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Carney complex with PRKAR1A mutation: A case report

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CASE STUDY

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ABSTRACT

Carney complex is a multiple endocrine neoplasia syndrome with various features which include myxomas, endocrine tumours and lentigines lesions. We report a case of Carney complex with components of lentigines, ACTH independent adrenal Cushing's syndrome (with a paradoxical increase in 24 hour urinary cortisol following the high dose (8mg) dexamethasone suppression test – and is likely to be due to primary pigmented nodular adrenal hyperplasia) positive for a protein kinase A type 1A regulatory subunit (PRKAR1A) gene mutation.

Key Words
Carney complex, PRKAR1A genetic mutation, cushing's syndrome

Implications for Practice:

1. What is known about this subject?
Carney complex is an uncommon multiple endocrine neoplasia syndrome with around 500 cases having been reported in literature till now.

2. What new information is offered in this case study?
A case with a clinical suspicion of Carney complex was confirmed by genetic testing which was carried out using Next generation sequencing (NGS) based methodology.

3. What are the implications for research, policy, or practice?
NGS methodology has been shown to be cost effective and could be used to screen a panel of genes involved in Carney complex.

Background

Carney complex is an uncommon autosomal dominant multiple endocrine neoplasia syndrome with around 500 cases having been reported in literature.[1]

It is characterised by characteristic spotty pigmentation of the skin, endocrinopathy, multiple endocrine and non-endocrine tumours.[2] We report a case of Carney complex with components of ACTH independent adrenal Cushing's syndrome, lentigines over face and mutation being positive for protein kinase A type 1A regulatory subunit (PRKAR1A).

Case details

A 11-year-old girl was referred to endocrinology clinic for the evaluation of weight gain and failure to gain height for four years. She was diagnosed to have recent onset hypertension and diabetes mellitus, without a significant family history. Her height was less than the 5th percentile (128cm). She had multiple lentigines over the face, a moon face, supra-clavicular pad of fat, truncal obesity, diffuse hyperpigmentation of the skin and easy bruisability. There was no proximal myopathy and the rest of systemic examination was within the normal limits.

Biochemical evaluation showed an elevated 8 am serum cortisol and 24 hour urinary cortisol, loss of circadian rhythm of cortisol secretion and failure to suppress endogenous plasma cortisol following a low dose dexamethasone suppression test, along with suppressed plasma adrenocorticotropic hormone (ACTH). Hence a diagnosis of ACTH independent Cushing's syndrome was confirmed (Table 1). A paradoxical increase in 24 hour

urinary cortisol was demonstrated after the high dose (8mg) dexamethasone suppression test which is characteristically described in primary pigmented adrenal nodular hyperplasia due to increased expression of glucocorticoid receptor in the nodules.[3]

Therefore, a diagnosis of Carney complex was suspected with an ACTH independent Cushing's syndrome with a paradoxical increase in 24 hour urinary cortisol after high dose (8mg) dexamethasone suppression test with multiple lentigines over face (Figure 1). Whole body Fluro-Deoxy Glucose Positron Emission Tomography/Computerized Tomography (FDG PET/CT) showed bilateral bulky adrenal glands (Figure 2) without any sellar lesion (Figure 3) or other tumours seen in Carney complex. The Echocardiogram did not show any atrial myxomas. In view of her young age, surgery was deferred and medical treatment with ketoconazole was initiated.

She was followed up for 3 years and responded well to ketoconazole and her 24 hour urinary cortisol levels remaining within normal limits. Secondary diabetes mellitus and hypertension also resolved with the medical treatment with ketoconazole. Currently her age is 14 years with a height of 145cm and Tanner stage (Breast stage 4, Pubic hair stage 3) with regular menstrual cycles on a dosage of Ketoconazole of 600mg per day. Once she reaches the target midparental height, she will be planned for a bilateral adrenalectomy as the definitive management for probable PPNAD (cause of adrenal Cushing's syndrome).

Genetic analysis

Genetic analysis was performed on the DNA which was extracted from the blood using a QIAamp blood mini kit (QIAGEN) according to the manufacturer's protocol.

The PRKAR1A gene mutation analysis was performed using polymerase chain reaction (PCR)-based enrichment followed by NGS utilizing Ion Torrent™ Personal Genome Machine (PGM) as described previously.[4] A single PRKAR1A gene amplicon sequencing was performed using NGS. The coding regions of the PRKAR1A gene with 11 exons (additional >50bp were included around the intron/exon junctions to capture the splice variants) were amplified with 8 pairs of novel primers designed using Primer 3 software. Following PCR-based target enrichment, library preparation and NGS was performed on the Ion torrent™ Personal Genome Machine (PGM) using 314 chips and an Ion PGM™ 200 Sequencing Kit (Ion Torrent, Life Technologies, Carlsbad, CA).

The data was analysed using the Ion torrent suit software and DNASTAR SEQMAN NGEN software (Madison, Wisconsin). Using the 314 chip, more than 50MB Q20 data were generated for various multiplexed samples and around 5 MB data were generated for the test sample.

The target PRKAR1A gene was sequenced at an average coverage more than 500x with more than 99 per cent of the target sequenced with a minimum coverage of 20x. Using this approach, the patient was found to be positive (44.4 per cent of reads demonstrated the allele T) for a reported nonsense germ line mutation in exon 7 c.682C>T p.R228X in the PRKAR1A gene resulting in a non-sense mediated mRNA decay (NMD), leading to PRKAR1A haploinsufficiency.[5] These findings were further confirmed by Sanger sequencing (Figure 4).

Discussion

We are reporting this case of Carney complex based on the recent diagnostic criteria as per Correa et al.,[2] with major criteria being skin manifestations (lentigines), paradoxical increase in 24 hour urinary cortisol after the high dose (8mg) dexamethasone suppression test (likely due to primary pigmented nodular adrenal hyperplasia, PPNAD) and supplemental criteria being inactivating mutation of PRKAR1A gene. This patient did not have any other manifestations or tumours which are described in Carney complex.

Carney complex was first described by J. Aiden Carney in 1985 as an autosomal dominant disorder with multiple neoplasia involving heart, central nervous system and endocrine organs along with skin lesions like spotty skin pigmentation and lentigines.[6] Though there have been only around 500 reported cases in literature, there may be an increase in the detection of Carney complex due to the recent alteration in diagnostic criteria.

As per Correa et al.,[2] the activating mutations of PRKACA and PRKACB have been included in supplemental criteria in the recent diagnostic criteria for Carney complex updated in 2015 which was not there in the previous criteria.[7] Also there are several added minor criteria which may be suggestive of or possibly associated with CNC, but not diagnostic of the disease. These may increase the detection rates of Carney complex. For diagnosis, a patient must have either two of the major criteria confirmed by histology, imaging or biochemical testing or one major criterion and one supplemental criterion as mentioned by Correa et al.[2,7]

The manifestations of Carney complex can be variable and may appear over a span of many years. It can be diagnosed as early as in the second year of life and as late as in the fifth decade of life, with a median age of detection being 20 years with most cases being familial in nature. There seems to be a bimodal age distribution of PPNAD among Carney complex patients with a minority of patients presenting during the first 2–3 years and majority of them manifesting in the second and third decade of life.[7] Carney complex has a slightly female predominance.[2]

Carney complex is characterized by cutaneous features (spotty skin pigmentation or lentigines), myxomas (cutaneous and/or cardiac), breast tumours, growth hormone secreting pituitary adenomas (acromegaly), large cell calcifying sertoli cell testicular tumour (LCCSCT), PPNAD or paradoxical positive response of urinary glucocorticoid excretion to dexamethasone administration during high dose dexamethasone suppression test, thyroid carcinoma, schwannomas and osteochondromyxoma.[2]

Carney complex is caused by mutations in the PRKAR1A gene which is situated at the 24.2–24.3 locus of the long arm of chromosome 17. These PRKAR1A gene (CNC1 locus) mutations are seen in more than 70 per cent of the patients with Carney complex and up to 80 per cent for those with Cushing's syndrome due to PPNAD.[8,9] PRKAR1A mutations are seen in 37 per cent of sporadic patients and in 80 per cent of familial patients with Carney complex. The PRKAR1A genetic mutation is detected less common in the first decade of life and the mutation detection frequency increases steadily as age advances among various manifestations of Carney complex.[8] The various PRKAR1A pathogenic mutations include single-base substitutions, deletions/insertions, combined rearrangements and large deletions.

A second genetic locus 'CNC2' locus is present in the chromosome 2p16 locus that has been detected in PRKAR1A- negative patients with Carney complex.[10] Two more recent studies have demonstrated that PRKACA and PRKACB gene defects are associated with the elements of the CNC Phenotype.[11,12] Therefore, the amplicon based target enrichment followed by NGS with its multiplexing option has shown to be cost effective even for the purpose of sequencing a single gene. Furthermore, this technology has been shown to be flexible and robust enough to include the increasing number of genes needed to be tested in clinical settings.

Conclusion

Carney complex is an uncommon disorder with various manifestations involving endocrinopathy, endocrine and non-endocrine tumours. NGS based genetic testing with its multiplexing options has been shown in this case to be a robust and inexpensive platform for screening even single-gene disorders. Screening for the various manifestations of the disease is important in view of the diverse presentation. The most common mutation seen in patients with Carney complex involves the PRKAR1A gene.

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PEER REVIEW

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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PATIENT CONSENT

The authors, Satyaraddi A, Chapla A, Shetty S, Thomas N declare that:

1. They have obtained written, informed consent for the publication of the details relating to the patient(s) in this report.
2. All possible steps have been taken to safeguard the identity of the patient(s).
3. This submission is compliant with the requirements of local research ethics committees.

Figure 1: Multiple Lentigines over the face (arrow marks) and moon face

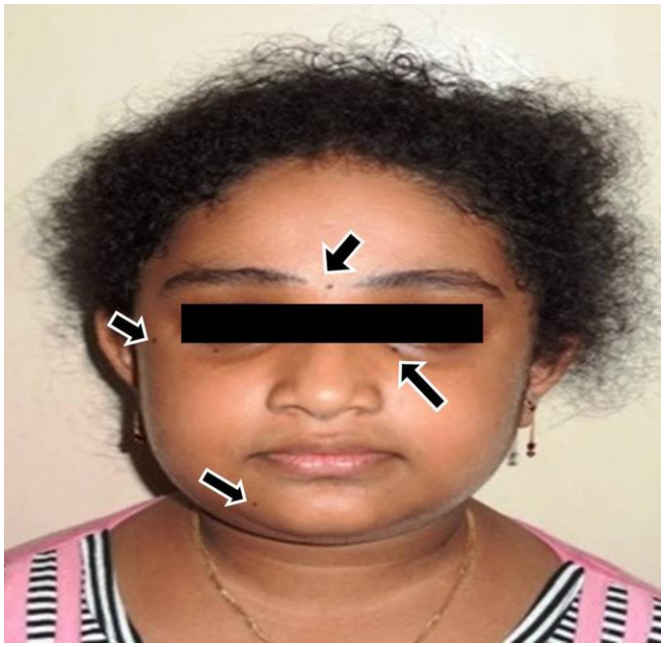

Figure 2: CT Abdomen showing bilateral bulky adrenal glands (arrow marks)

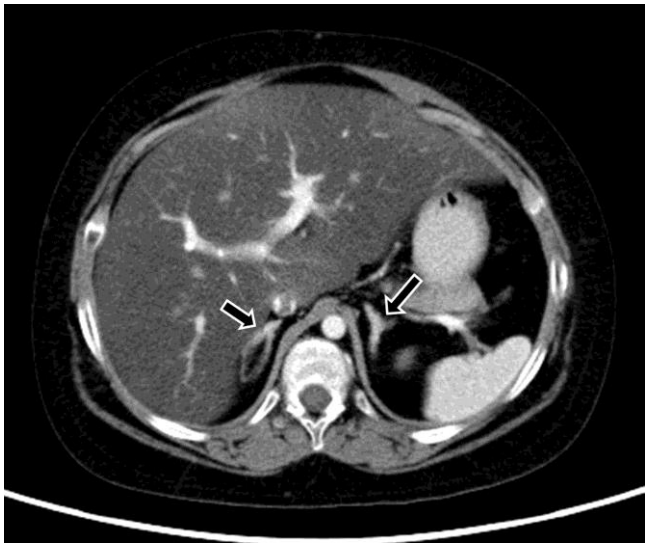

Table 1: Biochemical tests for evaluation of Cushing's syndrome
ACTH: Adrenocorticotropic hormone
ONDST: Overnight dexamethasone suppression test
HDDST: High dose dexamethasone suppression test

	Serum Cortisol (ug/dl) (N=7-25)	**24 hour urinary cortisol** (ug/day) (N= 10-100)	**Plasma ACTH** (pg/ml) (N= 0-46)
Basal (8 am)	18.78	190	6.36
Midnight	8.87	-	5.32
ONDST (Post 1 mg)	23.25	-	-
HDDST (Post 8mg)	23.5	472	-

Figure 3: FDG PET/CT – Absence of sellar lesion (arrow marks)

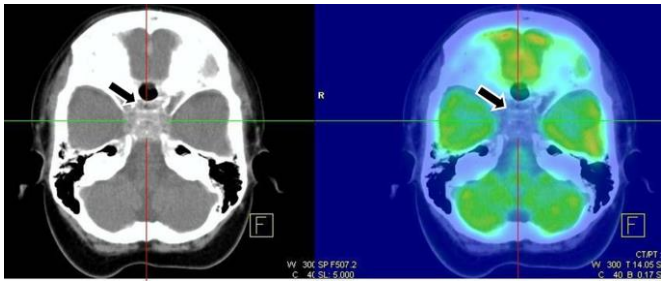

Figure 4:
Next-generation sequencing reads with the nonsense mutation c.682C>T p.R228X in the PRKAR1A gene
PRKAR1A gene mutation c.682C>T p.R228X - Next-generation sequencing reads with the Sanger confirmation