Rare association of acromegaly with left atrial myxoma in Carney’s complex due to novel PRKAR1A mutation

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Summary
Carney complex (CNC) is a rare autosomal dominant syndrome characterized by pigmented lesions of the skin and mucosae along with cardiac, endocrine, cutaneous, and neural myxomatous tumors. Mutations in the PRKAR1A gene have been identified in ~70% of the CNC cases reported worldwide. A 30-year-old male was referred to the endocrinology clinic with suspected acromegaly. He had a history of recurrent atrial myxoma for the past 8 years for which he underwent repeated surgeries. Presently, he complained of having headache, excessive snoring, sweating, and also noticed increase in his shoe size. Evaluation for acromegaly revealed elevated levels of GH in random as well as in suppressed condition. Magnetic resonance imaging scan revealed enlarged sella with microadenoma in the left anterior pituitary. Screening of PRKAR1A gene was carried out for the patient, his parents and siblings who were available and willing to undergo the test. The patient was diagnosed to have the rare CNC syndrome characterized by recurrent atrial myxoma and acromegaly due to a novel 22 bp insertion mutation in PRKAR1A which was predicted to be deleterious by in silico analysis. Screening the available family members revealed the absence of this mutation in them except the elder brother who also tested positive for this mutation. The present study reports on a novel PRKAR1A insertion mutation in a patient with acromegaly and left atrial myxoma in CNC.

Learning points:
- Identification of a novel deleterious PRKAR1A insertion mutation causing CNC.
- It is important that patients with cardiac myxoma be investigated for presence of endocrine overactivity suggestive of CNC.
- PRKAR1A mutation analysis should be undertaken in such cases to confirm the diagnosis in the patients as well as first degree relatives.
- This case highlights an important aspect of diagnosis, clinical course, and management of this rare condition.

Background
Carney complex (CNC) is a rare syndrome characterized by neoplasia involving the heart, CNS, and endocrine organs (1). It is a genetically heterogeneous syndrome and is inherited as an autosomal dominant trait. The regulatory subunit (R1A) of the protein kinase A (PRKAR1A) gene is implicated in its causation. PRKAR1A (OMIM # 188830) is a tumor suppressor gene that encodes a protein kinase A (PKA) regulatory 1-alpha subunit and is located on chromosome 17q24.
The PRKAR1A mutations causing CNC have been identified in \( \sim 70\% \) of the total cases reported worldwide (2). Majority of PRKAR1A mutations are reported to be nonsense, frameshift, and splicesite, which undergo nonsense-mediated mRNA decay (NMD) with resultant haploinsufficiency (3).

We report here a novel deleterious genetic finding in a patient with CNC who presented with recurrent left atrial myxoma and acromegaly.

**Case presentation**

A 30-year-old male, surgically operated for recurrent left atrial myxoma, presented to the Endocrine out-patient department of the All India Institute of Medical Sciences, New Delhi, with suspected acromegaly.

The history of present illness goes back to 2004 when the patient developed an episode of left hemiparesis and left upper motor neurone facial nerve palsy. He was managed at another medical care facility and recovered within a week. Echocardiography revealed left atrial myxoma (45×20 mm) for which he was operated in same year, followed by a repeat surgery after 1 year for a recurrence. The patient was on irregular follow-up after the second surgery, till the year 2011 when he was referred to the endocrine clinic for suspected acromegaly.

There was no history suggestive of increase in acral size, headache, galactorrhoea, abdominal striae, easy bruisability, proximal weakness, skin pigmentation, nor any family history of such illness.

**Clinical investigation**

Cardiac examination revealed normal sinus rhythm with heart rate of 90/min, blood pressure of 150/70 mmHg, and a mid systolic click was audible in mitral area. Acanthosis nigricans was present in axilla and neck.

Echocardiography showed left atrial myxoma (2.2×2.4 cm) attached to the inter atrial septum. Hormonal investigation showed prolactin level of 21.5 ng/ml (normal range 4.6–21.4 ng/ml) and total T4 level of 6.4 µg/dl (normal range 5.1–14.1 µg/dl). Dynamic testing of the pituitary axis revealed growth hormone values of 16 ng/ml at baseline, and 12.6 ng/ml, 60 min after administering 75 g glucose orally (normal growth hormone post 75 g glucose challenge should be <1 ng/ml). Morning serum cortisol level was 8 µg/dl (normal range 6.2–19.2 µg/dl) and salivary cortisol value at 2300 h was 0.1 µg/dl (normal <0.43 µg/dl). Magnetic resonance imaging (MRI) of sella (Fig. 1) showed pituitary microadenoma (9×6 mm) of the left half anterior pituitary. Contrast enhanced CT of the adrenal area revealed normal sized adrenal glands.

The patient underwent pituitary surgery through transnasal transphenoidal route. Histopathology and immunohistochemistry showed growth hormone staining pituitary adenoma. Post-operative follow-up at 5 months after surgery showed growth hormone levels to be 0.65 ng/ml fasting basal and 0.58 ng/ml 1 h after 75 g glucose load. Pre and post-operative IGF1 values were 599 and 173 ng/ml respectively (normal reference range from 26 to 30 years 75 to 275 ng/ml).

**Genetic screening**

Detailed family history and pedigree information was collected (Fig. 2). Peripheral blood sample was drawn in EDTA tubes for molecular investigations after taking informed consent from the patient, his parents, and siblings who were available and willing to undergo the test. Genomic DNA was extracted using standard salting out protocol and subjected to PCR amplification of the PRKAR1A gene with primers described previously (2) using 70–100 ng DNA, 1.25 mM MgCl2, 0.25 mM of each of the dNTPs (Invitrogen), 5 pM of each primer and 0.5 units of Taq Polymerase (Invitrogen) in a 25 µl volume mixture using thermocycler ABI 9700 (Applied Biosystems).

**Sequencing and mutation analysis**

All the amplified products were purified using the Qiagen kits (Qiagen, GmbH), sequenced using BigDye Terminator
Mix version 3.1 (Applied Biosystems) and analyzed on an ABI-3100 Genetic Analyzer (Applied Biosystems). The nucleotide sequences were compared with the published cDNA sequences of PRKAR1A (GenBank accession number ENSG00000108946) gene.

In silico analysis

Prediction of functional effects of the novel mutation was done using softwares PROVEAN (http://provean.jcvi.org/index.php) and MutationT@ster (http://www.mutation-taster.org/) (4) (5).

Investigation

Direct sequencing of the PRKAR1A gene in the patient revealed a heterozygous 22 bp insertion mutation in exon 6 (Fig. 3). Screening of the available family members revealed the absence of this mutation in them except the elder brother who is positive for this mutation.

Assessment for pathogenicity using PROVEAN showed it to be deleterious (score = 19.027) and MutationT@ster predicted it to be disease causing (score = 1) leading to frameshift thereby causing splice site changes which might have resulted in affecting the protein features and NMD.

Discussion

CNC, as described by J Aiden Carney in 1985, is an autosomal dominant disorder characterized by neoplasia involving heart, CNS, and endocrine organs. Presence of pigmented skin and mucosal lesions along with these tumors is an important hallmark of this syndrome (1) (6). Most of the cases are familial and the median age of presentation is 20 years. CNC can manifest itself as spotty cutaneous pigmentation, cutaneous myxomas, cardiac myxomas, psammomatous melanotic schwannoma, acromegaly, large cell calcifying Sertoli cell tumor, thyroid carcinomas or nodules, and breast adenomas (7).

The most common endocrine gland manifestations are acromegaly, thyroid and testicular tumors, and adrenocorticotropic hormone-independent Cushing’s syndrome due to primary pigmented nodular adrenocortical disease (PPNAD).

Cushing’s syndrome due to PPNAD is observed in children and young adults, with a peak during the second decade of life. It is rare but can occur before the age of 4 years and it is rarely diagnosed after the age of 40 years. Diagnosis of Cushing’s syndrome due to PPNAD is often difficult because hypercortisolism can develop progressively over years (3).

It is generally established that a patient presenting with two or more of the manifestations given in Table 1 would be diagnosed as having CNC. If the patient has a germline PRKAR1A mutation and/or a first-degree relative affected by CNC, a single manifestation is sufficient for the diagnosis (3).

PRKAR1alpha is a tumor suppressor gene, which is found to be mutated in almost 50% of CNC cases demonstrating an autosomal dominant transmission (8). When a PRKAR1A mutation is identified, genetic analysis should be undertaken in first-degree relatives. Routine echocardiography screening of the first degree relatives is appropriate for searching for familial myxomas (8). Interestingly, in the present report, at the time of sampling for molecular studies the elder brother (III.1) was apparently normal and did not show any symptoms of...
CNC but only had a history of infertility similar to the index case (III.2). Shortly afterwards he had repeated episodes of embolization with hemiparesis. Echocardiography revealed bilateral atrial myxoma, for which he underwent surgery.

The cAMP-signaling pathway is crucial for normal functioning of the endocrine cells. Any disruption in this pathway may result in tumorigenesis. PKA, the main mediator of cAMP signalling, is a tetramer consisting of two regulatory and two catalytic subunits.

In humans, four different regulatory (PRKAR1A, PRKAR1B, PRKAR2A, and PRKAR2B) and three catalytic subunits (PRKACA, PRKACB, and PRKACG) have been identified till date. The two molecules of cAMP bind to the regulatory PKA dimer under high cAMP concentration in the cellular environment. This produces a reversible conformational change that disrupts the PKA tetramer and results in dissociation of its catalytic subunits. These dissociated catalytic subunits regulate phosphorylation of different effector enzymes, ion channels, and transcription of specific genes that mediate metabolism, cell proliferation, differentiation, and apoptosis (9). Maximum numbers of mutations have been found in PRKAR1A, one of the four regulatory PKA subunits (10). Majority of PRKAR1A mutations result in frameshift changes generating a premature stop codon. Such mutant mRNA molecules are unstable and are degraded by the process of NMD.

The insertion of heterozygous 22 bp exon 6 PRKAR1A identified in the patient disrupts codon 228, leading to frameshift downstream and creating a premature stop codon at position 239 in the mRNA which undergoes NMD. This results in 50% reduction in normal cellular PRKAR1A levels because one copy of the PRKAR1A gene is mutated and the other is normal. This reduction in regulatory component level results in a higher proportion of catalytic subunits causing increased signaling and activation of the downstream cellular processes of proliferation and differentiation leading to tumorigenesis.

The mutation identified in the index case was also seen in his elder brother (III.1). Absence of the mutation in the parental DNA suggests its de novo occurrence. Previous studies have reported high-frequency occurrence of sporadic cases with de novo PRKAR1A mutations (11). Several reports have described this as a heterogeneous disorder with the presence of small indels and large gene rearrangements (12).

The 22 bp insertion mutation found in this study is a duplication of an 18 bp region intervened by a stretch of four nucleotides. To the best of our knowledge, the sequence change is novel and is not reported or found associated with any other disorder.

To rule out the possibility of the change to be a polymorphism, 100 chromosomes from healthy individuals who acted as controls were screened for the presence of the same. The change was not identified in any of them, proving this to be a mutation and not a polymorphism. This is in concordance with the results of the in silico analysis which predicted the sequence change to be pathogenic resulting in the disease phenotype.

Infertility is an important phenotypic presentation in CNC cases. Previous studies have suggested the presence of several unknown reasons affecting fertility in CNC males other than Sertoli cell tumors (13). The report presents evidence for this important observation as infertility was present in both the brothers who were positive for the mutation. Clinical investigations ruled out the presence of Sertoli cells tumors in both.

Although the same mutation was identified in the two brothers, the elder one showed late onset of the disease as well as absence of acromegaly proving the heterogeneous nature of this condition. This is in accordance with other dominant disorders showing both inter- and intra-familial variable expressivity of the mutant allele.

### Conclusion

In conclusion, we have identified a novel deleterious PRKAR1A 22 bp insertion mutation in a familial case of CNC. The significance of this study is the identification of the new insertion mutation leading to CNC with unique clinical manifestations of infertility without Sertoli cell tumors, acromagaly, and cardiac myxoma. This novel change adds to the universal mutation pool of PRKAR1A causing CNC. The characteristic presentation documented
in this family of infertility without Sertoli cell tumors along with acromegaly and cardiac myxoma in future will aid the clinicians in timely diagnosis and appropriate treatment management of this rare condition.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Patient consent
Written informed consent has been obtained from the patient on the journal’s consent form.

Author contribution statement
S Birla carried out the molecular genetic studies, literature search, data analysis, and manuscript preparation. S Aggarwal was involved in the clinical diagnosis, literature survey, and manuscript preparation. A Sharma supervised the genetic studies and manuscript editing. Clinical diagnosis and management was performed under N Tandon’s supervision.

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