amplified by polymerase chain reaction (PCR) using primers described previously [6]. Semi-nested PCR analysis of exon 2 in the DNA obtained from the paraffin-embedded adrenal tissue was performed 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 3730 XL DNA Analyzer (Applied Biosystems, Foster City, CA). The study protocol was approved by the institutional review board, and informed consent was obtained from each of the seven family members.

Results

Family member studies Case II-2 (index patient)

The detailed history of this index patient was reported by Tung et al., 2005 [7]. Briefly, a 41-year-old man (Figure 1), born in November 1949, came to our outpatient department in October 1990 with cushingoid appearance. The patient’s father also had cushingoid features, but due to his death, his clinical characteristics were not analyzed as part of this study. Endocrine studies of this index patient revealed adrenal CS (Table 1). A paradoxical rise of 24-h UFC to 2-day HDDST was observed [8]. Both abdominal CT and adrenal scintigraphy showed tracer uptake in the left adrenal gland and non-visualization of 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 gland was yellow-tan without obvious black pigmentation. Microscopically, non-pigmented adrenocortical adenoma was the major portion and focal PPNAD was the minor portion of this left adrenocortical nodule [8]. The cushingoid features of the patient gradually disappeared thereafter. A very low baseline morning plasma cortisol level (0.97 μg/dL at 0800 h) was measured 1.5 months after left adrenalectomy. Six months later, the baseline plasma cortisol and ACTH levels at 0800 h were normal (8.7 μg/dL and 38.8 pg/mL, respectively). Apparent cushingoid features still had not returned 12 months later, despite non-suppression of the plasma cortisol level (7.12 μg/dL) by overnight LDDST.

In May 2011, the patient was re-admitted because of a 13 kg weight gain during the preceding 6 months. A paradoxical rise of 24-h UFC during the 2-day HDDST was observed [8]. Both abdominal CT and MRI showed a normal-sized right adrenal gland. Subtotal right adrenalectomy (about 80% of the right adrenal gland resected) via the traditional thoracoabdominal approach was performed on May 25, 2011. The cut surface of the right adrenal gland was grossly observed to be brown-black. Diffuse PPNAD was confirmed microscopically [8]. Analysis of this patient’s genomic DNA obtained from peripheral blood leukocytes and bilateral adrenal tissues both showed a c.46C>T mutation (p.R16X nonsense amino acid mutation) in the PRKAR1A gene [8].
Case III-3

This 23-year-old woman (the third daughter of the index patient; born in March 1978) (Figure 1) came to our outpatient department in December 2000 for screening of CS. Her height was 156 cm; weight, 45 kg; and blood pressure, 90/60 mm Hg. Cushingoid features were not found during physical examination. Plasma cortisol level was suppressed to 0.90 μg/dL by overnight LDDST. Analysis of genomic DNA obtained from peripheral blood leukocytes demonstrated no mutation of the PRKAR1A gene.

Case III-4

This man aged 21 years and 5 months (the fourth child of the index patient; born in November 1979) (Figure 1) came to our outpatient department in April 2001 for screening of CS because of his father’s PPNAD. His height was 169 cm; weight, 60 kg; and blood pressure, 120/80 mm Hg. Cushingoid features were not observed during physical examination. Baseline plasma cortisol and ACTH levels at 0800 h were 5.32 μg/dL and 42.1 pg/mL, respectively. Plasma cortisol was suppressed to 3.23 μg/dL and 4.57 μg/dL (Table 1) by overnight LDDST in April 2001 and December 2006, respectively. Image studies and adrenalectomy were not performed because of the absence of clinical manifestations of CS. However, analysis of this patient’s genomic DNA obtained from peripheral blood leukocytes identified a c.46C>T mutation (p.R16X) in the PRKAR1A gene.

<table>
<thead>
<tr>
<th>Tests</th>
<th>Case II-2</th>
<th>Case II-4</th>
<th>Case III-2</th>
<th>Case III-7</th>
<th>Case III-4</th>
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</thead>
<tbody>
<tr>
<td>Baseline plasma cortisol level (µg/dL)</td>
<td></td>
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<tr>
<td>0800 h (normal range, 9-23)</td>
<td>37.9</td>
<td>26.1</td>
<td>17.5</td>
<td>17.3</td>
<td>5.32</td>
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<td>1600-2200 h (normal range, 3-13)</td>
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<td>20.4</td>
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<td>14.1</td>
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<tr>
<td>Baseline 24-h urinary free cortisol level</td>
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<tr>
<td>(µg/24-h) (normal range, 34-122)</td>
<td>1008</td>
<td>786</td>
<td>952</td>
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<tr>
<td>Baseline plasma ACTH level (pg/mL)</td>
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<td>8.29</td>
<td>2.05</td>
<td>14.6</td>
<td>42.1</td>
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<td>0800 h (normal range, 9-52)</td>
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<tr>
<td>Overnight low-dose dexamethasone suppression test*</td>
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<tr>
<td>Plasma cortisol level at 0800 h</td>
<td>32.3</td>
<td>24.3</td>
<td>20.9</td>
<td>13.8</td>
<td>4.57</td>
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<td>Two-day low-dose dexamethasone suppression test**</td>
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<tr>
<td>Plasma cortisol level at 0800 h</td>
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<td>762</td>
<td>1066</td>
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<td>Overnight high-dose dexamethasone suppression test</td>
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<td>Plasma cortisol level at 0800 h</td>
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<tr>
<td>Plasma cortisol level at 0800 h</td>
<td>33.7</td>
<td>20.0</td>
<td>22.9</td>
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<tr>
<td>24-h urinary free cortisol level</td>
<td>1470</td>
<td>676</td>
<td>1489</td>
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</tbody>
</table>

Table 1: Endocrinological studies (before adrenalectomy) in the five patients with primary pigmented nodular adrenocortical disease. *Overnight low-dose dexamethasone suppression test: 1 mg dexamethasone orally at 2300 h and blood sampling for cortisol level the following morning at 0800 h. ** Two-day low-dose dexamethasone suppression test: 0.5 mg dexamethasone 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 free cortisol level from 0800 h on the second day to 0800 h on the third day after dexamethasone administration. §Overnight high-dose dexamethasone suppression test: 8 mg dexamethasone orally at 2300 h and blood sampling for cortisol level the following morning at 0800 h. §§Two-day high-dose dexamethasone suppression test: 2 mg dexamethasone 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 free cortisol level from 0800 h on the second day to 0800 h on the third day after dexamethasone administration.

Case II-4

The detailed history of this patient was reported by Tung et al., 2009 [9]. Briefly, in October 2001, a 45-year-old man (the younger brother of the index patient; born in June 1956) (Figure 1) presented to our clinic with a cushingoid appearance. His height was 157 cm; weight, 64 kg; and blood pressure, 146/94 mm Hg. The results of endocrine studies revealed adrenal CS (Table 1). An adrenal scintigrapy using NP-59 showed uptake of the tracer in the left adrenal on the second day and in the right adrenal gland on the fourth day after NP-59 injection [9]. CT of the abdomen revealed a nodule (2 cm diameter) on the left adrenal and a normal-appearing right adrenal gland [9]. A laparoscopic left adrenalectomy was performed in November 2001. The cut surface of the excised left adrenal nodule showed multiple...
small black nodules. Diffuse PPNAD was confirmed microscopically [9].

In December 2001, baseline plasma cortisol levels still demonstrated a loss of normal circadian rhythm. An overnight LDDST failed to suppress plasma cortisol (14.0 μg/dL). In January 2002, a repeated CT of the abdomen revealed a residual nodule (1.5 cm diameter) on the left adrenal gland. Thus, a left total adrenalectomy.

Sixty-nine months after the second procedure, cushingoid appearance of the patient was still absent, although his overnight LDDST revealed a non-suppressible plasma cortisol concentration (12.7 μg/dL). The patient reports via a telephone conversation that the remission of cushingoid features continues 12 years after the second procedure. Analysis of genomic DNA obtained from peripheral blood leukocytes and the paraffin-embedded left adrenal tissues both identified a c.46C>T mutation (p.R16X) in the PRKAR1A gene.

Case III-7

A 25-year-old man (the third son of case II-4; born in July 1981) (Figure 1) came to our outpatient department in August 2006 due to a moon-faced appearance for 1 year and weight gain of 5-6 kg in the preceding 1.5 years. As noted above, his father had PPNAD. His height was 157 cm; weight, 58 kg; and blood pressure, 128/72 mm Hg. Physical examination revealed moon face with an excess of acne, buffalo hump, and abdominal obesity. An absence of normal circadian plasma cortisol fluctuations was observed (baseline plasma cortisol levels of 17.3 μg/dL at 0800 h and 14.1 μg/dL at 1600 h) (Table 1). Baseline plasma ACTH level was 14.6 pg/mL. In September 2006, an adrenal scintigraphy using NP-59 showed bilateral adrenal uptake of the tracer on the fifth day after NP-59 injection. Abdominal CT did not find any adrenal nodule in September 2006, August 2007, or April 2010. Left total adrenalectomy via a left flank approach was performed in June 2010. Diffuse PPNAD was proved microscopically. The patient refused right adrenalectomy despite persistent manifestations of CS after left total adrenalectomy. Analysis of this patient’s genomic DNA from peripheral blood leukocytes identified a c.46C>T mutation (p.R16X) in the PRKAR1A gene.

Discussion

Since the first description in 1985 of “the complex of myxomas, spotty pigmentation, and endocrine overactivity” [10], now known as “Carney complex”, more than 500 CNC patients have been reported from all ethnicities, and the majority of them (almost 70%) presented with a positive family history [11,12]. CNC is an autosomal dominant disorder featuring skin lesions, cardiac and other myxomas, and different types of endocrine tumors including PPNAD, growth hormone-secreting pituitary tumors, gonadal and thyroid neoplasms, and rarely, benign and malignant psammomatous melanotic schwannomas [13]. The most frequent endocrine manifestation of CNC is PPNAD [14]. There seems to be a bimodal age distribution of PPNAD among CNC patients, a minority present during the first 2-3 years, whereas the majority manifest in the second and third decade of life [2]. Our patients in case II-2, II-4, III-2, and III-7, with PPNAD and mutation of the PRKAR1A gene, meet the diagnostic criteria of CNC set by Stratakis et al. [2]. The case III-4 patient has a biological father afflicted with CNC and is a case of PPNAD in the latent phase of CS with mutation of the PRKAR1A gene. Therefore, the case III-4 patient is also classified as having CNC.
The clinical manifestations of CS in patients with PPNAD can be divided into three stages: (a) overt (CS present clinically and biochemically), (b) subclinical (no clinical features of CS, but detectable adrenocortical hyperfunction or autonomy or both), or (c) latent (no clinical or laboratory evidence of CS, but the genetic trait is present) [9]. Our index patient (case II-2) still presented overt CS after unilateral total adrenalectomy (Table 2). In case III-2, the patient progressed through all three stages of CS (latent, subclinical, and overt) after unilateral (left) total adrenalectomy, and this progression from latent to overt CS occurred over a period of >11 years in the right PPNA [8]. For case III-4, the patient remains in the latent CS stage despite carrying the PRKAR1A gene mutation common to his afflicted family members. In case II-4, the first two stages of CS (latent and subclinical) are observed and the remission period of CS persists for >12 years after unilateral total adrenalectomy (Table 2). In case III-7, overt CS persists after unilateral total adrenalectomy (Table 2). Thus, all the five family members with PPNAD displayed various clinical manifestations of CS even though they each had the same mutation (c.46C>T) in the PRKAR1A gene.

Establishing the diagnosis of PPNA can be challenging, particularly when PPNA is the only manifestation of the disease and there are no other afflicted family members. In addition, patients with the disease not only may present atypical forms of CS, such as cyclical or episodic CS, but they may also have normal radiologic imaging or show only subtle nodularity [15,16]. Beyond 18 years of age, a unilateral 2-3 cm diameter adrenal macronodule is common in PPNA (as in our case II-2, II-4, and III-2) [5,17]. This can make it difficult to distinguish CS due to PPNA from ACTH-independent unilateral adrenocortical adenoma based on radiological criteria only [9]. A paradoxical increase in 24-h UFC during the Liddle test can be used to identify PPNA and distinguish it from other causes of adrenal CS [18,19].

The hypercortisolism due to PPNA resolves after bilateral adrenalectomy [5,15]; however, lifelong glucocorticoid replacement is required after this form of surgery. Mortality and morbidity because of adrenal insufficiency after bilateral total adrenalectomy were reported in patients with PPNA [20-22]. Carney and Young reported that 6 out of 17 PPNA patients that underwent unilateral adrenalectomy exhibited remission of their clinical features of CS [15]. Seven other patients had persistence of CS, two died postoperatively, and two were lost to follow-up. These authors also speculate that less than total adrenalectomy may have a place in some selected PPNA patients who are asymptomatic or only minimally symptomatic [15]. We previously reported a case of PPNA with remission of CS after unilateral adrenalectomy in 2009 (case II-4) [9]. Here we present additional objective data. For example, from case III-2 and II-4 (Table 2), the observation of larger size (by abdominal CT) and increased function (by adrenal scintigraphy) of the affected left adrenal gland compared to the right may assist in the decision to perform unilateral adrenalectomy as a choice for PPNA patients to avoid lifelong glucocorticoid replacement therapy. Such a management approach for patients with PPNA represents a form of patient-centered care.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Sex</th>
<th>Age (yr) at first surgery</th>
<th>Manifestations of Cushing's syndrome (CS)</th>
<th>Computed tomography findings</th>
<th>Adrenal scintigraphy*</th>
<th>Adrenalectomy</th>
<th>Pathological findings of adrenal glands</th>
<th>Features of Carney complex</th>
<th>Gene mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>II-2</td>
<td>Male</td>
<td>42</td>
<td>Overt CS</td>
<td>Right tiny adrenal nodule, left adrenal nodule (2 cm)</td>
<td>Bilateral adrenal uptake</td>
<td>Bilateral total adrenalectomy</td>
<td>Bilateral diffuse PPNA</td>
<td>PPNA, bilateral papillary thyroid carcinoma</td>
<td>PRKAR1A? (c.46 C&gt;T)</td>
</tr>
<tr>
<td>III-2</td>
<td>Female</td>
<td>24</td>
<td>Three stages of CS on different dates, Remission of CS for &gt;11 years after left total adrenalectomy</td>
<td>Left adrenal nodule (3 x 2 cm), normal-sized right adrenal gland</td>
<td>Left adrenal uptake</td>
<td>Left total adrenalectomy, right subtotal adrenalectomy (80%)</td>
<td>Left focal PPNA, right diffuse PPNA</td>
<td>PPNA, bilateral thyroid cysts</td>
<td>PRKAR1A? (c.46 C&gt;T)</td>
</tr>
<tr>
<td>III-4</td>
<td>Male</td>
<td>32§</td>
<td>Latent CS</td>
<td>Not done</td>
<td>Not done</td>
<td>No</td>
<td>Unknown</td>
<td>No screened</td>
<td>PRKAR1A? (c.46 C&gt;T)</td>
</tr>
<tr>
<td>II-4</td>
<td>Male</td>
<td>45</td>
<td>Overt CS Persistent remission of CS for &gt;12 years after left total adrenalectomy</td>
<td>Left adrenal nodule (2 cm), normal-sized right adrenal gland</td>
<td>Earlier uptake of left adrenal than right</td>
<td>Left total adrenalectomy</td>
<td>Left diffuse PPNA</td>
<td>PPNA, bilateral thyroid cysts</td>
<td>PRKAR1A? (c.46 C&gt;T)</td>
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<tr>
<td>III-7</td>
<td>Male</td>
<td>29</td>
<td>Overt CS</td>
<td>Normal-sized bilateral adrenal glands</td>
<td>Bilateral adrenal uptake</td>
<td>Left total adrenalectomy</td>
<td>Left diffuse PPNA</td>
<td>PPNA</td>
<td>PRKAR1A? (c.46 C&gt;T)</td>
</tr>
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</table>

Table 2: Clinical characteristics in a family with primary pigmented nodular adrenocortical disease (PPNA). *Adrenal scintigraphy: 131I-6iodomethyl-19-norcholesterol adrenal scintigraphy. ‡PRKAR1A: regulatory subunit R1A of protein kinase A. §Three stages of CS: overt, subclinical, and latent Cushing's syndrome. 32: The patient was 32 years old when the gene study was performed in October 2011.
Conclusions

The clinical manifestations of CS and imaging features in our intrafamilial PPNAD patients are heterogeneous. The three stages of CS (overt, subclinical, and latent) should be kept in mind in the management of PPNAD. Detailed adrenal imaging of PPNAD patients is necessary to assist with the decision to perform unilateral adrenalectomy.

References