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Phenotypic Variability in a Family with Carney Complex Accompanied by a Novel Mutation Involving *PRKAR1A*

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Carney complex is a rare, autosomal dominant disease accompanied by multiple endocrine neoplastic syndromes. Mutations in the *PRKAR1A* gene have recently been reported as a cause of Carney complex, but genotype-phenotype correlations vary widely. A 15-year-old Japanese man (Case 1) with short stature visited our hospital with suspected Cushing's syndrome. Biochemical investigations suggested corticotropin-independent Cushing's syndrome. Computed tomography revealed multiple bilateral adrenal tumors, and a two-staged partial adrenalectomy was performed. Pathological findings revealed primary pigmented nodular adrenocortical disease (PPNAD). The patient also exhibited distinctive spotty skin pigmentation. Based on these features, the patient was diagnosed as Carney complex. Cascade screening of family members was performed, and the mother (Case 2) and elder brother (Case 3) were diagnosed as Carney complex. Case 2 showed cardiac myxoma, acromegaly, spotty skin pigmentation, and mammary myxoid fibroadenoma. Case 3 exhibited gigantism, spotty skin pigmentation, and thyroid nodules. Target gene testing in Case 1 and 2 revealed the same novel mutation in *PRKAR1A* gene (c.503G>T, p.Gly168Val). This mutation was predicted as a pathogenic variant by multiple *in silico* analyses. Here, we present a family of Carney complex cases with a novel *PRKAR1A* pathogenic variant exhibiting varied clinical phenotypes within each case. In these cases, some specific phenotypes of Carney complex, such as pigmentary disorders, myxomas, and PPNAD are important as clues for diagnosis and prognostic factors. Clinicians should consider further examination in patients with Carney complex-specific phenotypes.

Keywords: Carney complex; heart myxoma; primary pigmented nodular adrenocortical disease; PRKAR1A gene; spotty skin pigmentation
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Introduction

Carney complex (CNC) is a rare autosomal dominant disease accompanied by multiorgan disorders such as Cushing's syndrome (CS) associated with primary pigmented nodular adrenocortical disease (PPNAD), spotty skin pigmentation, cardiac myxomas, and various other tumors, including growth hormone (GH)-producing adenomas as acromegaly, mammary myxoid fibroadenomas, large-cell calcifying Sertoli cell tumors, psammomatous melanotic schwanomas, and thyroid nodules (Lowe et al. 2017; Bouys and Bertherat 2021). Skin disorders are the most common manifestation, followed by cardiac myxomas and PPNAD (Stratakis et al. 2001). Additionally, PPNAD has a high specificity for CNC (Almeida and Stratakis 2010; Bouys and Bertherat 2021).

In 2000, a *PRKAR1A* gene mutation was identified as a factor responsible for > 60% of CNC patients (Kirschner et al. 2000; Sahut-Barnola et al. 2010). A part of the phenotype-genotype correlation was clarified by this gene (Takano et al. 2009). However, the genotype-phenotype correlation in CNC cases remains unknown. For example, CNC phenotypes can vary among patients with the same mutation in *PRKAR1A* gene, even within members of the

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same family (Bertherat et al. 2009; Bouys and Bertherat 2021). Therefore, it is difficult to diagnose CNCs in clinical practice.

Here, we report a family that exhibited considerably different CNC phenotypes accompanied by a novel mutation in *PRKAR1A*. When pathognomonic symptoms such as spotty skin pigmentation, myxoma, and PPNAD are present, a possible diagnosis of CNC should be considered by clinicians.

Case Presentation

Case 1

A 15-year-old Japanese adolescent (Case 1) with short stature visited our hospital for examination of suspected CS. He had noticed symptoms 2 years previously. His height was 147.0 cm [−3.9 standard deviations (SDS)] and body weight was 47.2 kg on admission. He showed features of CS, including a moon face, a buffalo hump, weight gain, and subcutaneous purpura. Biochemical data showed loss of diurnal variation on the hypothalamic-pituitary-adrenal axis with suppressed ACTH levels (plasma ACTH and cortisol levels: < 1.0 pg/mL and 26.3 μg/dL in the morning, and < 1.0 pg/mL and 28.4 μg/dL at the late-night, respectively). The 1 mg dexamethasone suppression test (DST) showed a high cortisol level (26.1 μg/dL). His urinary 24-hour cortisol excretion was 1,810 μg/day. Computed tomography (CT) and magnetic resonance imaging (MRI) revealed multiple bilateral adrenal adenomas (Fig. 1A-C). Adrenal scintigraphy with ^{131}I-adosterol showed bilateral adrenal uptake (Fig. 1D). There were no pituitary, cardiac, or thyroid tumors. Based on these data, he was diagnosed with CS due to bilateral multiple adrenal adenomas, and 1,000 mg metyrapone was administered. For the bilateral cortisol-producing adenomas, we discussed therapeutic options with the patient and his family. In particular, they were concerned about the patient's mortality with post-operative adrenal insufficiency after bilateral adrenalectomy, as previously reported (Ragnarsson et al.

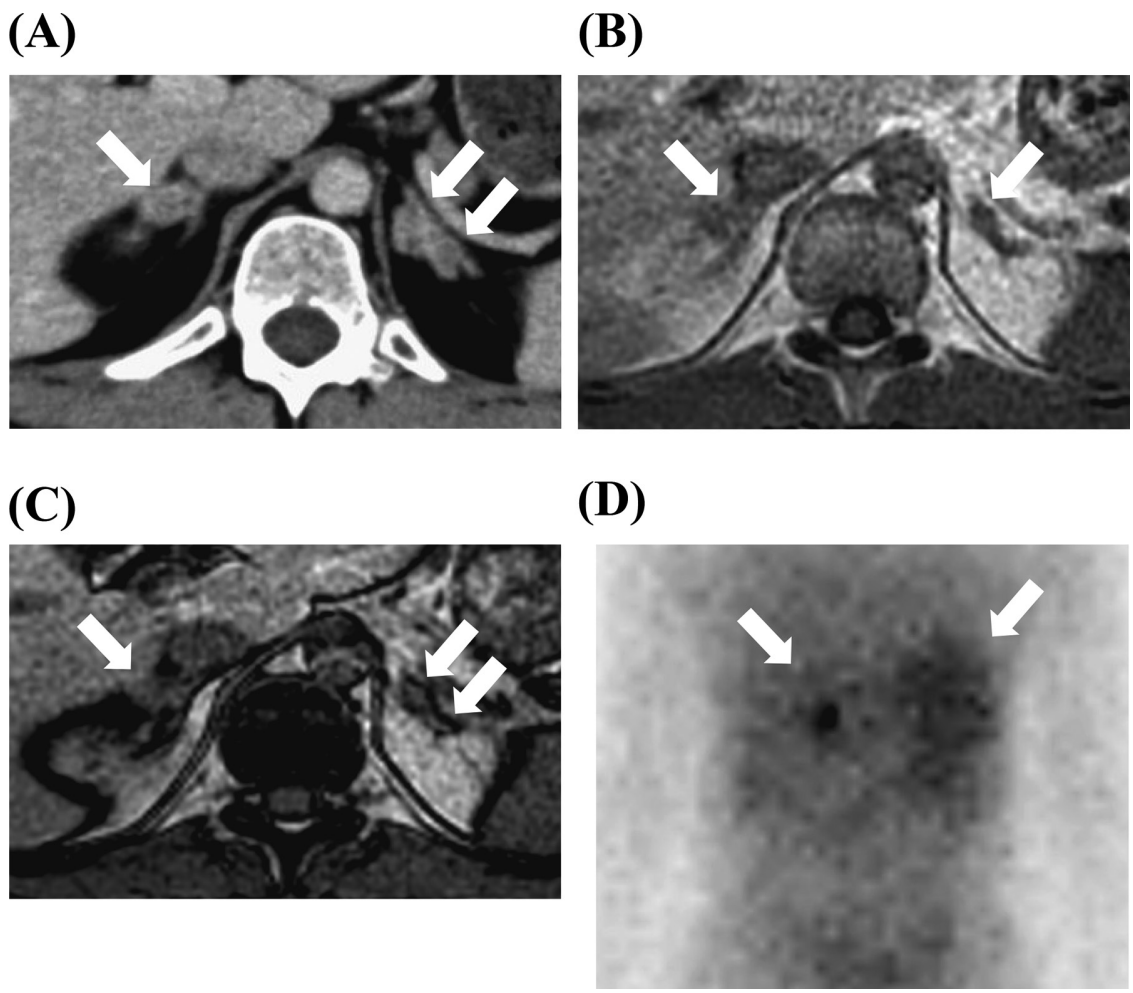

Fig. 1. Clinical images of Case 1.
Images obtained from contrast-enhanced computed tomography (CT) (A), plain magnetic resonance imaging (MRI) [T1-weighted in-phase (B) and out-of-phase (C)], and ^{131}I-adosterol scintigraphy (D) of the propositus on admission. MRI revealed nodules with slightly low signal intensity in the T1 weighted out-of-phase image compared with in-phase image, suggesting primary pigmented nodular adrenocortical disease. White arrows indicate bilateral adrenal tumors.

2019). Considering this, laparoscopic partial adrenalectomy of the right adrenal gland was performed at the age of 15 years. The lateral side of the right adrenal gland, including the largest tumor (approximately 16 mm in diameter), was resected to preserve the central vein and the adrenal function. Macroscopically, small tumors were found in the spared ipsilateral adrenal gland. An additional secondary partial adrenalectomy of the left adrenal gland was performed at the age of 16 years because cortisol secretion was not sufficiently normalized after the first operation (cortisol level after 1 mg-DST, 12.1 μg/dL; 24-hour urinary cortisol excretion, 202 μg/day). On the second operation, the cranial side of the adrenal gland appeared intact macroscopically. Therefore, the lateral and caudal parts were resected while preserving central vein, though some macroscopic pigmented micronodules were found in the margin of the tissue surface. The diagnosis of PPNAD was confirmed pathologically from inspection of the adrenal glands (Fig. 2A, B). After the secondary surgery, his symptoms diminished without adrenal insufficiency. Thereafter, his final height reached 153.0 cm (−2.4 SDS) at the age of 20 years (target height 169.0 cm; paternal height 165.0 cm and maternal height 160 cm) (Barstow and Rerucha 2015), with relatively controlled cortisol excretion [cortisol level after 1 mg-DST, 8.2 μg/dL (one month after secondary operation), and 10.3 μg/dL (four years after secondary operation)].

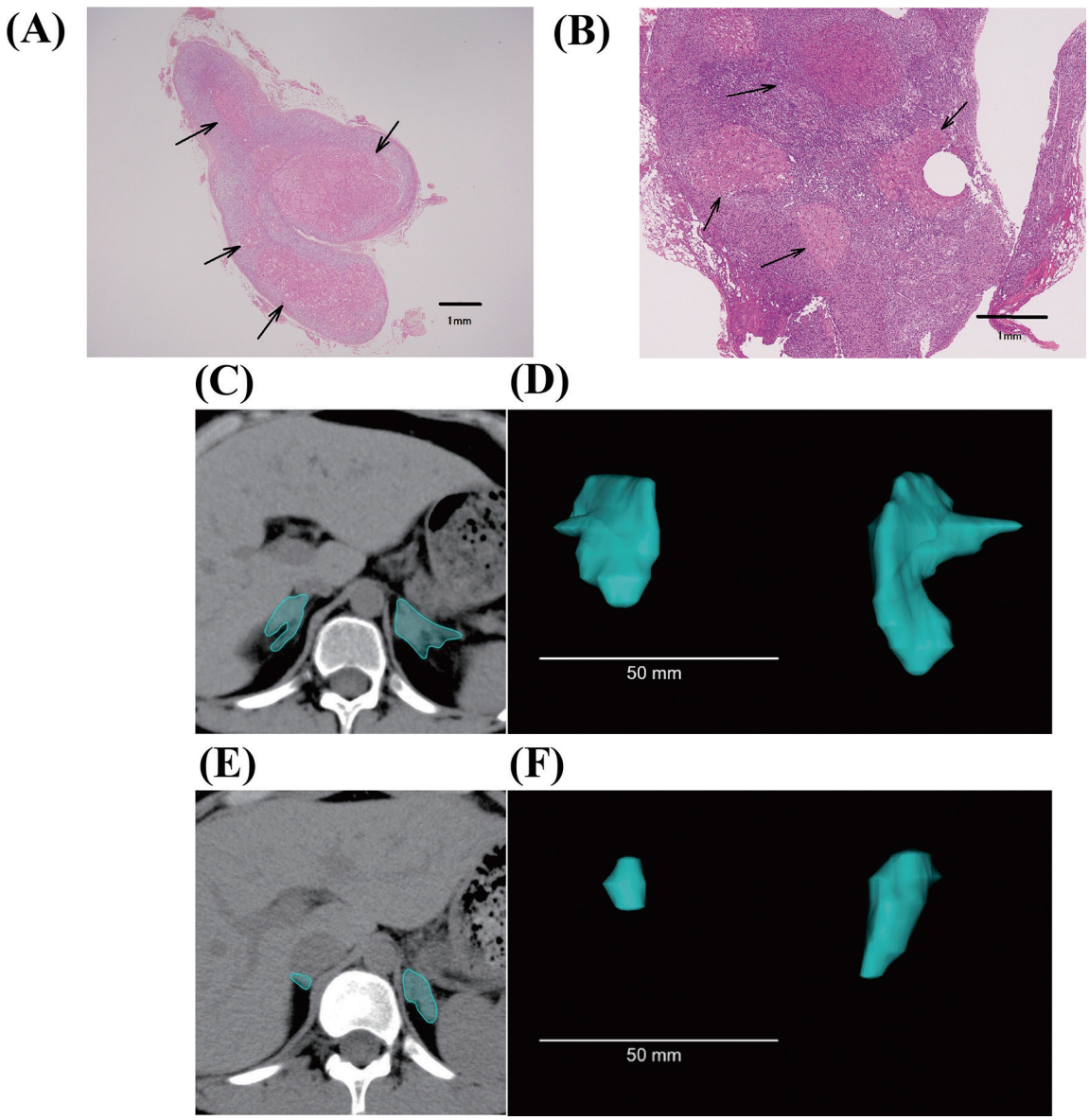

Fig. 2. Pathological findings and adrenal computed tomography (CT) volumetry in Case 1.
Pathological findings in the right (A) and left (B) adrenal glands of Case 1 with hematoxylin and eosin-stained specimen. Well-circumcised nodules (shown with black arrows) suggestive of primary pigmented nodular adrenocortical disease are shown. Demonstrable pre-operative (C and D) and four years post-operative (E and F) adrenal CT volumetry images show decrease in adrenal volume after operation. CT volumetry was conducted with plain CT images.

Table 1. Clinical manifestations in Case 1, 2, and 3.

Manifestation	Percent frequency of manifestation	Case 1	Case 2	Case 3
Spotty skin pigmentation	77%	○	○	○
Cardiac myxoma	53%	×	○	×
PPNAD	45-70%	○	×	×
Acromegaly/Gigantism	10%	×	○	○
Thyroid nodule	5%	×	×	○
Ductal breast adenoma	3% (female)	×	○	×
LCCSCT	33% (male)	×	×	×
PMS	10%	×	×	×

Phenotype of family members and the percent frequency of manifestation are shown (Kirschner et al. 2000; Lowe et al. 2017; Bouys and Bertherat 2021). ○, present; ×, absent.
PPNAD, primary pigmented nodular adrenocortical disease; LCCSCT, large-cell calcifying Sertoli cell tumors; PMS, psammomatous melanotic schwanoma.

Adrenal CT volumetry performed with reference to the previous report (Matsunaga et al. 2021) using the Eclipse treatment planning system version13.6 (Varian California, USA) (Fig. 2C-F) revealed decreased adrenal volumes (pre-operation, 3.7 mL and 7.8 mL; post-operation, 0.4 mL and 3.4 mL; 89.2% and 56.4% reduction in the right and left adrenal glands, respectively).

Based on his pathognomonic symptoms, including spotty skin pigmentation and PPNAD, he was diagnosed as CNC. Then, we conducted cascade screening. First, his mother (Case 2) was revealed to have CNC, as described below. Furthermore, his elder brother was found to be extremely tall at 195.0 cm (+3.6 SDS) upon visiting the hospital, and he was diagnosed with CNC (Case 3). Each phenotype of family members compared with those in previous reports is shown in Table 1.

Case 2

Case 2 showed spotty skin pigmentation, ductal breast adenoma, left atrial myxoma (Fig. 3A) and acromegaly with the high levels of insulin-like growth factor-1 (IGF-1) and GH [413 ng/mL (+5.7 SDS) and 15.7 ng/mL]. A 75 g oral glucose tolerance test (OGTT) revealed unsuppressed GH levels (GH nadir; 12.2 ng/mL at 90 min), and pituitary MRI showed a micropituitary adenoma shifting the pituitary stalk to the left (Fig. 3B). Firstly, surgical resection of the cardiac myxoma was performed. Thereafter, transsphenoidal pituitary surgery (TSS) for acromegaly was performed. However, a lack of GH suppression in OGTT (GH nadir; 1.28 ng/mL at 90 min) persisted after operation. Thereafter, we advised an additional operation, but she rejected. Therefore, we proposed somatostatin analogs (SSAs) as the therapy for acromegaly. However, she denied SSA treatment because of economic limitation, and we discussed additional treatment, resulting in the choice of cabergoline administration with informed consent. Cabergoline treatment was commenced at a dose of 0.25 mg/week.

Case 3

Case 3 recognized his growth in height from the age of 13 years. At 16 years of age, he was 195.0 cm (+3.6 SDS) when we first encountered in the outpatient. He was also examined for the probability of CNC due to proband's diagnosis. Ultrasound examination revealed no heart myxoma, but pituitary MRI revealed a pituitary tumor (4 × 9 × 4 mm, Fig. 4). The patient also had spotty skin pigmentation and thyroid nodules. Laboratory data revealed high IGF-1 and GH levels [780 ng/mL (+3.8 SDS) and 6.8 ng/mL respectively] and a lack of GH suppression in the OGTT (GH nadir; 6.4 ng/mL at 60 min), though he did not show other acromegalic phenotypes, such as thickening of soft tissue, diabetes, hypertension, and sleep apnea syndrome. Based on these data, the patient was diagnosed with gigantism due to a GH-secreting pituitary adenoma, and TSS was performed. Because his GH nadir in the OGTT was not normalized after surgery (GH nadir; 3.3 ng/mL at 30 min), 20 mg octreotide long-acting release per four weeks was continued.

Informed consent on genetic analysis of PRKAR1A

Because informed consent was obtained from Case 1 and 2, we performed genome sequencing analysis of blood cells for *PRKAR1A* gene in order to investigate phenotype-genotype correlations in this family.

Materials and Methods

DNA extraction and sequencing procedure

Genomic DNA was extracted from whole blood samples of Case 1 and 2. In addition, genomic DNA was extracted from the left adrenal gland tissue of Case 1. The resected tissue specimens were frozen in liquid nitrogen immediately after excision and stored at −80°C. To sequence *PRKAR1A, PRKACA, PDE11A, ARMC5, PDE8B, GNAS*, and *CTNNB1*, 1.0 μL of cDNA was subjected to PCR using Tks Gflex DNA polymerase (Takara Bio,

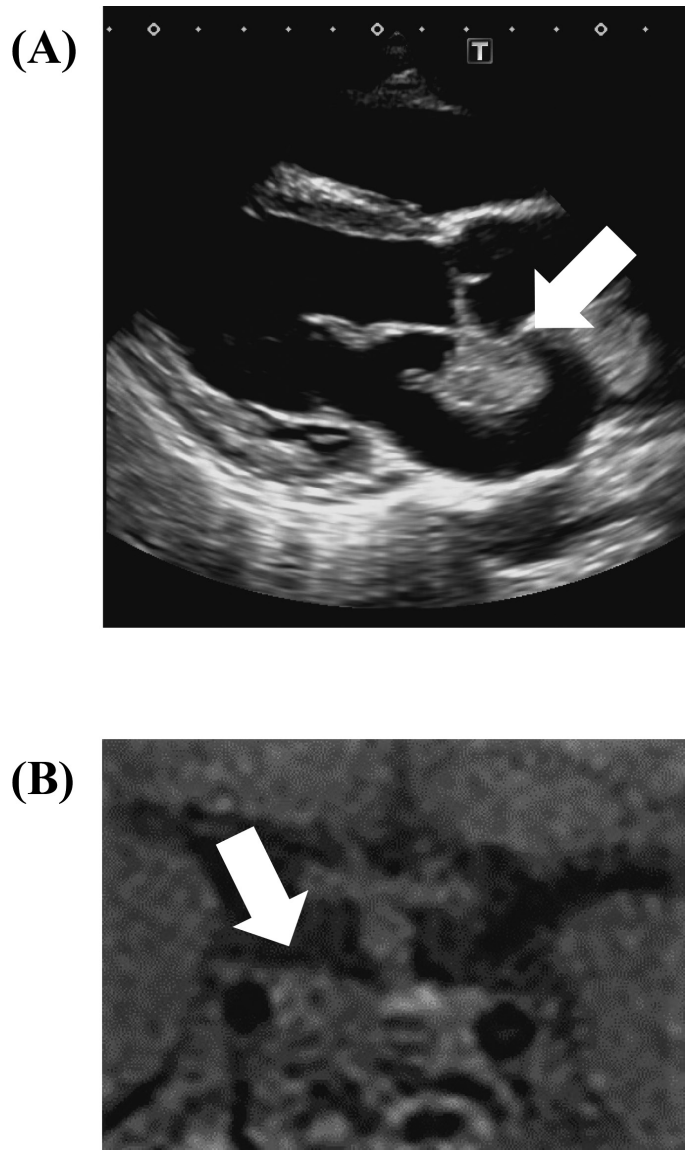

Fig. 3. Clinical images of Case 2.
(A) Ultrasonography showed left atrial myxoma measuring 32 × 19 × 23 mm. (B) In T1-weighted plain magnetic resonance imaging (MRI), adenoma was identified and the pituitary stalk is shifted to the left. A white arrow indicates arterial myxoma and pituitary adenoma, respectively.

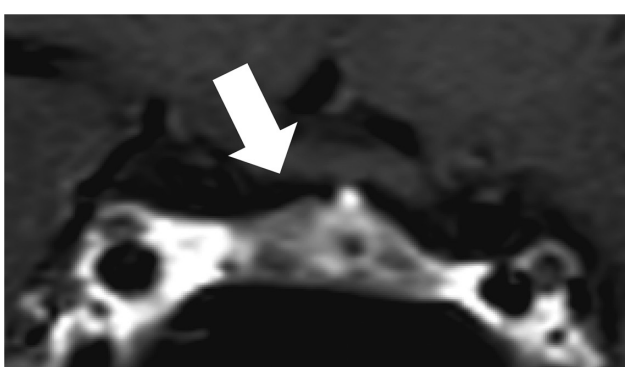

Fig. 4. A clinical image of Case 3.
Pituitary image from T1-weighted, contrast-enhanced magnetic resonance imaging (MRI) of Case 3. A partially cystic adenoma is indicated by the white arrow.

Kusatsu, Japan). Primers were used according to previous studies (Libé et al. 2008; Tadjine et al. 2008; Almeida and Stratakis 2010; Almeida et al. 2012; Maria et al. 2020) (Table 2).

In silico analysis procedure

The functional effects of *PRKAR1A* mutations were evaluated using PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), and the sorting intolerant from tolerant algorithm (http://sift.jcvi.org/).

Consent for publication

Written informed consent was obtained from the patients (Case 1, 2, and 3) for the publication and images contained in this case report.

Table 2. Primer sequences used in this study.

Gene Symbols	Exon area	Forward primer (5' to 3')	Reverse primer (3' to 5')
PRKAR1A	Exon 1	AGTCGCCCACCTGTCATCT	CACTTCTCCTTTCCGCAGTC
	Exon 2	CATTGACGTCAGTAGCCGAA	ATCTTGGATCGGTCCAGCTC
	Exon 3	CCTAGTCCCCACTTCCCTGT	ATCACCTCATCATCTCCCCA
	Exon 4	CATGCCGAAGGATCTCATTT	ATGGATGAAGTTCCACCCTG
	Exon 5	TTGCTTGATTTTCTTTCCCC	ATTCTTATTGCTCGGAAGCG
	Exon 6	CAGGTTGCAAACGTGAAATG	CTGCGATAAAGGAGACCGAA
	Exon 7	AGCCAAAGCCATTGAAAAGA	GCCTCCTCTCCCGTAACAAT
	Exon 8	TTGCTTGATTTTCTTTCCCC	ATTCTTATTGCTCGGAAGCG
	Exon 9	TCATTTAACTCGTCAGAAATCACC	TTCTAAATCACACTCTCAAACACCA
	Exon 10	GGCATAATATTGGCGGAAAA	AAGGCTTTTCCCAAGTCCAT
	Exon 11	AGAATGTTGAATGGGCATGG	TTAGCCCACTCTTTCCCTCTT
	Exon 12	CACCCTGGGTTTGAGAGTGT	TTCCCTCTCAGAGCCAAAAA
	Exon 13	CCCATCTTTGCTTTCTCCAG	AACAGACAGGAAGCTGCGAT
PRKACA	Exon 7	CGCTGGATCTCATCTACAGGG	CGAAGAAGGGCGGGTAGC
PDE11A	Exon 1	GGGTGAACATGTGCAGGAAC	GGGGATACCGAGGCAGATTC
	Exon 1-4	CAGCCCATATTCTCAGTGCG	GCGTTCACATTTCAGCAGAGT
	Exon 3-11	AGAGCTTTGCTAGAGGTGGT	CACCAGAGGGATGTTGGCTG
	Exon 9-15	TCAAGTGAAGAAGTCCTGGGC	TACAGCGTGAGGTCTGTTGC
	Exon 15-20	GCTAACCTGTCCTCCAAGGAA	TGCTGGACTGAAAATGGCCC
ARMC5	Exon 1-2	CACCAAGCATCAGGTTTCCG	CTTGGGACGGACTCCGAGAA
	Exon 3	TGTGCAAGGACAGACTTCCG	GACTGCCCGGGTCTAGAGAG
	Exon 4-5	CTCAGGCCACATTCTCCAGG	GGTCGTGTGGGAATCAGGAG
	Exon 6	TCACGCCTCTTGGACTCTGC	GACAGGTGAGTGGGAAGGTG
	Exon 7-8	CTCACCCAGAGGTTTCACCC	TGGGCTGTTCTGCTTCTCTG
PDE8B	Exon 1	GAGGAAGATGGCCCAAAAG	GCTCCCATCATCTCCACAAA
	Exon 2	AGTGCACACGGTGGCATAAT	GAGCCGAGACTGAGCCTCTA
	Exon 3	GGCCTGGTCTTTTGGTTGTT	ACACTGCTTAAAAACATCAACGC
	Exon 4	TCCTAATCCACAAGGGCATC	AACAAACAAAACCCCCAAAG
	Exon 5	CTGCTGGAGCTTTCTCTGCT	GTCCTGGGGCTTAATTTCCT
	Exon 6	CAAGCACTTTGAACACCTTGA	TGCCATTCATTGCCTGTTTA
	Exon 7	TTGGGAGACATCAGCATTCA	TCAGTATTCTTTGCACAGCTTGA
	Exon 8	CTTCCTACGGGGCACACA	CCAGATTCACTTGAGTTCCAAA
	Exon 9	AGGCATTGGGAAATGTAACG	GCTCATACTGGCATTTCAAGC
	Exon 10	ATGTGTGGGCTCTGTGTGAA	AAAGATTTGTCAGAGGAACCAAA
	Exon 11	TGGTGTATGTCTTTCATCTGTTCA	TGCCATAAAGGAAGATTCAAGG
	Exon 12	GCCCGGCCTAATGTTTATTT	CCCTCCAAAGAGACGACAAA
	Exon 13	CTCCTGCCTCCAAAGTTCC	CTCTCACAGAACCCGCTTG
	Exon 14	TCTTTTGGATTCTGGGCATA	CAGCCTCATGGAAAGACAAA
	Exon 15	ATCCCAGTTCTCCACGTTCT	TGCAATTCTTACTCTAACTGTGCTC
	Exon 16	CTTCCAGCTAGTCCCATTTGA	CAGGGGCCATAGTCTCTCTG
	Exon 17	CCCCTGTGTGTGTGAAGCTA	TGGCAGAAAGGTCCATGTC
	Exon 18	CGCCTCTCCACATCTAGGTC	GAACTCACTGAAGACCAAATGAGAT
	Exon 19	CTGGGGAAAATGGAATAGCA	TTCATCCCCAAGGAAAACAA
	Exon 20	ACAGGTGGTGACAGGGACTT	AAGGAACCAGAAGCCTACCC
	Exon 21	AGGCTTGAGAGTGGGAAGGT	CTGCTGGGGATGCAAAGAAT
	Exon 22	GATCCCAAACTTGTCCCAGA	CAGGATGACAGCAGAGCAAA
GNAS	Exon 1	TTCACCCTAGTTCGGTTGGG	AGCAAGTTCTTCCCGCCTTT
	Exon 2	GTCAAGGAAAGTTGCAAGTCTGT	AGCCCTTCCCAGGATTTTCTAA
	Exon 3	AATTGCCGGGAGGATGGATG	TGCCAATATGGCTGATGGTCC

	Exon 4-5	CGAACCCACAACTCCCTGAA	AGTTTTGCCTGAAGTGTGGT
	Exon 6	GGGCTCAAAATTCAAAATCACACC	AGAACTTTCTGCCAGTGGGG
	Exon 7-9	GTGCTGCATAACTGTGGGAC	AGAGAGCAAAGCCAAGAGCG
	Exon 10-11	GCAAGTGGATCCAGTGCTTC	GGGTTTTCAGCCTGACCGTT
	Exon 12-13	GCAAGAAAAACGCACTCCCA	CATGCCCTATGGTGGGTGAT
CTNNB1	Exon 2	GACCATGAGGTCTGCGTTTC	GCCATTAGGAGGAGTGAGCAG
	Exon 3	ACCCTGGCTATCATTCTGCTT	ACTCACTATCCACAGTTCAGCA
	Exon 4	TGTTGTGGTGAAGAAAAGAGAGT	AGACATTCTGAAACTACTCCCC
	Exon 5-6	GACGAGGACCAGGTAAGCAAT	TCCCTGGTGCCAAAAAGGTTA
	Exon 7	TGAGTGATGGGGTCCAGGAA	TTCAGTAGTTAAAGTTCTACCACCT
	Exon 8-9	CAGAAGGACACCTCCTAAGGC	ACAGCCATCCAACAGCTAGAG
	Exon 10	AGTATGGCTGCGATAGGGGT	GGGGGAACCAATGACCAAAG
	Exon 11	AGGAGGCCTCTTTTCAGTGAC	TCCTCCCTCTTCTCAAGTCTCAA
	Exon 12-13	ACACCCCAAGACATAAAATTCAGAG	TTGAGAGGAAAGAACAAGCTGC
	Exon 14	AGCATTTGTGTAATGTTGGAGTT	AGCAAACCGGCTCTTCTGAT
	Exon 15	GGTTGAAGAGGCTAGAAAGCG	CCCACCCTACCAACCAAGTC

Sets of primers used to amplify and sequence the coding region of each gene are shown.

Results

A novel heterozygous mutation in exon 5 of *PRKAR1A* gene (c.503G>T, p.Gly168Val) was identified in both cases (Fig. 5). This mutation involving *PRKAR1A* gene was identified as a pathogenic variant via *in silico* analyses (Table 3). This mutation is located on the cyclic adenosine monophosphate (cAMP)-binding domain A.

Discussion

We describe a CNC family with a novel mutation in *PRKAR1A* gene. Interestingly, the individual phenotypes differed considerably from each other.

CNC was reported as a syndrome accompanied by myxomas, spotty pigmentation, and endocrine overactivity by Carney et al. (1985). In particular, spotty pigmentation is the most frequent phenotype observed in patients with CNCs. On the other hand, heart myxoma is known to be a prognostic factor in CNCs because of sudden death. Particularly, the diagnosis of proband fortunately led to early detection and treatment of heart myxoma in Case 2. Additionally, PPNAD is the most common and specific endocrine feature found in CNCs, present in 45-70% of patients (Lowe et al. 2017; Bouys and Bertherat 2021). To support PPNAD specificity, patients with isolated PPNAD, or patients diagnosed with CNCs without a family history can also present *de novo* germline *PRKAR1A* mutations (Groussin et al. 2005; Tsurutani et al. 2022). Therefore, heart myxoma (the prognostic factor), spotty skin pigmentation (the most frequent phenotype), and PPNAD (the most specific phenotype) are important phenotypes in CNC management.

In terms of genetic insights, > 60% of CNCs exhibit mutations in the *PRKAR1A* gene (Kirschner et al. 2000). More than 125 *PRKAR1A* pathogenic mutations have been reported (https://www.ncbi.nlm.nih.gov/clinvar/?gr=0&term=PRKAR1A[gene]). Inactivating mutations involving *PRKAR1A* cause tumor development via a lack of tumor-suppressing function, especially in adrenocortical nodules (Groussin et al. 2005; Bertherat et al. 2009; Sahut-Barnola et al. 2010). In another study, in-frame deletions of the *PRAKAR1A* gene, including exon 5, resulted in the expression of proteins with defective binding to the catalytic subunits, which led to increased activity of the enzyme to produce glucocorticoids (Bataille et al. 2014). This suggests the importance of the cAMP-binding domain A located in exon 5, as in Case 1 and 2. Nevertheless, the genotype-phenotype correlation in CNC remains unclear because the same mutation in *PRKAR1A* shows phenotypic variability, even in the same family (Bertherat et al. 2009). This could complicate the diagnosis of CNCs in clinical practice.

Recently, additional somatic gene modifiers associated with adrenal cortisol production have been reported to explain phenotypic variability in CNCs. We analyzed previously reported gene modifiers [*PRKACA* (Carney et al. 2015), *PDE11A* (Libé et al. 2008), *ARMC5* (Maria et al. 2020), *PDE8B* (Almeida and Stratakis 2010), *GNAS* (Almeida et al. 2012), and *CTNNB1* (Tadjine et al. 2008)] in adrenal tumor genomic DNA in Case 1 with informed consent, but there were no significant mutations. Primers used for sequence analysis are shown in Table 2. This suggests that other gene modifiers or factors are associated with phenotypic variability in CNC. To support this, an association between haploinsufficiency of CNCs and nonsense-mediated mRNA decay (NMD) was described in 2006 (Groussin et al. 2006). This phenomenon is the degradation of mutated mRNAs recognized by ribosomes. This complex mechanism may be associated with specific and variable phenotypes in CNCs, and further advances are awaited.

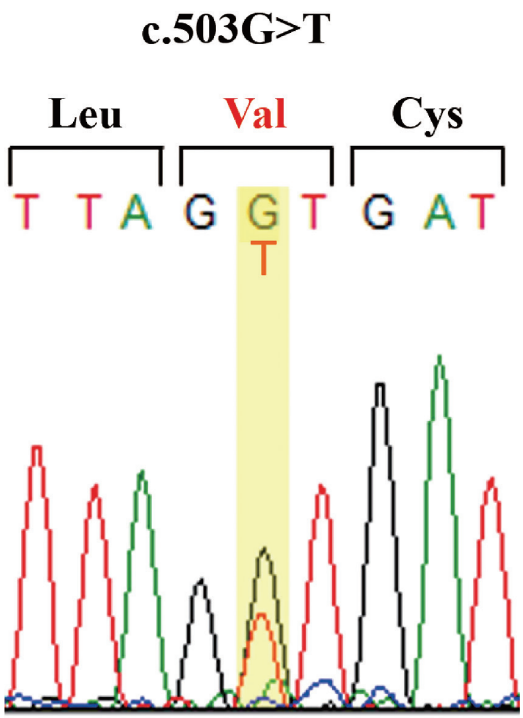

Fig. 5. Genetic analysis of *PRKAR1A* in Case 1 and 2. Chromatogram shows heterozygous mutation involving *PRAKAR1A*. A resected adrenal specimen from Case 1 exhibited the same mutation.

Finally, we discuss PPNAD-treatment. Partial adrenalectomy was established with the aim of preservation of adrenocortical function for multiple adrenal tumors (Diner et al. 2005) avoiding postoperative adrenal insufficiency. Indeed, the patients with CS who had undergone bilateral adrenalectomy showed higher hazard ratios for mortality and chronic glucocorticoid administration was associated with a higher rate of mortality derived from infection (Ragnarsson et al. 2019). Considering this report, total adrenalectomy has the risk of increased mortality, and partial adrenalectomy has a distinct benefit in Case 1. Alternatively, unilateral total adrenalectomy has also been reported to be effective for long-term control of PPNAD (Tsurutani et al. 2022). It remains unclear which procedure is superior, but unilateral total adrenalectomy can be conducted after partial adrenalectomy. Therefore, partial adrenalectomy may be considered the first step in treatment of PPNAD if the surgical tolerance for multiple operations allows. Additionally, he had anxiety about growth of height. Short stature was known as the feature of pediatric CS, but catch-up of growth after treatment of pediatric CS is often underwhelming (Magiakou et al. 1994). However, some patients show continuous growth after treatment (Savage et al. 2001, 2008). The effect of treatment for pediatric CS on height remains unclear and requires further study. Fortunately, as a result, Case 1 showed additional growth in height after partial adrenalectomy (−3.4 to −2.4 SDS after the operation, +6.0 cm) without adrenal insufficiency. This report is the first successfully treated PPNAD with (two-staged) partial adrenalectomy in childhood as far as we know. In summary, treatment for PPNAD should be determined based on the patient's wishes and future quality of life.

Table 3. Results of bioinformatic programs regarding a novel mutation in *PRKAR1A*.

Bioinformatic Tool	Score	Pathogenicity prediction
PolyPhen-2	1	Probably Damaging
SIFT	0	Damaging

Analyses were performed using PolyPhen-2 and the SIFT algorithm.
SIFT, Sorting Intolerant from Tolerant.

In conclusion, we report a family with CNC accompanied by a novel mutation in the *PRKAR1A* gene. Their phenotypes varied even among family members. To avoid missing CNC cases, clinicians should remain alert to pathognomonic signs of CNCs, such as heart myxoma, spotty skin pigmentation, and PPNAD.

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Conflict of Interest

The authors declare no conflict of interest.

References

Almeida, M.Q., Azevedo, M.F., Xekouki, P., Bimpaki, E.I., Horvath, A., Collins, M.T., Karaviti, L.P., Jeha, G.S., Bhattacharyya, N., Cheadle, C., Watkins, T., Bourdeau, I., Nesterova, M. & Stratakis, C.A. (2012) Activation of cyclic AMP signaling leads to different pathway alterations in lesions of the adrenal cortex caused by germline PRKAR1A defects versus those due to somatic GNAS mutations. *J. Clin. Endocrinol. Metab.*, **97**, E687-693.

Almeida, M.Q. & Stratakis, C.A. (2010) Carney complex and other conditions associated with micronodular adrenal hyperplasias. *Best Pract. Res. Clin. Endocrinol. Metab.*, **24**, 907-914.

Barstow, C. & Rerucha, C. (2015) Evaluation of short and tall stature in children. *Am. Fam. Physician*, **92**, 43-50.

Bataille, M.G., Rhayem, Y., Sousa, S.B., Libé, R., Dambrun, M., Chevalier, C., Nigou, M., Auzan, C., North, M.O., Sa, J., Gomes, L., Salpea, P., Horvath, A., Stratakis, C.A., Hamzaoui, N., et al. (2014) Systematic screening for PRKAR1A gene rearrangement in Carney complex: identification and functional characterization of a new in-frame deletion. *Eur. J. Endocrinol.*, **170**, 151-160.

Bertherat, J., Horvath, A., Groussin, L., Grabar, S., Boikos, S., Cazabat, L., Libe, R., René-Corail, F., Stergiopoulos, S., Bourdeau, I., Bei, T., Clauser, E., Calender, A., Kirschner, L.S., Bertagna, X., et al. (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. *J. Clin. Endocrinol.*

Metab., **94**, 2085-2091.
Bouys, L. & Bertherat, J. (2021) MANAGEMENT OF ENDOCRINE DISEASE: Carney complex: clinical and genetic update 20 years after the identification of the CNC1 (PRKAR1A) gene. *Eur. J. Endocrinol.*, **184**, R99-R109.
Carney, J.A., Gordon, H., Carpenter, P.C., Shenoy, B.V. & Go, V.L. (1985) The complex of myxomas, spotty pigmentation, and endocrine overactivity. *Medicine* (*Baltimore*), **64**, 270-283.
Carney, J.A., Lyssikatos, C., Lodish, M.B. & Stratakis, C.A. (2015) Germline PRKACA amplification leads to Cushing syndrome caused by 3 adrenocortical pathologic phenotypes. *Hum. Pathol.*, **46**, 40-49.
Diner, E.K., Franks, M.E., Behari, A., Linehan, W.M. & Walther, M.M. (2005) Partial adrenalectomy: the National Cancer Institute experience. *Urology*, **66**, 19-23.
Groussin, L., Cazabat, L., René-Corail, F., Jullian, E. & Bertherat, J. (2005) Adrenal pathophysiology: lessons from the Carney complex. *Horm. Res.*, **64**, 132-139.
Groussin, L., Horvath, A., Jullian, E., Boikos, S., Rene-Corail, F., Lefebvre, H., Cephise-Velayoudom, F.L., Vantyghem, M.C., Chanson, P., Conte-Devolx, B., Lucas, M., Gentil, A., Malchoff, C.D., Tissier, F., Carney, J.A., et al. (2006) A PRKAR1A mutation associated with primary pigmented nodular adrenocortical disease in 12 kindreds. *J. Clin. Endocrinol. Metab.*, **91**, 1943-1949.
Kirschner, L.S., Carney, J.A., Pack, S.D., Taymans, S.E., Giatzakis, C., Cho, Y.S., Cho-Chung, Y.S. & Stratakis, C.A. (2000) Mutations of the gene encoding the protein kinase A type I-alpha regulatory subunit in patients with the Carney complex. *Nat. Genet.*, **26**, 89-92.
Libé, R., Fratticci, A., Coste, J., Tissier, F., Horvath, A., Ragazzon, B., Rene-Corail, F., Groussin, L., Bertagna, X., Raffin-Sanson, M.L., Stratakis, C.A. & Bertherat, J. (2008) Phosphodiesterase 11A (PDE11A) and genetic predisposition to adrenocortical tumors. *Clin. Cancer Res.*, **14**, 4016-4024.
Lowe, K.M., Young, W.F., Lyssikatos, C., Stratakis, C.A. & Carney, J.A. (2017) Cushing syndrome in Carney complex: clinical, pathologic, and molecular genetic findings in the 17 affected Mayo Clinic patients. *Am. J. Surg. Pathol.*, **41**, 171-181.
Magiakou, M.A., Mastorakos, G. & Chrousos, G.P. (1994) Final stature in patients with endogenous Cushing's syndrome. *J. Clin. Endocrinol. Metab.*, **79**, 1082-1085.
Maria, A.G., Tatsi, C., Berthon, A., Drougat, L., Settas, N., Hannah-Shmouni, F., Bertherat, J., Faucz, F.R. & Stratakis, C.A. (2020) ARMC5 variants in PRKAR1A-mutated patients modify cortisol levels and Cushing's syndrome. *Endocr. Relat. Cancer*, **27**, 509-517.
Matsunaga, H., Tezuka, Y., Kinoshita, T., Ogata, H., Yamazaki, Y., Shiratori, B., Omata, K., Ono, Y., Morimoto, R., Kudo, M., Seiji, K., Takase, K., Kawasaki, Y., Ito, A., Sasano, H., et al. (2021) The potential of computed tomography volumetry for the surgical treatment in bilateral macronodular adrenal hyperplasia: a case report. *Tohoku J. Exp. Med.*, **253**, 143-150.
Ragnarsson, O., Olsson, D.S., Papakokkinou, E., Chantzichristos, D., Dahlqvist, P., Segerstedt, E., Olsson, T., Petersson, M., Berinder, K., Bensing, S., Höybye, C., Edén-Engström, B., Burman, P., Bonelli, L., Follin, C., et al. (2019) Overall and disease-specific mortality in patients with Cushing disease: a Swedish nationwide study. *J. Clin. Endocrinol. Metab.*, **104**, 2375-2384.
Sahut-Barnola, I., de Joussineau, C., Val, P., Lambert-Langlais, S., Damon, C., Lefrançois-Martinez, A.M., Pointud, J.C., Marceau, G., Sapin, V., Tissier, F., Ragazzon, B., Bertherat, J., Kirschner, L.S., Stratakis, C.A. & Martinez, A. (2010) Cushing's syndrome and fetal features resurgence in adrenal cortex-specific Prkar1a knockout mice. *PLoS Genet.*, **6**, e1000980.
Savage, M.O., Chan, L.F., Afshar, F., Plowman, P.N., Grossman, A.B. & Storr, H.L. (2008) Advances in the management of paediatric Cushing's disease. *Horm. Res.*, **69**, 327-333.
Savage, M.O., Lebrethon, M.C., Blair, J.C., Ho, J.T., Johnston, L.B., Lienhardt, A., Clark, A.J. & Chaussain, J.L. (2001) Growth abnormalities associated with adrenal disorders and their management. *Horm. Res.*, **56** Suppl 1, 19-23.
Stratakis, C.A., Kirschner, L.S. & Carney, J.A. (2001) Clinical and molecular features of the Carney complex: diagnostic criteria and recommendations for patient evaluation. *J. Clin. Endocrinol. Metab.*, **86**, 4041-4046.
Tadjine, M., Lampron, A., Ouadi, L., Horvath, A., Stratakis, C.A. & Bourdeau, I. (2008) Detection of somatic beta-catenin mutations in primary pigmented nodular adrenocortical disease (PPNAD). *Clin. Endocrinol.* (*Oxf.*), **69**, 367-373.
Takano, K., Yasufuku-Takano, J., Morita, K., Mori, S., Takei, M., Osamura, R.Y., Teramoto, A. & Fujita, T. (2009) Evidence that PKA activity is constitutively activated in human GH-secreting adenoma cells in a patient with Carney complex harbouring a PRKAR1A mutation. *Clin. Endocrinol.* (*Oxf.*), **70**, 769-775.
Tsurutani, Y., Kiriyama, K., Kondo, M., Hasebe, M., Sata, A., Mizuno, Y., Sugisawa, C., Saito, J. & Nishikawa, T. (2022) Carney complex complicated with primary pigmented nodular adrenocortical disease without Cushing's syndrome recurrence for five years after unilateral adrenalectomy. *Intern. Med.*, **61**, 205-211.