Neuroendocrinology Letters Volume 38 No. 4 2017
ISSN: 0172-780X; ISSN-L: 0172-780X; Electronic/Online ISSN: 2354-4716
Web of Knowledge / Web of Science: Neuroendocrinol Lett
Pub Med / Medline: Neuro Endocrinol Lett

CASE REPORT

Unusual presentations of Carney Complex in patient with a novel PRKAR1A mutation

Safak Akin [1], Senem Noyan [2], Selcuk Dagdelen [1], Ilhan Pasaoglu [3], Volkan Kaynaroglu [4], Melike Mut Askun [5], Cenk Yucel Bilen [6], Hayyam Kiratli [7], Dilek Ertoy Baydar [8], Sevgen Onder [8], Cenk Sokmensuer [8], Kudret Aytemir [9], Gul Erkin [10], Pinar Ozgen Kiratli [11], Mehmet Alikasifoglu [12], Tomris Erbas [1]

1 Hacettepe University, Faculty of Medicine, Department of Endocrinology and Metabolism, Ankara, Turkey
2 Damagen, Ankara, Turkey
3 Hacettepe University, Faculty of Medicine, Department of Cardiovascular Surgery, Ankara, Turkey
4 Hacettepe University, Faculty of Medicine, Department of General Surgery, Ankara, Turkey
5 Hacettepe University, Faculty of Medicine, Department of Neurosurgery, Ankara, Turkey
6 Hacettepe University, Faculty of Medicine, Department of Urology, Ankara, Turkey
7 Hacettepe University, Faculty of Medicine, Department of Ophthalmology, Ankara, Turkey
8 Hacettepe University, Faculty of Medicine, Department of Pathology, Ankara, Turkey
9 Hacettepe University, Faculty of Medicine, Department of Cardiology, Ankara, Turkey
10 Hacettepe University, Faculty of Medicine, Department of Dermatology, Ankara, Turkey
11 Hacettepe University, Faculty of Medicine, Department of Nuclear Medicine, Ankara, Turkey
12 Hacettepe University, Faculty of Medicine, Department of Medical Genetics, Ankara, Turkey

Correspondence to: Safak Akin, MD.
Hacettepe University, Faculty of Medicine
Department of Endocrinology and Metabolism
Ankara, Turkey.
TEL: +90 312 305 12 88; FAX: +90 312 311 67 68; E-MAIL: safakcavus@gmail.com

Submitted: 2016-09-29 Accepted: 2017-05-03 Published online: 2017-08-28

Key words: **Carney Complex; novel PRKAR1A mutation; reccurrent myxoma; primary pigmented nodular adrenocortical disease; large-cell calcifying Sertoli cell tumor**

Neuroendocrinol Lett 2017; **38**(4):248–254 **PMID:** 28871709 NEL380417C02

Abstract

Carney Complex (CNC) is a multiple neoplasia syndrome characterized by skin tumors and pigmented lesions, myxomas, and various endocrine tumors. The aim of this case report was to describe a case of CNC with a novel *PRKAR1A* mutation. A man aged 46 years with a medical history of surgery for cardiac myxomas at the age of 39 was admitted to our hospital because of four newly-developed heart masses. The histologic examination confirmed cardiac myxomas. He had many presentations of CNC such as growth hormone (GH) and prolactin (PRL)-secreting mixed pituitary adenoma, benign thyroid nodule, large-cell calcifying Sertoli cell tumor (LCCST), and superficial angiomyxoma. A bilateral adrenalectomy was performed because the laboratory findings suggested primary pigmented nodular adrenocortical disease (PPNAD). The pathologic examination revealed a focal unilateral PPNAD, unilateral nonpigmented adrenocortical nodule, and bilateral adrenal medullary hyperplasia. Two years after the second cardiac operation, an interatrial septum-derived tumor was detected. An atrial myxoma was confirmed with histologic studies. Based on these findings, the patient was confirmed to have CNC. A novel insertion mutation in the type 1A regulatory subunit of the cAMP-dependent protein kinase A gene (*PRKAR1A*) in exon 2 was detected in our patient through genetic analysis. The presence of multiple myxomas and

endocrine abnormalities should be an indication to physicians to further investigate for CNC. Herein, we described a case of CNC with a novel mutation in exon 2 of the *PRKAR1A* gene with typical and atypical clinical features.

INTRODUCTION

Carney complex (CNC) is a multiple neoplasia syndrome characterized by skin tumors and pigmented lesions, myxomas, and various endocrine tumors, and was first described in 1985 by J. Aidan Carney (Carney *et al.* 1985). The most common endocrine gland manifestations are thyroid and testicular tumors, acromegaly, and adrenocorticotropic hormone (ACTH)-independent Cushing's syndrome (CS) due to primary pigmented nodular adrenocortical disease (PPNAD). It is a genetically heterogenous autosomal dominant disease, but the disease is sporadic in a significant number of patients (Salpea & Stratakis 2014). In this report we describe a case of CNC with a novel *PRKAR1A* mutation. Our case emphasizes both the classic presenting symptoms of CNC and uncommon diagnoses of the complex.

CASE PRESENTATION

A man aged 46 years with a medical history of cardiac myxoma excision from the left atrium when he was aged 39 years old was admitted our hospital because of three newly-developed cardiac masses. On transesophageal echocardiography, masses in the right atrium measured 4.5×5.8 cm and the atrium was prolapsing through the tricuspid valve into the right ventricle. The largest of the three masses (1.7×1.2 cm) was in the left atrium. Preoperative thoracic computerized tomography (CT) demonstrated multiple bilateral pulmonary emboli. The

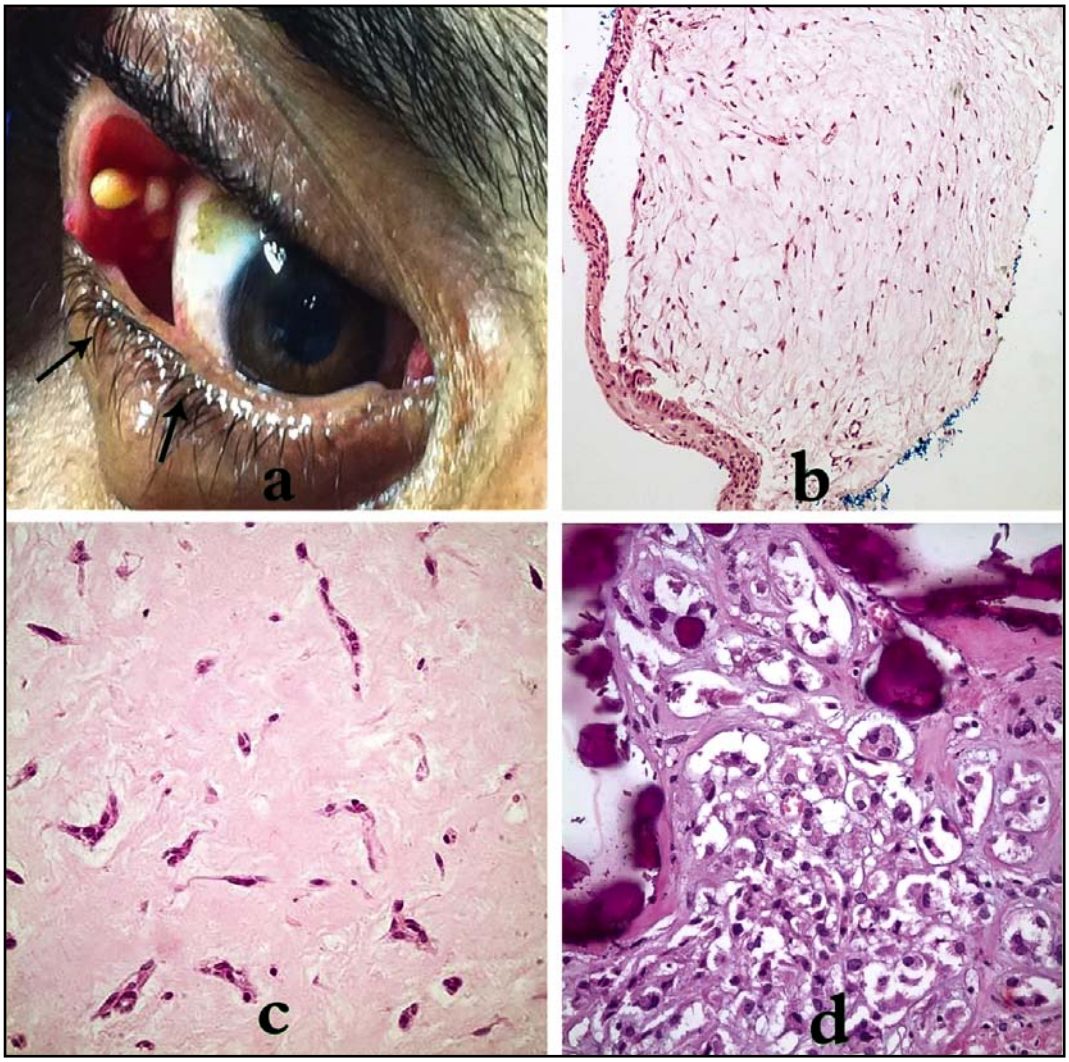

Figure 1.a. Solid papules on upper eyelid **b**. Eyelid superficial angiomyxoma consisting of polypoid shape, hypocellular, myxoid neoplasm without atypia and quite a few vessel **c.** Myxoma cells are seen as elongated, fusiform or stellate with oval or elongated nuclei and eosinophilic cytoplasm, **d.** Eosinophilic large neoplastic cells consisting of myxoid matrix calcification on testes.

cardiac masses and pulmonary emboli were resected via an open thoracotomy in the same surgery. The histologic diagnosis of all cardiac and pulmonary masses was myxoma (Figure 1c).

The patient's past medical history comprised neurofibroma excision from the left axillary region and skin tag excision from the right nasolabial sulcus. He was using metoprolol and amlodipine treatment for hypertension. He had twin boys through assisted reproductive techniques. There were no relatives with any history suggestive of CNC in the family.

On physical examination, the skin findings were as follows: a cutis verticis gyrate on the brow, amelanotic nevus on the right side of the brow, approximately 1 cm erythematous limited lesion on the right upper and lower conjunctival mucosa; solid papules on the upper eyelid (Figure 1a); multiple spotty brown pigmented lesions (lentigines) on the lower lip, palm and fingers; and a small and smooth papule lesion on the left ear helix. Additionally, he had an acromegalic appearance. His cardiac examination revealed normal sinus rhythm with heart rate 92/min, blood pressure of 140/90 mmHg, and a mid systolic murmur (2/6 degree) was audible in the mitral area. A nodule was palpable in the left thyroid lobe. He had an enlargement of his right testis, and masses detected in the right inguinal region and medial side of the thigh.

Hormonal and biochemical results are shown in Table 1. There was no GH suppression in the oral glucose tolerance test (OGTT). The pituitary magnestic resonance imaging (MRI) showed a 12×7.5-mm diameter pituitary macroadenoma. His pituitary adenoma was removed through transsphenoidal pituitary surgery. Histopathology findings showed a GH and PRL-secreting mixed adenoma. Following the pituitary surgery, he underwent total thyroidectomy, right orchiectomy, and soft tissue excision of the right inguinal region during the same surgery. The right testicular mass was identified as a large-cell calcifying Sertoli cell tumor (LCCST) (Figure 1d), and the right inguinal mass was consistent with superficial angiomyxoma. On pathologic examination of the thyroid, a hyalinizing trabecular adenoma and non-nodular thyroid tissue was noted to be more pronounced in the C cells (Figure 2a–d).

Evaluation of CS and pheochromocytoma was shown in Table 2. A two-day low-dose- followed by a two-day high-dose dexamethasone suppression test (DST) was performed for PPNAD, which showed a paradoxical increase of free cortisol in the urine from baseline 122 µg/d to 820 µg/d 6 days later. There were no adrenal lesions on the abdominal CT scan. We also diagnosed CS due to PPNAD, thus a bilateral adrenalectomy was planned. The preoperative echocardiogram findings were dilated cardiomyopathy, and the ejection fraction at that time was 36%. There was no myxoma in the cardiac chamber. A cardioverter defibrillator was implanted. During folllow-up, the patient presented with transient bilateral upper extremity weakness. A new cardiac mass (2.2×2.2 cm) was detected. A third cardiac operation was performed two years after his first admission to our unit. The postoperative course was again without complication. Histologically, a myxomatous tissue was identified. A laparoscopic bilateral adrenalectomy, right eyelid mass excision, and right conjunctival mucosal lesion excision were performed during the same operation after the cardiac surgery. In pathology, specimens were diagnosed as unilateral focal PPNAD, a unilateral nonpigmented adrenocortical nodule, and bilateral adrenal medullary hyperplasia (Figure 2e, f). Histologically, benign skin adnexal tumors showing follicular differentiation were identified on the conjunctiva, which were consistent with mantle cell hyperplasia, trichodiscomas, and fibrofolliculomas. In addition, a superficial angiomyxoma was identified on the eyelid (Figure 1b).

Tab. 1. Laboratory findings.

Biochemical results	Hormonal results
FPG: 96 mg/dL	GH: 5.35 ng/mL (0–3)
BUN: 13.3 mg/dL, Cr: 0.8 mg/dL	IGF–1: 522 ng/mL (64–336, age–sex matched)
ALT: 29 U/L, AST: 20 U/L	ACTH: 22.1 pg/mL (0–46)
Na: 145 mEq/L, K: 4.5 mEq/L	Cortisol: 18.8 µg/dL (>18)
Ca: 9.3 mg/dL, P: 3.6 mg/dL	FSH: 17.4 mIU/mL (1.2–19.2)
PTH: 56.4 pg/mL (0–75)	LH: 6.86 mIU/mL (1.2–8.6)
25 (OH) D vitamin: 6.8 µg/L	Testosterone: 199 ng/dL (254– 1600)
	PRL: 16.9 ng/mL (2.5–18.1)
	DHEAS <15 µg/dL

Tab. 2. Evaluation of Cushing Syndrome and Pheochromocytoma.

Urinary free costisol: 132 and 151 µg/day (13–75)	Vanillyl–mandelic acid: 6.7 mg/day (3.3–6.6)*
Low dose DST, cortisol: 12.5 µg/dL	Dopamin: 555.6 µg/day (190–480)*
High dose DST, cortisol: 17.6 µg/dL	Normetanephrine: 633 µg/day (105–354)*

DST: dexametasone supression test, *24 hr excretion of urine.

An evaluation of this patient's presentation revealed several features consistent with the diagnosis of CNC. Having met clinical criteria for a diagnosis of CNC, sequencing of the *PRKAR1A* gene was undertaken. An analysis showed that a novel deletion mutation (c171insT) in exon 2 of the *PRKAR1A* gene was present in our patient.

Genetic testing

Genomic DNA was extracted from white blood cells in accordance with standard procedures. Polymerase chain reaction (PCR) conditions were established to

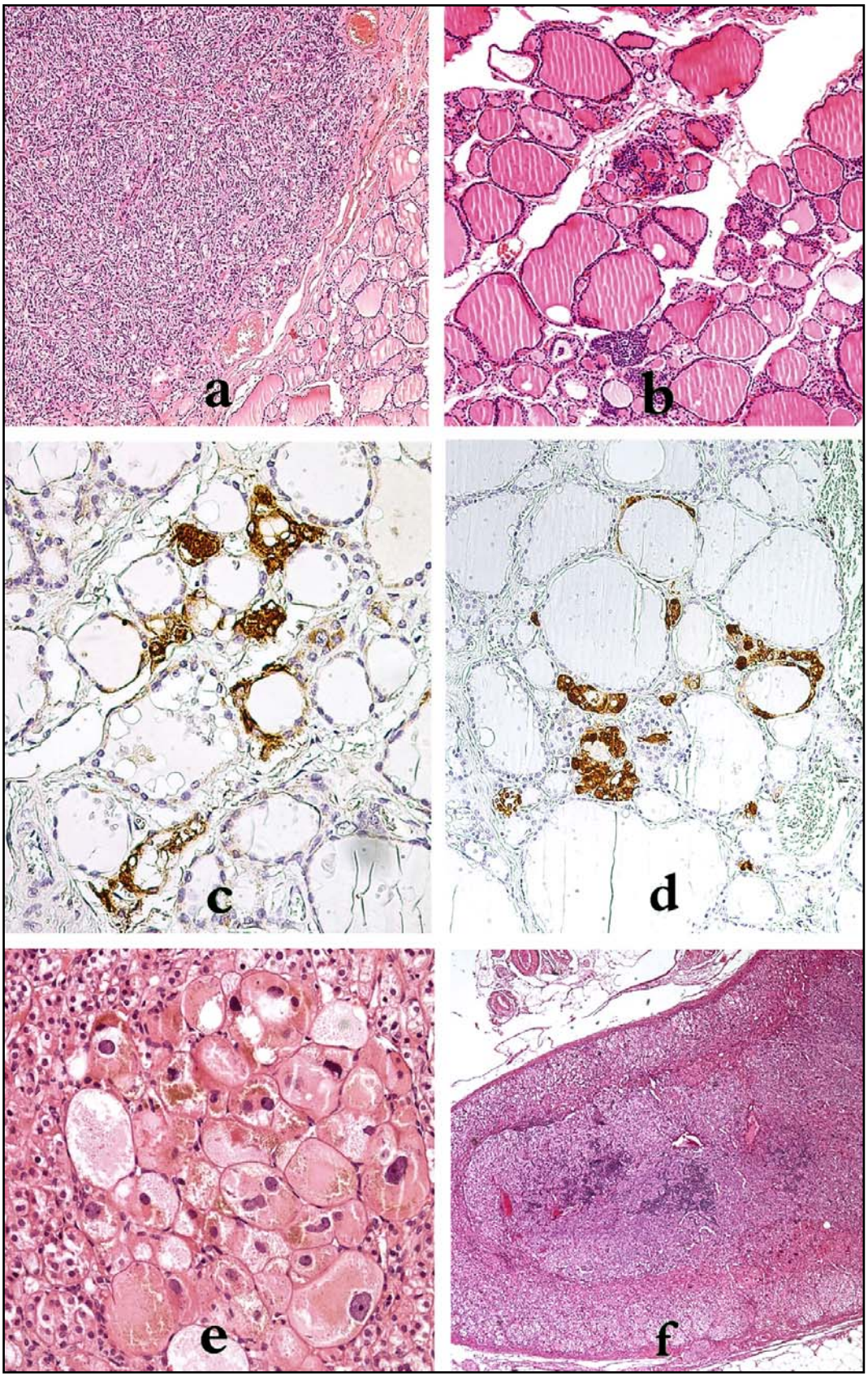

Figure 2 a. Thyroid nodule consisting of nested neoplastic follicular cells exhibiting trabecular arrangement in a hyalinized/sclerotic stroma **b.** Solid nodular proliferation of round to polygonal C cells in intrafollicular location with CEA **(c)** and calcitonin (**d)** immunoreactivity on thyroid **e.** Nodule composed of eosinophilic, similar to normal zone reticularis, cells with abundant brown pigment and aberration was seen in unilateral adrenal gland. **f**. The cortex to medulla ratio is much less than 10:1 ratio which is abnormal and consistent with adrenal medullary hyperplasia.

separately amplify all exons and exon-flanking intron sequences of the *PRKAR1A* gene for the patient sample. PCR amplifications were performed in 25 mL reaction volumes containing 100 ng of genomic DNA, 0.2 mM each of dNTP and 2U Taq DNA polymerase in the reaction buffer supplied by the manufacturer. As the standard procedure, 35 PCR cycles were performed with 1 min denaturation at 94 °C, 1 min annealing at 50 or 58 °C, depending on the primer pair, and 1 min elongation at 72 °C (Kirschner, Sandrini *et al.* 2000). The PCR products were purified and sequenced on both strands using dye-terminator chemistry (ABI BigDye Terminator v3.1) and subsequently resolved on an ABI3130 (Applied Biosystems, Foster City, USA). NC 000017 was used as the reference sequence for *PRKAR1A*. The genetic analysis found a novel insertion mutation (c.171-172insT) (p.L57FfsX70) in the *PRKAR1A* gene in exon 2 (Figure 3).

DISCUSSION

We presented a case of unusual manifestations of CNC. The diagnosis of CNC can be made if two or more major manifestations of the syndrome are confirmed in histology, biochemical testing or imaging. If the patient has a demonstrated germline *PRKAR1A* gene mutation and/or a first-degree relative affected by CNC, a single manifestation is sufficient for the diagnosis (Stratakis *et al.* 2001; Sandrini & Stratakis 2003; Boikos & Stratakis 2006).

Biatrial myxomas are rare in patients with CNC. Presenting signs and symptoms derive from cardiac and embolic events. In our patient, cardiac myxoma was detected in both atria, which presented with pulmonary emboli and transient ischemic attack. Recurrent myxoma are not uncommon with recurrence rates approaching 21% in familial cases (Turhan *et al.* 2008). We scanned our patient in terms of recurrent myxoma with transthoracic echocardiography every six months and detected a new cardiac myxoma.

Various endocrine tumors accompany CNC. Clinical acromegaly due to rapidly growing adenoma is rare and may be seen in only 10–20% of patients (Rothenbuhler & Stratakis 2010). The pituitary adenomas usually stain positive for GH and PRL (Courcoutsakis *et al.* 2013). The most frequent testicular manifestation in CNC is LCCSCT. It can cause obstruction of seminiferous tubules and lead to reduced fertility. The hypergonadothropic hypogonadism and LCCST may explain our patient's infertility. Our patient's thyroid pathology was consistent with hyalinizing trabecular adenoma, and the C cells in non-nodular thyroid tissue had a striking appearance. However, both preoperative and postoperative serum calcitonin and carcinoembryonic antigen levels were normal. Thyroid carcinoma is unusual among patients with CNC, but when present, it is follicular or papillary in type (Stratakis *et al.* 1997). To our knowledge, there have been no reports of medullary thyroid carcinoma together with CNC.

PPNAD is the most frequent endocrine manifestation in patients with CNC (Rothenbuhler & Stratakis 2010). PPNAD is a rare cause of ACTH-independent CS and may be isolated or occur as part of CNC. It affects between 25% and 60% of individuals with CNC (Bertherat *et al.* 2009; Rothenbuhler & Stratakis 2010; Courcoutsakis *et al.* 2013). Diagnosis of CS due to PPNAD is often difficult because hypercortisolism can develop progressively over years and may be periodic among patients with CNC. Interestingly, most patients with CNC and PPNAD have a paradoxical cortisol response after dexamethasone administration (Stratakis *et al.* 1999). In our patient, a paradoxical increase in

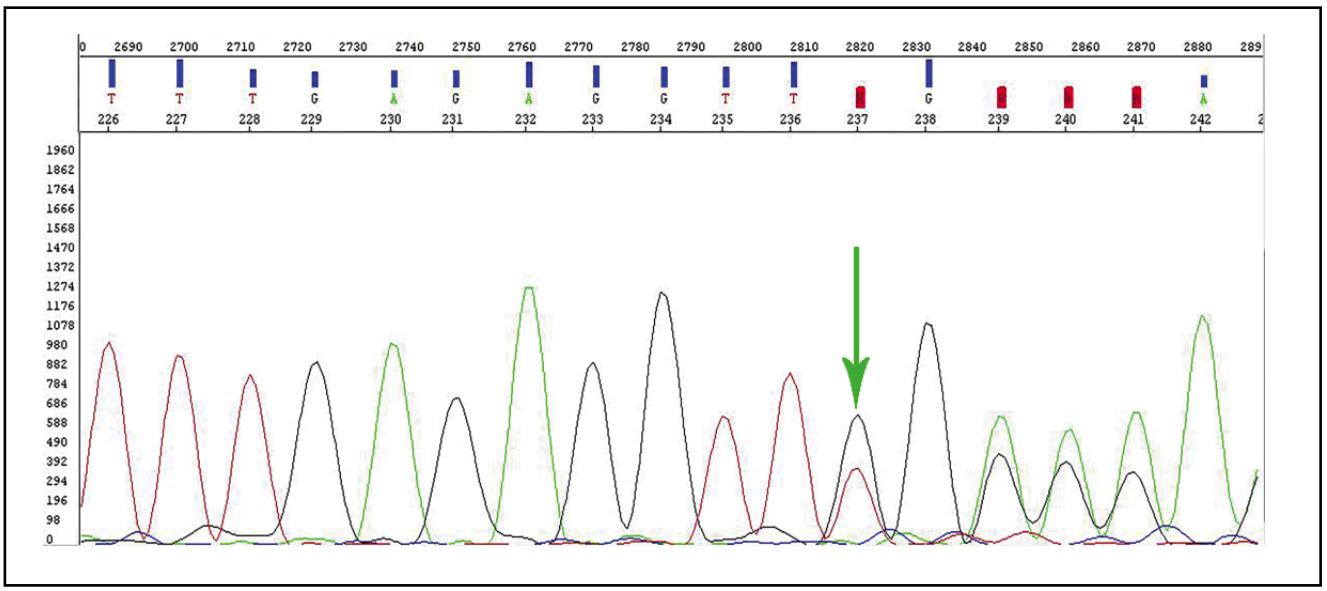

Figure 3. The genetic analysis found a novel insertion mutation (c.171-172insT) (p.L57FfsX70) in the *PRKAR1A* gene in exon 2.

cortisol secretion was exhibited in response to the Liddle's test so we suspected PPNAD and a focal PPNAD was identified in the histopathologica findings of the adrenal glands. Unusual pathologic features of patients with CNC have been reported in the literature; one nonpigmented nodule and multiple pigmented nodules on one side of the adrenal glands (Travis *et al.* 1989) and a nonpigmented adrenocortical adenoma and focal PPNAD in one adrenal gland and a diffuse PPNAD in the other (Tung *et al.* 2012). To our knowledge, this is the first case of CNC caused by a unilateral focal PPNAD, a unilateral nonpigmented adrenocortical nodule and bilateral adrenal medullary hyperplasia with the novel mutation of PRKAR1A gene to be reported in the literature. On the other hand, we do not know if the bilateral adrenal medullary hyperplasia contributed to the increased glucocorticoid responsiveness in our case. Increased urinary catecholamine excretion due to possible coexistence of adrenal medullary hyperplasia has previously been reported in a patient with adrenal CS (Kazama *et al.* 1985). Moreover, glucocorticoid binding activity has been shown in patients with pheochromocytoma (Kontula *et al.* 1985). The clinical signs of CS in our patient may have been associated with both bilateral adrenal medullary hyperplasia and the focal unilateral PPNAD.

More than 70% of patients who are diagnosed as having CNC carry mutations on the *PRKAR1A* gene (Correa *et al.* 2015). Heterozygous inactivating mutations of PRKAR1A gene have been reported in aproximately 45% to 65% of families with CNC. Patients with CNC and PPNAD have a higher percentage of *PRKAR1A* mutations, which have been reported to reach 80% (Groussin *et al.* 2002). Patients with *PRKAR1A* mutations had myxomas, skin lesions, thyroid tumors, and Sertoli cell tumors, and schwannomas more frequently, and these clinical signs appeared at an earlier age compared with those with CNC who did not have *PRKAR1A* mutations (Bertherat *et al.* 2009). Heterogeneity in genetic and clinical manifestations of patients with CNC have previously been reported (Pack *et al.* 2000; Stratakis *et al.* 2001; Bertherat *et al.* 2009; Horvath *et al.* 2010). To date, more than 120 different PRKAR1A mutations have been identified in patients with CNC (Schernthaner-Reiter *et al.* 2015).

In conclusion, we have described a case of CNC with a novel mutation in exon 2 of the *PRKAR1A* gene. The significance of this study is the identification of the new insertion mutation that lead to CNC with unusual clinical manifestations. These included recurrent cardiac biatrial myxomas; GH and PRL-secreting mixed pituitary adenoma; infertility with large-cell calcifying Sertoli cell tumor of the testis; hyalinizing trabecular adenoma; striking C cells in the thyroid; superficial angiomyxoma in inguinal region and eyelid; mantle cell hyperplasia; trichodiscomas and fibrofolliculomas on the conjunctiva; CS due to a focal unilateral PPNAD; and possible unilateral adrenocortical nodule or bilateral adrenal medullary hyperplasia. To our knowledge, this is the first report of a patient with CNC who also had bilateral adrenal medullary hyperplasia and a focal PPNAD on the adrenal and the presence of marked appearing C cells on the thyroid.

***Conflict of Interest**: The authors declare that they have no conflict of interest.*

REFERENCES

1 Bertherat J, Horvath A, Groussin L, Grabar S, Boikos S, Cazabat L, Libe R, Rene-Corail F, Stergiopoulos S, Bourdeau I, Bei T, Clauser E, Calender A, Kirschner LS, Bertagna X, Carney JA, Stratakis CA (2009). Mutations in regulatory subunit type 1A of cyclic adenosine 5'-monophosphate-dependent protein kinase (PRKAR1A): phenotype analysis in 353 patients and 80 different genotypes. The Journal of Clinical Endocrinology and Metabolism **94**: 2085–2091.

2 Boikos SA, Stratakis CA (2006). Carney complex: pathology and molecular genetics. Neuroendocrinology **83**: 189–199.

3 Carney JA, Gordon H, Carpenter PC, Shenoy BV, Go VL (1985). The complex of myxomas, spotty pigmentation, and endocrine overactivity. Medicine **64**: 270–283.

4 Correa R, Salpea P, Stratakis CA (2015). Carney complex: an update. European journal of endocrinology / European Federation of Endocrine Societies **173**: M85–97.

5 Courcoutsakis NA, Tatsi C, Patronas NJ, Lee CC, Prassopoulos PK, Stratakis CA (2013). The complex of myxomas, spotty skin pigmentation and endocrine overactivity (Carney complex): imaging findings with clinical and pathological correlation. Insights Into Imaging **4**: 119–133.

6 Groussin L, Kirschner LS, Vincent-Dejean C, Perlemoine K, Jullian E, Delemer B, Zacharieva S, Pignatelli D, Carney JA, Luton JP, Bertagna X, Stratakis CA, Bertherat J (2002). Molecular analysis of the cyclic AMP-dependent protein kinase A (PKA) regulatory subunit 1A (PRKAR1A) gene in patients with Carney complex and primary pigmented nodular adrenocortical disease (PPNAD) reveals novel mutations and clues for pathophysiology: augmented PKA signaling is associated with adrenal tumorigenesis in PPNAD. American Journal of Human Genetics **71**: 1433–1442.

7 Horvath A, Bertherat J, Groussin L, Guillaud-Bataille M, Tsang K, Cazabat L, Libe R, Remmers E, Rene-Corail F, Faucz FR, Clauser E, Calender A, Bertagna X, Carney JA, Stratakis CA (2010). Mutations and polymorphisms in the gene encoding regulatory subunit type 1-alpha of protein kinase A (PRKAR1A): an update. Human Mutation **31**: 369–379.

8 Kazama Y, Noguchi T, Kawabe T, Shirosu N, Takei K, Kobayashi K, Ueno A, Onaya T (1985). A case of Cushing's syndrome associated with possible adrenomedullary hyperplasia. Endocrinologia japonica **32**: 355–359.

9 Kirschner LS, Sandrini F, Monbo J, Lin JP, Carney JA, Stratakis CA (2000). Genetic heterogeneity and spectrum of mutations of the PRKAR1A gene in patients with the carney complex. Human Molecular Genetics **9**: 3037–3046.

10 Kontula K, Pomoell UM, Gunsalus GL, Pelkonen R (1985). Glucocorticoid receptors and responsiveness of normal and neoplastic human adrenal cortex. The Journal of Clinical Endocrinology and Metabolism **60**: 283–289.

11 Pack SD, Kirschner LS, Pak E, Zhuang Z, Carney JA, Stratakis CA (2000). Genetic and histologic studies of somatomammotropic pituitary tumors in patients with the "complex of spotty skin pigmentation, myxomas, endocrine overactivity and schwannomas" (Carney complex). The Journal of Clinical Endocrinology and Metabolism **85**: 3860–3865.

12 Rothenbuhler A, Stratakis CA (2010). Clinical and molecular genetics of Carney complex. Best practice & research Clinical Endocrinology & Metabolism **24**: 389–399.

13 Salpea P, Stratakis CA (2014). Carney complex and McCune Albright syndrome: an overview of clinical manifestations and human molecular genetics. Molecular and Cellular Endocrinology **386**: 85–91.
14 Sandrini F, Stratakis C (2003). Clinical and molecular genetics of Carney complex. Molecular genetics and metabolism **78**: 83–92.
15 Schernthaner-Reiter MH, Trivellin G, Stratakis CA (2015). MEN1, MEN4, and Carney Complex: Pathology and Molecular Genetics. Neuroendocrinology.
16 Stratakis CA, Courcoutsakis NA, Abati A, Filie A, Doppman JL, Carney JA, Shawker T (1997). Thyroid gland abnormalities in patients with the syndrome of spotty skin pigmentation, myxomas, endocrine overactivity, and schwannomas (Carney complex). The Journal of Clinical Endocrinology and Metabolism **82**: 2037–2043.
17 Stratakis CA, Kirschner LS, Carney JA (2001). Clinical and molecular features of the Carney complex: diagnostic criteria and recommendations for patient evaluation. The Journal of Clinical Endocrinology and Metabolism **86**: 4041–4046.
18 Stratakis CA, Sarlis N, Kirschner LS, Carney JA, Doppman JL, Nieman LK, Chrousos GP, Papanicolaou DA (1999). Paradoxical response to dexamethasone in the diagnosis of primary pigmented nodular adrenocortical disease. Annals of Internal Medicine **131**: 585–591.
19 Travis WD, Tsokos M, Doppman JL, Nieman L, Chrousos GP, Cutler GB, Jr., Loriaux DL, Norton JA (1989). Primary pigmented nodular adrenocortical disease. A light and electron microscopic study of eight cases. The American Journal of Surgical Pathology **13**: 921–930.
20 Tung SC, Hwang DY, Yang JW, Chen WJ, Lee CT (2012). 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. Endocrine Journal **59**: 823–830.
21 Turhan S, Tulunay C, Altin T, Dincer I (2008). Second recurrence of familial cardiac myxomas in atypical locations. The Canadian Journal of Cardiology **24**: 715–716.