Male Infertility associated with a Novel *PRKAR1A* Mutation in Carney Complex

Clinical Medicine Insights:
Endocrinology and Diabetes
Volume 17: 1–7

DOI: 10.1177/11795514241293073

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ABSTRACT: Carney Complex (CNC) is a rare syndrome characterized by spotty skin pigmentation and multiple neoplasms, notably cardiac myxomas, schwannomas, and endocrine tumours. It is often inherited in an autosomal dominant manner with *PRKAR1A* gene mutations found in the majority of cases. Male infertility is established as part of the CNC phenotype and is largely associated with Large cell calcifying Sertoli cell tumours (LCCSCT). We describe a case of a 30-year-old male patient with Carney Complex, presenting with severe oligoasthenozoospermia and primary pigmented nodular adrenocortical disease (PPNAD). During follow-up consults, the severe oligozoospermia and impaired semen motility persisted and the patient was also diagnosed with a recurring cardiac myxoma and LCCSCT. Molecular testing identified a novel *PRKAR1A* mutation involving a deletion of exons 4 to 7. Our findings suggest this mutation causes *PRKAR1A* haploinsufficiency, which may be directly linked to male infertility, irrespective of the presence of testicular tumours. Accordingly, in male patients with CNC, detection of a *PRKAR1A* gene mutation may serve as a predictive marker for infertility. This case report illustrates the importance of early consideration and management of infertility in male patients diagnosed with CNC.

PLAIN LANGUAGE SUMMARY

Male infertility associated with a novel *PRKAR1A* mutation in carney complex

Carney Complex (CNC) is a rare genetic disorder that leads to the development of various tumours, including in the heart and endocrine glands. Most cases are linked to mutations in a gene called *PRKAR1A* and are inherited in an autosomal dominant manner. This case report presents a detailed examination of a patient diagnosed with Carney Complex (CNC), followed over nearly 10 years. The patient initially consulted due to infertility, which led to extensive investigations and diagnosis of several type of tumours: Primary pigmented nodular adrenocortical disease (PPNAD) in the adrenal glands, a cardiac myxoma, and Large cell calcifying Sertoli cell tumours (LCCSCT) in the testes - all tumours are characteristic features of CNC. Notably, genetic testing revealed a novel mutation in the *PRKAR1A* gene. Through this case, we highlight how this genetic mutation might cause *PRKAR1A* haploinsufficiency and be solely responsible for infertility in CNC irrespective of testicular tumours, providing valuable insights into a condition where male infertility is rarely documented. This report contributes important new information to the limited existing research on the connection between CNC and male infertility.

KEYWORDS: Carney complex, male infertility, oligoasthenozoospermia, ***PRKAR1A*** gene deletion, ***PRKAR1A*** haploinsufficiency, PPNAD, LCCSCT

RECEIVED: June 17, 2024. **ACCEPTED:** October 3, 2024.

TYPE: Case Report

FUNDING: The author(s) received no financial support for the research, authorship, and/or publication of this article.

COMPETING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Introduction

Carney Complex (CNC) is a rare, multiple neoplasia syndrome characterized by spotty skin pigmentation, myxomas, schwannomas and a variety of endocrine tumours. It is inherited in an autosomal dominant manner, but approximately 30% of cases are sporadic.[1,2] The mutations causing CNC have been mapped to two loci: 2p16 and 17q22-24.[3,4] Recent studies have uncovered new genes that could also be involved in Carney complex.[5,6] The majority of patients have a mutation on the *PRKAR1A* gene which is located on chromosome 17. The *PRKAR1A* gene encodes protein kinase A (PKA) regulatory subunit-1-alpha and is considered to be a tumour suppressant gene.[2]

CNC is diagnosed clinically based on the presence of major clinical criteria where at least two of them need to be met to establish a diagnosis. Molecular testing is used as an auxiliary tool that affirms the clinical criteria.[1,4]

Male patients with CNC are at risk of having reduced fertility that has predominantly been linked to the presence of Large cell calcifying Sertoli cell tumours (LCCSCT) which are frequently observed in these patients.[2] However, *PRKAR1A* haploinsufficiency can also lead to male infertility by affecting sperm count, motility and morphology in the absence of LCCSCT.[7,8]

Here, we review a case of a male patient with CNC and infertility. The patient initially presented with severe

oligozoospermia and impaired semen motility in the absence of testicular tumours. The molecular testing uncovered a novel *PRKAR1A* gene mutation, specifically a large deletion involving exons 4 to 7. After several years, the patient developed LCCSCT. We propose that the novel mutation prompts *PRKAR1A* haploinsufficiency and we further posit that *PRKAR1A* haploinsufficiency may be a sole cause for male infertility in CNC by causing persistent, severe oligoasthenozoospermia. This case illustrates the importance of evaluating male infertility in CNC patients and emphasizes addressing the associated infertility challenges.

Case Description

A 30-year-old male patient consulted the Endocrinology department at our hospital because of infertility. He had been in a childless marriage for 3 years.

His medical history included acute onset cardiac arrhythmia 5 years before his first consultation. He had been admitted to the Cardiology department at a different hospital where a transthoracic echocardiogram (TTE) revealed a mass in the left atrium. The patient underwent surgical treatment, and histopathological evaluation of the mass revealed a cardiac myxoma.

He was also diagnosed with Diabetes mellitus type 1 four years before his first consultation, based primarily on his age of onset. Consequently basal-bolus insulin regimen was promptly initiated. It is important to note he was not screened for the presence of insulin antibodies at the time of diagnosis. The patient also complained of poor glycaemic control, despite insulin therapy adherence, and of low back pain and muscle weakness.

During physical examination, the patient was alert, but in evident pain. He was using crutches for mobility. He was well-built due to being a former bodybuilder. His height was 182 cm, his weight 86 kg and his BMI was 26.

The patient had facial plethora. Small, brown spots were visible on the skin of the patient's face and back. On the upper thorax at the midsternal line, there was a visible healed surgical scar from a previous sternotomy. Violet stretch marks 20 cm long and 2 to 3 cm wide were noticeable on both sides of the lower abdomen.

Cardiac auscultation revealed an accentuated first heart sound and no cardiac murmurs. The blood pressure was 150/100 mmHg, the pulse rate was 67 beats per minute and the respiratory rate was 20 breaths per minute. The andrological examination revealed a normally virilized male patient with an adult-sized penis. The volume of the testes was measured with a Prader orchidometer. Both testes had a volume of 20 ccm. The patient was at Tanner stage 5. The scrotal content was normal, and there was no varicocele.

Cushing's syndrome was suspected based on clinical findings including, low back pain, muscle weakness, facial plethora, violet stretch marks and hypertension. Additional laboratory tests were performed to confirm the diagnosis. Basal plasma cortisol was 1450 nmol/L (118.6-618), and basal plasma ACTH was 10 pg/mL (10-60). Urinary free cortisol was 468 nmol/24 h urine (55-250), and 1 week later 580 nmol/24 h urine. Liddle's low dose dexamethasone test showed a paradoxical positive response of urinary cortisol excretion (cortisoluria of 540 nmol/24 h). Other hormone levels and tumour markers: alpha – fetoprotein, carcinoembryonic antigen and CA 19-9 were in normal ranges. Further laboratory test results are shown in Table 1.

Table 1. Pertinent laboratory results.

TEST	PATIENT RESULT	REF. RANGE
ESR	46 mm/h	(12-19)
C-reactive protein	8.9 mg/L	(0-6)
Haemoglobin	169 g/L	(135-169)
Haematocrit	48%	(40-52)
Leucocyte count	8.5×10^9/L	(4-11)
Lymphocytes	30%	(10-48)
Eosinophils	0.2%	(0-7)
Monocytes	8%	(3-10)
Platelet count	290×10^9/L	(150-400)
Fasting plasma glucose	14.8 mmol/L	(3.88-5.55)
HbA1c	8.1%	(<5.7%)
LDL-cholesterol	4.8 mmol/L	(2.2-3.7)
HDL-cholesterol	0.8 mmol/L	(>1.036)
Triglycerides	3.1 mmol/L	(up to 2.0)
Urea (serum)	5.9 mmol/L	(2.7-7.8)
Creatinine	100 µmol/L	(45-109)
Total serum protein	5.9 g/dL	(6.4-8.3)
Albumin	2.5 g/dL	(3.4-4.7)
Globulin	2.7 g/dL	(2.7-3.5)
Sodium	147 mmol/L	(136-145)
Potassium	3.4 mmol/L	(3.3-5.1)
AST	19 U/L	(0-37)
ALT	32 U/L	(0-41)
TSH	3.075 mIU/L	(0.25-4.55)
IGF-1	125 ng/mL	(120-330)
FSH	6.0 mIU/mL	(1.0-10)
LH	5.4 mIU/mL	(1.0-10)
Testosterone	12.5 nmol/L	(9-35)
Prolactin	9.8 ng/mL	(2.7-16.9)

Semen analysis (two samples taken 6 weeks apart) revealed sperm volume of 3.0 and 3.4 ccm respectively; sperm

concentration of 2×10^6/mL and 1×10^6/mL; 20% and 10% progressive motility; 25% and 15% total motility; 10% and 15% normal morphology, according to strict Kruger criteria.

A series of imaging examinations were then performed. Thyroid and testicular ultrasonography had normal findings. A spinal X-ray revealed osteoporotic thoracic and lumbar vertebrae and an anterior wedge fracture of L1. Osteoporosis was confirmed with a DXA scan: At AP spine (L2-L4) *Z*-score was −3.1, and total femur *Z*-score was −2.8.

A pituitary magnetic resonance imaging (MRI) scan revealed a pituitary gland normal in size, shape and position. Unenhanced computed tomography (CT) of the abdomen, revealed ill-defined masses that filled both adrenal compartments with an estimated Hounsfield Unit of 40 (Figure 1).

The patient underwent bilateral adrenalectomy. Histopathological examination of the adrenal glands confirmed primary pigmented nodular adrenocortical disease (PPNAD) (Figure 2, Panels A and B).

Subsequently, after the histopathological confirmation of PPNAD a diagnosis of Carney complex was established. The patient met 3 major clinical criteria: spotty skin pigmentation, cardiac myxoma and PPNAD.

In the months following the bilateral adrenalectomy his diabetes resolved, allowing for the complete discontinuation of insulin therapy. This confirmed that the patient's diabetes had been secondary to his Cushing's syndrome, rather than type 1 diabetes. He was also pain-free and was no longer using crutches. Five years after the initial consultation his control transthoracic echocardiogram revealed a 2.2 cm × 1.2 cm × 1.0 cm mass in the left atrium arising from the interatrial septum (histopathology upon surgical excision confirmed that the lesion was a myxoma) (Figure 3). Nine years after the initial consultation, control testicular ultrasound revealed abnormalities for the first time. Subcentimeter calcifications typical for LCCSCT were noted on both testes, the largest being 4 mm in diameter (Figure 4). In the context of the testicular abnormalities revealed by the testicular ultrasound indicative of LCCSCT, germ cell tumour markers AFP and β-hCG were tested and found to be negative, effectively ruling out the presence of other testicular germ cell tumours.

During this timeframe, genetic analyses were performed. No *PRKAR1A* gene mutation was revealed using Sanger DNA sequencing of all *PRKAR1A* exons and exon-intron boundaries. However, whole exome sequencing (WES) analysis using the

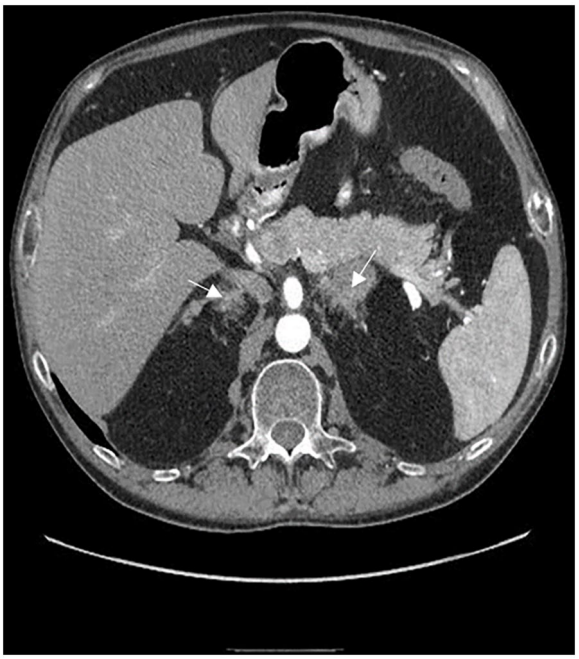
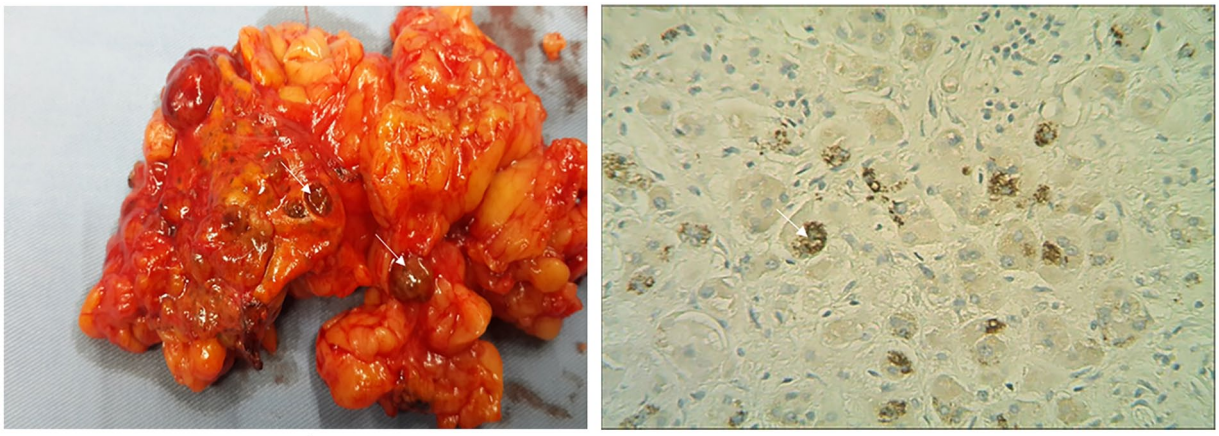


Figure 1. CT of the abdomen with ill-defined masses in both adrenal compartments (white arrows).



A B

Figure 2. Panel A: Gross appearance of the resected specimen of the adrenal gland in our patient with CNC and PPNAD. The adrenal gland is enlarged (7 cm × 6 cm) in size. Multiple brown-coloured nodules are observed (white arrows). Panel B: Immunohistochemical analysis using anti Chromoganin A specific antibody. Note presence of brown precipitates (lipofuscin) within the cytoplasm of large cells indicating a positive reaction (white arrow).

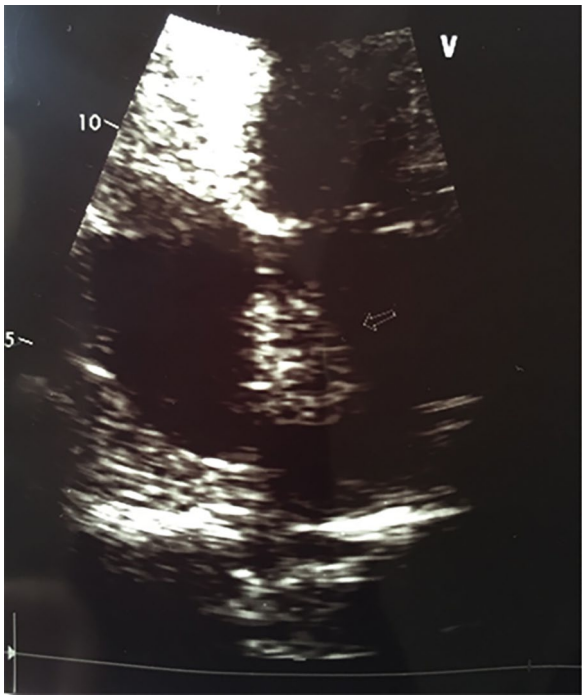


Figure 3. Cardiac myxoma arising from the interatrial septum (empty arrow).

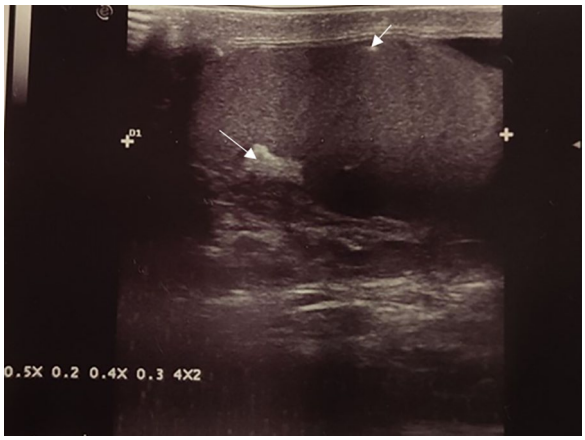


Figure 4. Testicular ultrasound with calcifications indicative of LCCSCT (white arrows).

Endocrine neoplasia panel (PanelApp UK, Version 3.3), suggested a novel, large deletion involving exons 4 to 7 (Figure 5, Panel A), which was confirmed by Multiple Ligation Probe-dependent Amplification (MLPA) analysis (Figure 5, Panel B). These molecular tests confirmed the CNC diagnosis that had been established 9 years earlier.

Additionally, the most common Y-chromosome deletions were excluded using a protocol based on two multiplex polymerase chain reactions[9] and Quantitative Fluorescent (QF)-PCR detection of sex chromosome aneuploidies and AZF deletions/duplications.[10] Furthermore, the patient's WES data were analysed for mutations in genes included in the Male Infertility Panel (FER16, 167 Genes, CeGat, Germany[11]), as well as for copy number variations (CNVs). Apart from the *PRKAR1A* gene variant, no other pathogenic variant associated with male infertility has been detected.

In regard to his infertility, the treatment plan after making the clinical diagnosis of Carney Complex started with counselling on the implications of this condition. Although the *PRKAR1A* gene mutation was confirmed through genetic testing 9 years later, the patient and his wife were informed about the inheritance pattern of CNC, potential health impacts and available treatment methods. The couple decided to pursue assisted reproductive technique (ART) procedures, specifically in vitro fertilization with donor sperm (IVF-D). Given the severe oligozoospermia and impaired motility did not show any improvement in control semen analyses (Figure 6) they opted to use a donor sperm to increase their chances of successful fertilization and to mitigate the risk of transmitting the condition. Chronological timeline of significant medical events experienced by the patient is shown in Figure 6.

Discussion

Carney Complex has been first described by Carney et al[12] as a syndrome characterized by myxomas, spotty skin pigmentation and endocrine overactivity. Most of CNC cases are inherited in an autosomal dominant fashion, but CNC can also occur sporadically by a de novo mutation.[2,4] It is a rare condition, and the exact prevalence is unknown. To date, around 750 cases have been reported through a joint effort by the NIH-Mayo clinic and the Cochin Hospital in France.[2,13] The clinical phenotype of Carney complex is diverse. However, skin myxomas and lentigines, cardiac myxomas and primary pigmented nodular adrenocortical disease (PPNAD) are the most frequent clinical features.[3] The clinical manifestations have been solidified into major and supplemental clinical criteria. Two major criteria or one major and one supplemental criterion are needed for the confirmation of a CNC diagnosis.[1]

The majority of CNC patients have small, intragenic mutations in the *PRKAR1A* gene. Most of the mutations generate a non-sense mRNA, thus activating a process called non-sense mediated mRNA decay (NMD) which causes *PRKAR1A* haploinsufficiency.[14] It is believed that *PRKAR1A* haploinsufficiency leads to unopposed cell proliferation and tumourigenesis through increased cAMP signalling and increased protein kinase A (PKA) activity. The genotype-phenotype correlation is quite variable, although some studies have shown an association between certain mutations and the age of presentation, severity of disease and development of certain types of tumours.[6] Larger deletions are linked to a more severe disease.[15] Sanger sequencing uncovers the small mutations and is usually performed as the first genetic test. If single-nucleotide sequencing is negative, the next step in molecular testing should be a MLPA analysis which uncovers deletions/duplications on the *PRKAR1A* gene. Another approach would be to perform a WES analysis which can identify mutations in *PRKAR1A* and mutations in other genes associated with

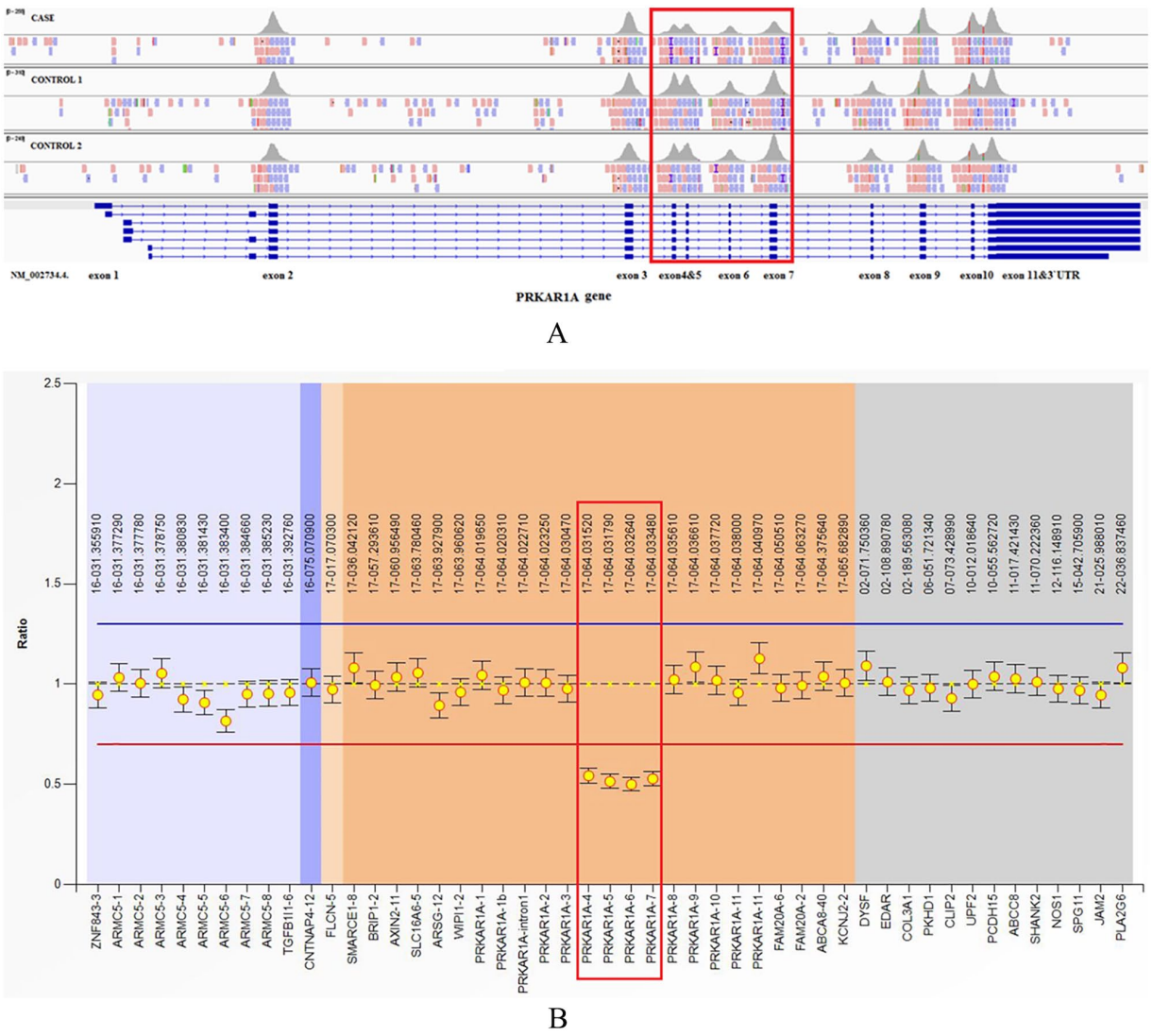


Figure 5. Panel A: *PRKAR1A* gene coverage from the whole exome sequencing (WES) analysis, visualized in the Integrated Genomic Viewer (IGV) showing the deleted exons (4-7) of the *PRKAR1A* gene in our case compared to controls. Panel B: MLPA analysis in our patient with Carney complex showing a deletion of exons 4 to 7 of the *PRKAR1A* gene.

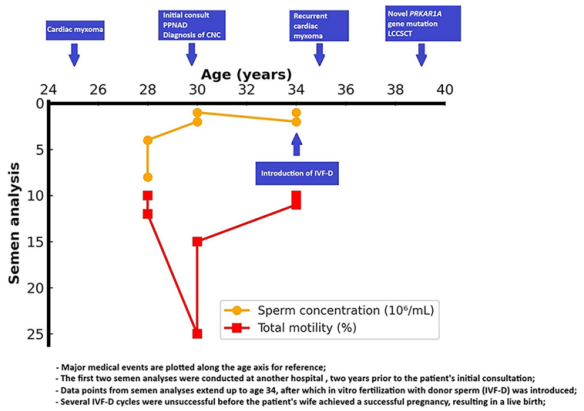


Figure 6. This figure shows the patient's semen analysis trends, with key medical events annotated.
Abbreviations: CNC, Carney complex; LCCSCT, Large cell calcifying Sertoli cell tumour; PPNAD, primary pigmented nodular adrenocortical disease.

CNC.[1] Over 125 unique mutations in the *PRKAR1A* gene have been identified with only a few mutation hotspots.[6]

The clinical diagnosis of CNC in our patient was unequivocal, based on his clinical features, biochemical analyses and imaging techniques. He had recurrent cardiac myxoma, lentiginines, PPNAD, oligoasthenozoospermia and LCCSCT. Genetic testing using WES and MLPA analyses confirmed a deletion involving exons 4 to 7 of the *PRKAR1A* gene. This is an in-frame deletion which removes 120 amino acid residues (aminoacids 117-236) of the PKA type I regulatory subunit alpha. This region includes almost the whole cAMP-binding domain A (aminoacids 137-254). To our knowledge, based on the available database on *PRKAR1A* gene mutations this is a novel mutation. Similar in-frame deletion involving exons 3 to 6 of the *PRKAR1A* gene has been already described[16] and shown to result in haploinsufficiency. Namely, functional analyses have

revealed that the deletion of exons 3 to 6 of the *PRKAR1A* gene generates a truncated protein which is expressed, but rapidly degraded, and is not able to bind to the protein kinase A catalytic subunit. We propose that the novel in-frame deletion of exons 4 to 7 also generates *PRKAR1A* haploinsufficiency.

The male as a sole factor of infertility is attributed in 20% of cases.[17] Oligozoospermia is a common cause of male infertility, with over 60% of cases being idiopathic and genetic causes accounting for less than 10%.[18] Male infertility has been scarcely documented as a component of Carney complex and it is generally connected to the presence of LCCSC tumours, as these tumours are present in 40% to 50% of patients with CNC.[3] Moreover, there have been just a small number of reports on infertility tied with oligozoospermia and CNC. Burton et al,[8] showed that in CNC mice *PRKAR1A* haploinsufficiency can cause oligozoospermia alongside impaired semen motility and morphology, in the absence of LCCSCT. They indicated that *PRKAR1A* haploinsufficiency leads to increased PKA activity which interferes with a pathway necessary for the structural integrity of the spermatozoon. This results in fragile sperm that is structurally abnormal, reduced in number and motility with diminished ability to fertilize eggs. Defects in the epididymis that may lead to spermatozoa leakage, inflammatory responses, and subsequent testicular atrophy is another proposed mechanism involved in infertility in CNC mice. The observed sperm defects in 7 CNC patients with *PRKAR1A* mutations in the same study were consistent with these findings. Additionally, sperm fragility and reduced fertility in males may contribute to the higher transmission rate of CNC by females, a phenomenon that has been observed but remains unexplained in terms of its precise mechanism.[19]

To our knowledge, there is only a single clinical report of oligozoospermia, with a confirmed *PRKAR1A* mutation and absence of LCCSCT.[20] Two other case reports have commented on oligo/azoospermia and CNC, however, in both cases genetic analysis had not been conducted, and in the latter bilateral testicular calcifications consistent for LCCSCT had also been identified[21,22].

Our patient presented with an inability to conceive with his wife after 3 years of marriage. The initial evaluation for his infertility included an andrological examination, semen analysis on 2 separate occasions, hormone testing for FSH, LH, Testosterone, Prolactin and a testicular ultrasound. All investigations were normal, except for the semen analyses, which revealed oligoasthenozoospermia. While impaired semen morphology was observed, the threshold for teratozoospermia was not surpassed according to Kruger and WHO criteria. At this stage, Cushing's syndrome and diabetes were considered potential contributors to the impaired semen parameters, as they have been shown to impact male reproductive function.[23,24] The patient underwent bilateral adrenalectomy, which resulted in the resolution of hypercortisolaemia and secondary diabetes; however, the oligoasthenozoospermia persisted. Genetic testing confirmed a novel *PRKAR1A* gene mutation, providing the first evidence linked to the patient's infertility, while sex chromosome aneuploidies and AZF deletions/duplications, which are also genetic causes of male infertility, were ruled out with negative results. The impaired semen parameters were consistent with the findings from the study by Burton et al. Since LCCSCT appeared 9 years after the patient's initial consultation, we propose that our novel *PRKAR1A* gene mutation through haploinsufficiency could be the primary factor for male infertility in other CNC patients. However, as demonstrated in our patient, LCCSCT can still develop in later stages of the condition, potentially exacerbating the existing oligoasthenozoospermia by obstructing the seminiferous tubules and possibly causing frank hypogonadism by destroying normal testicular tissue. In very rare cases, other types of testicular tumours in CNC may produce oestrogens, leading to hypogonadism and infertility.[2] Finally, there are various reasons for infertility in Carney complex. We believe this case report is an important addition to the limited literature suggesting that *PRKAR1A* haploinsufficiency is a direct mechanism causing oligozoospermia and impaired semen motility in humans, similar to what is observed in CNC mice. However, since the most common cause of oligozoospermia is idiopathic, we cannot rule out the possibility that other mechanisms may contribute to infertility in our case of Carney complex.

Conclusion

Our comprehensive case analysis of Carney complex underscores the imperative for a broader recognition of male infertility within the spectrum of this disorder. Our findings suggest that male infertility should be considered a pivotal characteristic when establishing a CNC diagnosis, even in the absence of LCCSCT. Genetic analysis with a positive *PRKAR1A* gene mutation, should prompt clinicians to further evaluate male infertility in CNC patients, extending beyond the tumour-centric approach. This also involves acknowledging the potential challenges posed by infertility and fostering informed discussions about various assisted reproduction methods.

Declarations

Ethics Approval and Consent to Participate

This case report was approved by the Ethics committee of the Medical faculty, Skopje under approval number 03-4422/4. Written consent was obtained from the patient for publication of this case report and any accompanying images.

Consent for Publication

Written informed consent was obtained from the patient for publication of this case report and any accompanying images.

Author Contributions

M.D-Conceptualization, Data curation, Formal analysis, Investigation, Resources, Supervision, Writing – original draft,

Writing – review & editing; D.P.K-Data curation, Formal analysis, Investigation, Methodology, Resources, Writing – review & editing; J.G-Data curation, Formal analysis, Investigation, Resources; T.M-Supervision, Validation, Writing – review & editing; G.B-Data curation, Formal analysis, Resources; C.D-Conceptualization, Data curation, Investigation, Supervision, Validation, Writing – review & editing.

Acknowledgements

Dr. Maja Dimitrovska would like to acknowledge Dr. Marko Dimitrovski for his invaluable support throughout the development of this manuscript. Although not affiliated with our case, his expertise and assistance greatly enhanced the quality of this work.

Data Availability Statement

Data generated during this study are included in this published article.

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