Pathol. Oncol. Res. (2012) 18:149–152
DOI 10.1007/s12253-012-9502-3

REVIEW

Novel Genetic Mutation in the Background of Carney Complex

Csaba Halászlaki · István Takács · Henriett Butz · Attila Patócs · Péter Lakatos

Received: 19 December 2011 / Accepted: 17 January 2012 / Published online: 2 February 2012

Abstract Carney complex is a rare disease inherited in an autosomal dominant manner. It is mostly caused by inactivating mutations of the subunit of protein kinase A. Carney complex is associated with atrial myxoma, nevi or myxomas of the skin, breast tumor and endocrine overactivity. Primary pigmented nodular adrenocortical disease is the specific endocrine manifestation. The authors present the history of a 53-year-old female patient who had undergone surgery for atrial myxomas, thyroid tumor and breast cancer. She was also operated for an adrenal adenoma causing Cushing's syndrome. Genetic study revealed a novel mutation in the regulatory subunit of protein kinase A (ivs2-1G>A splice mutation in intron 2). Her heterozygous twins were also genetically screened and one of them carried the same mutation. The authors emphasize that despite the absence of specific treatment for patients with Carney complex, confirmation of the diagnosis by genetic studies is important for the close follow-up of the patient and early identification of novel manifestations.

Keywords Carney complex · Endocrine overactivity · Genetic study · Regulatory subunit 1A of the protein kinase A · Splice mutation

C. Halászlaki · I. Takács · P. Lakatos
1st Department of Medicine, Semmelweis University,
Korányi Sándor u. 2/A,
Budapest 1083, Hungary

H. Butz
2nd Department of Medicine, Semmelweis University,
Szentkirályi u. 46,
Budapest 1088, Hungary

C. Halászlaki (✉)
4th Department of Internal Medicine, St. Margit Hospital,
Bécsi út 132,
Budapest 1032, Hungary
e-mail: drhalaszlaki@gmail.com

A. Patócs
Molecular Medicine Research Group,
Hungarian Academy of Sciences,
Szentkirályi u. 46,
Budapest 1088, Hungary

Introduction

J. Aiden Carney was the first to describe the syndrome named after him 26 years ago: Carney complex (CNC). This extremely rare disease, with an autosomal dominant inheritance, is primarily characterized by cardiac myxoma, benign tumors and/or brownish lesions on the skin, lentigines, and endocrine overactivity [1]. Earlier, two acronyms were used to attempt to summarize the essence of the disease: one was "LAMB syndrome": Lentigines, Atrial myxomas, Mucocutaneous myxomas, and Blue nevi; the other one was "NAME snydrome": Nevi, Atrial myxoma, Myxoid neurofibroma, and Ephelides (freckles), or, instead of the latter two, mucinous skin tumor and endocrine overactivity [2].

The clinical picture of CNC, however, is a lot more complex. The syndrome could be seen as a form of multiple endocrine neoplasia, as the disease can include two or more endocrine tumors, and in half of the cases, endocrine overactivity is also reported [3]. Primary pigmented nodular adrenocortical disease (PPNAD) belongs to this group, whose name comes from the brown hyperpigmented lesion on the cut surface of both adrenal cortices, usually accompanied by the clinical picture of Cushing's syndrome. In PPNAD, both adrenal glands are enlarged, but adrenocorticotropic hormone

(ACTH) levels remain in the normal range. PPNAD may occur in approximately 25% of affected individuals. Cushing syndrome caused by PPNAD is seen in 70% of affected females before age 45 years but in only 45% of affected males, likely reflecting the generally higher frequency of Cushing syndrome in females. Other endocrine abnormalities including follicular thyroid cancer, pituitary adenoma producing growth hormone or prolactin, or the Sertoli cell (or rarely, Leydig cell) tumor of the testis may also develop. Up to 75% of individuals with CNC have multiple thyroid nodules. Clinically evident acromegaly is a relatively frequent manifestation of CNC, occurring in approximately 10% of adults at the time of presentation. Subtle hyperprolactinemia may be present in up to 75% of individuals with CNC. Sertoli cell tumors are observed in one third of affected males at the time of presentation, which is often within the first decade.

In 70% of the cases, CNC involves atrial, in 80%, skin myxomas, and in 50%, breast fibroadenomas or myxomas [2, 4]. Cardiac myxomas occur at a young age and may occur in any or all cardiac chambers. Cutaneous myxomas appear between birth and the fourth decade. Breast myxomas, often bilateral, occur in females after puberty. Both males and females may develop breast nipple myxomas at any age. Pale brown to black lentigines are the most common presenting feature of CNC and may be present at birth. Typically, they increase in number around puberty. These lentigines tend to fade after the fourth decade, but may still be evident in the 70s. CNC may also involve the development of peripheral nerve schwannomas, rarely in the form of psammomatous melanotic schwannomas (PMS), occurring in approximately 10% of individuals with CNC [4, 5].

The disease may present in any gender and age, but mostly, it is diagnosed around 20 years of age. Most individuals with CNC have a normal life span. However, because some die at an early age, the average life expectancy for individuals with CNC is 50 years [4]. Symptoms are determined by tumors, skin lesions and endocrine overactivity. Diagnosis is based on physical exam, the demonstration of hormonal abnormalities, the histological evaluation of discovered tumors, and genetic tests.

The underlying mutation in the most frequent type of CNC (type 1) is the genetic mutation of the 1A regulatory subunit of protein kinase-A (*PRKAR1A*). Located in position 22–24 on the long arm of chromosome 17, this gene encodes the spatial form of one of the regulatory subunits of protein kinase A (PKA). Type 2 of CNC is caused by another genetic abnormality in chromosomal region 2p16. Currently, little is known about the clinical differences between the two types.

Today, treating the disease is not possible. After the surgical removal of tumors and the cardiac myxoma, and the surgical/pharmacological therapy of PPNAD, there is a significant risk for recurrence [6].

Case Presentation

The medical history of the 53-year-old female patient includes cardiac surgeries for an atrial myxoma in 1989, then in 1996 for its recurrence; in 1992, follicular thyroid cancer producing mucin, thyreoglobulin, and calcitonin; neoadjuvant chemotherapy, surgery and radioactive iodine treatment was used, which was followed by the development of hypothyroidism. In 1998, bilateral mastectomy was performed, because malignant tumors were found affecting both breasts. In 2002, traumatic fracture of the right upper arm, in 2003, uterus removal for uterine myoma, and treatment for hypertension, paroxysmal atrial fibrillation and right bundle branch block was initiated. In 2004, an adrenal adenoma causing Cushing's syndrome was removed, which was followed by Addisonian crisis. Afterwards, she had adrenal insufficiency, type II diabetes treated with oral antidiabetics, cholelithiasis and gastroesophagal reflux.

In May, 2009, because of hypertension and increased serum chromogranin A levels, tests were carried out to screen for pheochromocytoma and neuroendocrine tumors. In June, 2009, contrast-enhanced abdominal and pelvic CT scans were performed, which did not show any abnormalities in the adrenal region indicative of pheochromocytoma, but revealed a hyperattenuating structure at the fundus of the urinary bladder. The follow-up CT scan in March, 2010 showed that the size of the structure seen in the pelvis, at the fundus of the urinary bladder slightly decreased, but otherwise, the abdominal status was unchanged.

Because of the diseases in the medical history, genetic tests were run to confirm or rule out CNC and von Hippel-Lindau disease (vHL). The first disease was suspected because of the many malignant and benign tumors resulting from the genetic defect, and the second syndrome had to be ruled out because of the suspected pheochromocytoma. In November, 2009, DNA was isolated from peripheral blood, and polymerase chain reaction (PCR) followed by bidirectional DNA sequencing was used to test the *PRKAR1A* gene. In the 2nd intron, a heterozygous form of the ivs2-1G>A splice site mutation was confirmed in the patient. Detailed molecular genetic analysis clarified that this mutation causes on mRNA level the skipping of the 2nd exon, after transcription of exon 1 follows the exon 3. The resulted protein is a short (73 aminoacid long) and the premature Stop codon occurs at residue number 73 (Fig. 1.).

After the genetic diagnosis of CNC, genetic screening was performed on first-degree relatives. She had fraternal twin sons: one of them did, and one of them did not carry the genetic mutation.

Discussion

The tumor syndrome CNC is most frequently caused by the mutation of the *PRKAR1A* gene [7]. In our case, a novel

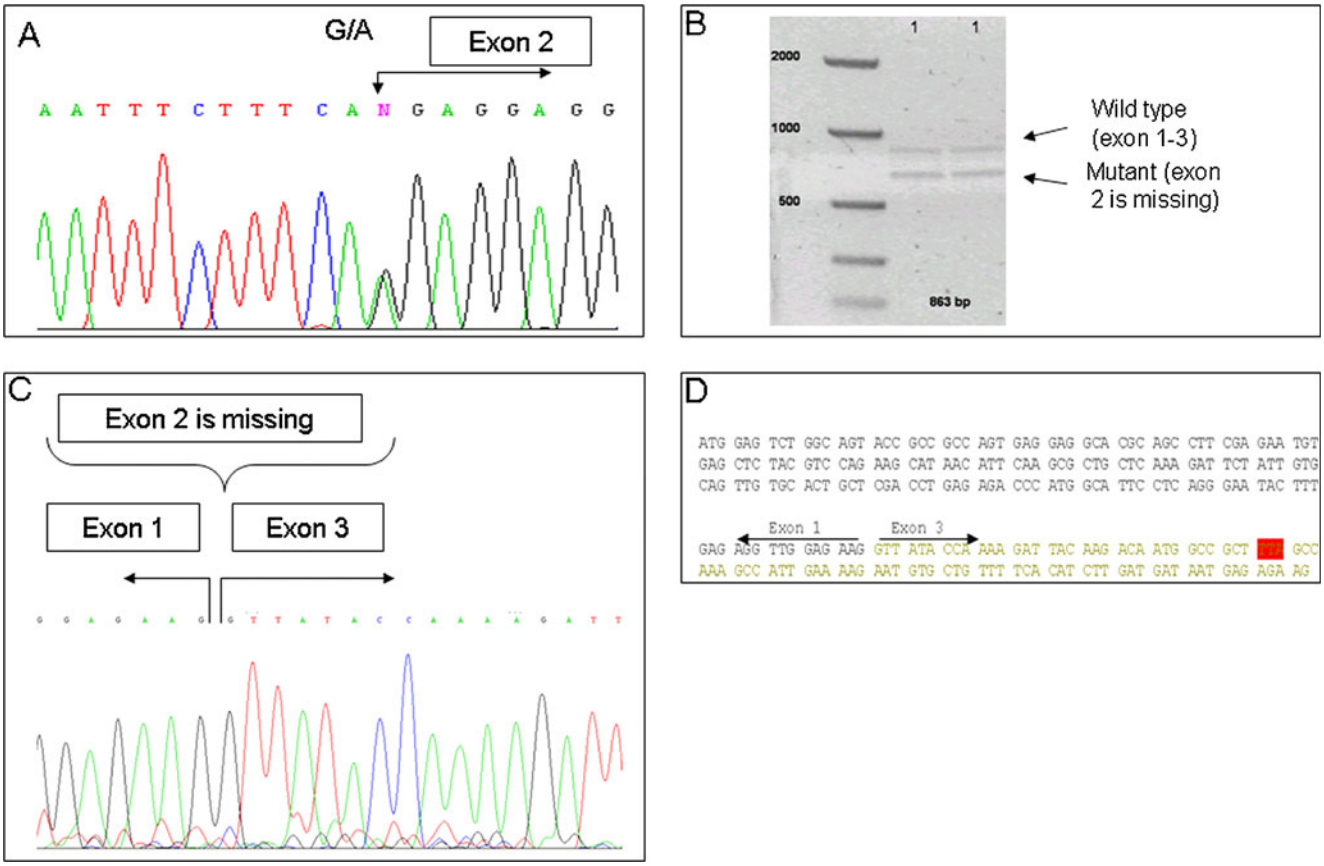

Fig. 1 ivs2-1G>A splice site mutation of the *PRKAR1A* gene. **a** Germline presentation of the ivs2-1G>A mutation in the proband; **b** gel electrophoresis of the PCR fragments resulted after RT-PCR of the *PRKAR1A*; **c** cDNA sequencing of the mutant fragment demonstrating the skipping of the 2nd exon during transcription; **d** the schematic representation of the resulted truncated protein

mutation of this gene was confirmed in the background of recurring atrial myxoma, thyroid cancer, bilateral breast tumors, and adrenal adenoma causing Cushing's syndrome.

Based on literature data, half of all *PRKAR1A* mutations causing CNC are splice site mutations, which result in the production of a dysfunctional protein. The PKA protein plays a role in the regulation of carbohydrate and lipid metabolism, and it is one of the components of the signal transmission route related to guanine nucleotide binding-protein coupled receptors (GPCRs). This enzyme is made up of two regulatory and two catalytic subunits. Cyclic adenosine monophosphate (cAMP) connects to the regulatory subunits, and afterwards, the released catalytic subunits phosphorize different target proteins. Besides ion channels and other enzymes, PKA activates the transcription factor: cAMP response element-binding (CREB) protein. The *PRKAR1A* gene encodes one of the regulatory subunits of this important enzyme [8].

In our case, a splice site mutation (ivs2-1G>A) was revealed by direct DNA sequencing of the DNA isolated from the peripherial blood sample obtained from the proband. Using RNA isolated from the peripherial leucocytes of the proband and performing reverse transcription PCR (RT-PCR) using primer pairs mapping to the cDNA of the *PRKAR1A* as expected two different fragments were observed. Direct DNA sequencing confirmed that one is the normal, wild type sequence while the shorter one is resulted from the mistranscription caused by the splice site mutation newly discovered (Fig. 1.). The resulted mutant protein is only 73 aminoacid long due to the premature Stop codon at position of 73th. As expected the novel splice mutation similarly to others; up to date, 28 types of *PRKAR1A* splice site mutations have been reported in the literature, cause a defective, functionally inactive protein. This high number of splice mutations has been associated with more than 600 cases of CNC registered [4, 9, 10]. Of course beside splice mutation other, more than 100 abnormal variants of the 1A regulatory subunit of PKA are known which can be demonstrated in about 60% of CNC patients [9, 11, 12].

The screening tests of the family revealed that this new, previously unreported ivs2-1G>A splice site mutation was present in one of the currently symptom-free fraternal twins. Genetic diagnosis is of great significance in currently symptom-free carriers because providing close monitoring and follow-up

allows for the early recognition of tumors and endocrine disorders and the timely initiation of treatment [13, 14].

Acknowledgements We thank members of our laboratory (Molecular Medicine Research Group) for scientific advice. We thank the patient and her family for contribution and co-operation.

Informed consent We state formally that an appropriate institutional review board approved the experiment, and that informed consent was obtained from the subjects and from parents or legal guardians for minors. We ensure the safety and dignity of human subjects.

Declaration of interest We fully declare neither financial nor potential conflict of interest and that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Author contribution statement (optional) We confirm that all of the named authors contributed substantially to the article, and agree to take public responsibility for the validity of its content. We also confirm that all major sources of funding have been acknowledged in the text of the article and that there is either (a) no conflict of interest that could be perceived as prejudicing its impartiality, or (b) a potential conflict of interest that is fully declared within the text of the article. We declare that there is no conflict of interest.

References

1. Carney JA, Gordon H, Carpenter PC, Shenoy BV, Go VL (1985) The complex of myxomas, spotty pigmentation, and endocrine overactivity. Medicine (Baltimore) 64(4):270–283
2. Stratakis CA, Kirschner LS, Carney JA (2001) Clinical and molecular features of the Carney complex: diagnostic criteria and recommendations for patient evaluation. J Clin Endocrinol Metab 86(9):4041–4046
3. Stratakis CA, Kirschner LS, Carney JA (1998) Carney complex: diagnosis and management of the complex of spotty skin pigmentation, myxomas, endocrine overactivity, and schwannomas. Am J Med Genet 80(2):183–185
4. Stratakis CA, Horvath A (1993–2003 Feb 05) Carney Complex. In: Pagon RA BT, Dolan CR, Stephens K (ed) GeneReviews [Internet]. Seattle (WA). http://www.ncbi.nlm.nih.gov/books/NBK1286/. Last update date:2010 Jun 22.
5. Carney JA, Stratakis CA (1998) Epithelioid blue nevus and psammomatous melanotic schwannoma: the unusual pigmented skin tumors of the Carney complex. Semin Diagn Pathol 15(3):216–224
6. Bertherat J (2006) Carney complex (CNC). Orphanet J Rare Dis 1:21
7. Libe R, Horvath A, Vezzosi D, Fratticci A, Coste J, Perlemoine K, Ragazzon B, Guillaud-Bataille M, Groussin L, Clauser E, Raffin-Sanson ML, Siegel J, Moran J, Drori-Herishanu L, Faucz FR, Lodish M, Nesterova M, Bertagna X, Bertherat J, Stratakis CA (2011) Frequent phosphodiesterase 11A gene (PDE11A) defects in patients with Carney complex (CNC) caused by PRKAR1A mutations: PDE11A may contribute to adrenal and testicular tumors in CNC as a modifier of the phenotype. J Clin Endocrinol Metab 96(1):E208–E214
8. Vezzosi D, Vignaux O, Dupin N, Bertherat J (2010) Carney complex: clinical and genetic 2010 update. Ann Endocrinol (Paris) 71 (6):486–493
9. Rothenbuhler A, Stratakis CA (2010) Clinical and molecular genetics of Carney complex. Best Pract Res Clin Endocrinol Metab 24(3):389–399
10. 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. Hum Mutat 31(4):369–379
11. Pan L, Peng L, Jean-Gilles J, Zhang X, Wieczorek R, Jain S, Levine V, Osman I, Prieto VG, Lee P (2010) Novel PRKAR1A gene mutations in Carney Complex. Int J Clin Exp Pathol 3(5):545–548
12. Storr HL, Metherell LA, Dias R, Savage MO, Rasmussen AK, Clark AJ, Main KM (2010) Familial isolated primary pigmented nodular adrenocortical disease associated with a novel low penetrance PRKAR1A gene splice site mutation. Horm Res Paediatr 73 (2):115–119
13. Landry CS, Waguespack SG, Perrier ND (2009) Surgical management of nonmultiple endocrine neoplasia endocrinopathies: state-of-the-art review. Surg Clin North Am 89(5):1069–1089
14. Szabo D, Zsippai A, Bendes M, Tombol Z, Szabo PM, Racz K, Igaz P (2010) Pathogenesis of adrenocortical cancer. Orv Hetil (Hungarian Medical Journal) 151(29):1163–1170 (In Hungarian)