Clinical and Molecular Genetics of Primary Pigmented Nodular Adrenocortical Disease

ABSTRACT

Carney complex (CNC) is a multiple endocrine neoplasia (MEN) syndrome associated with other, non-endocrine manifestations such as lentigines, cardiac myxomas and schwannomas. Primary pigmented nodular adrenocortical disease (PPNAD), leading to corticotrophin-independent Cushing's syndrome is the most frequent endocrine lesion in CNC. The complex has been mapped to 2p16 and 17q22-24, although additional heterogeneity may exist. The gene coding for the protein kinase A (PKA) type I-a regulatory subunit (RIa), PRKAR1A, had been mapped to 17q. Cloning of the PRKAR1A genomic structure and its sequencing showed mutations in CNC-, CNC with PPNAD- and sporadic PPNAD-patients. In CNC tumors, PKA activity showed increased stimulation by cAMP, whereas PKA activity ratio was decreased, and in CNC tumors, there is LOH of the normal allele, suggesting that normal PRKAR1A may be a tumor suppressor in these tissues. CNC is the first human disease caused by mutations of one of the subunits of the PKA enzyme, a critical component of the cAMP signaling system and a potential participant in many other signaling pathways. **(Arq Bras Endocrinol Metab 2004;48/5:637-641)**

Keyword: Carney complex; Primary pigmented nodular adrenocortical disease; PRKAR1A

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RESUMO

Clínica e Genética Molecular da Doença Nodular Adrenocortical Pigmentada Primária.
Complexo de Carney (CNC) é uma síndrome de neoplasia endócrina múltipla (MEN) associada com outras manifestações não endócrinas, como lentígenes, cardiomixomas e adenomas de células de Schwann. A doença nodular pigmentada primária da adrenal (PPNAD), que apresenta-se como síndrome de Cushing independente de corticotropina é a lesão mais freqüente observada em CNC. O CNC tem sido relacionados aos sítios cromossômicos 2p16 e 17q22-24, entretanto, heterogenicidade pode ocorrer. O gene codificador da proteína reguladora tipo 1A da proteína quinase A(RIa), PRKAR1A, tem sido localizado no cromossomo 17q. A clonagem da estrutura genômica e seqüenciamento do gene PRKAR1A revelou mutações em pacientes com CNC e em formas esporádicas de PPNAD. Em tumores de pacientes com CNC, a proteína quinase A apresenta uma resposta de atividade maior após o estímulo com AMPc. Também, nestes tecidos, é observada a perda de heterozigose do alelo normal. Isto sugere que o gene normal do PRKAR1A pode funcionar como um gene de supressão tumoral nos tecidos estudados. CNC é a primeira doença conhecida a ocorrer devido a mutações de uma das sub-unidades da proteína quinase A, um componente crucial na via de sinalização do AMPc e um potencial participante de outras vias de sinalização celular. **(Arq Bras Endocrinol Metab 2004;48/5:637-641)**

Descritores: Complexo de Carney; Doença nodular pigmentada primária da adrenal; PRKAR1A

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PRIMARY PIGMENTED NODULAR ADRENOCORTICAL DISEASE (PPNAD) is a rare cause of ACTH-independent Cushing's syndrome (CS) caused by autonomously functioning nodules of the adrenals. PPNAD is associated with Carney complex (CNC) (1), a multiple neoplasia syndrome; occasionally it may present as a sporadic and isolated disorder. The recent molecular elucidation of CNC (2) allowed the generation of new hypotheses for PPNAD tumorigenesis. In this paper, we are going to review the PPNAD and its involvement in CNC.

CNC (OMIM 160980) (3) is a multiple neoplasia syndrome, inherited in an autosomal dominant manner, characterized by lentigines, cardiac myxomas, and endocrine tumors as PPNAD, large-cell calcifying Sertoli cell tumor (LCCSCT), GH-producing adenoma and thyroid carcinomas. CNC has been considered a multiple endocrine neoplasia syndrome because most CNC patients have more than one endocrine tumor (4,5). PPNAD is the most frequent and characteristic endocrine tumor observed in CNC (1). CNC is frequently observed in families (6); however, approximately 50% of the cases are without any family history (7,8). Two syndromes that had previously been described as LAMB (lentigines, atrial myxomas and blue nevi) (9) or NAME (nevi, atrial myxomas and ephelides) (10), represented variants of CNC (6,11).

Diagnostic criteria for CNC (table 1) have been reviewed recently (6). The presence of 2 (or more than 2) of the following manifestations, spotty skin pigmentation, cutaneous or mucosal myxoma, cardiac myxoma, breast myxoma, PPNAD, GH-producing adenoma, primarily large-cell calcifying Sertoli cell tumor (LCCSCT), thyroid carcinoma, psammomatous melanotic schwannoma, blue nevus, breast ductal adenoma and/or osteochondromyxoma, makes the diagnosis of CNC.

More than 25% of CNC patients present with manifestations of PPNAD. Even this number is probably an underestimate: PPNAD was observed in almost all CNC patients who underwent to autopsy (6). PPNAD involves both adrenal glands. The adrenals are usually normal in size but contain several dark brown micro-nodules (12). These nodules are non-encapsulated and the surrounding cortex is normal, atrophic or even hypertesrophic (13,14). In PPNAD from CNC patients, the nodular cells stain positively for synaptophysin by immunohistochemistry. This neuroendocrine marker, which in normal glands is prent only in medullary cells, suggest a neuroendocrine role in PPNAD development or de-differentiation of adrenocortical cells in PPNAD. Other neuroendocrine markers such as chromogranin A and tyrosine hydroxylase do not stain PPNAD nodules (15).

PPNAD occurs isolated, but than 90% of all cases are associated with CNC (16). Familial cases of isolated PPNAD have also been reported (2). These families had no other clinical feature, which could have suggested CNC; however, subtle manifestations of the disorder could have been missed (17,18). A minority of PPNAD patients present during the first 2–3 yr, whereas the majority manifest in the second and third decade of life (6).

PPNAD is a non-malignant lesion; however, surgery is indicated because the mortality among patients with PPNAD is the same as those associated with Cushing's syndrome (CS) (6). Patients with PPNAD should be followed by screening for CNC and its potentially fatal components (13,19). Clinically, patients with PPNAD may present with signs of classic CS (moon face, central obesity, hypertension, myopathy); however, other patients present with atypical signs of cyclical or subtle hypercortisolism and no real stigmata of CS. These patients may have osteopenia, osteoporosis, mood or psychiatric changes and are difficult to diagnose (20,21).

Liddle's test is the most accurate test for the diagnosis of PPNAD. An increase in urinary free cortisol excretion of more than 50% on day 6 of the test identifies almost all CNC patients with PPNAD. This paradoxical response is rarely observed in patients with other primary adrenal diseases (21). It has been suggested that this phenomenon of increasing cortisol excretion in PPNAD under dexamethasone stimulation is related to the primary lesion; in other words, it is not associated with unusual responses of the hypothalamic-pituitary axis. This paradoxical response was observed *in vitro* with PPNAD tissues and was correlated with increased glucocorticoid receptor expression (22).

Two genetic *loci* have been identified for CNC, on chromosome 2p16 (23,24) and chromosome 17q22-24 (25). This second *locus* harbors the gene encoding the regulatory subunit alpha of the protein kinase A (PRKAR1A), which has recently shown to be mutated in CNC patients (26,27). The gene located on chromosome region 2p16 is still unknown. There is no clear genotype-phenotype correlation among patients with CNC. This was well demonstrated when 2 families sharing the same mutation presented with clearly different clinical manifestations (26). Cytogenetic studies in tumor tissues from CNC patients revealed the involvement of chromosome 2p16 even in patients who mapped to chromosome 17q22-24 (28).

The regulatory subunit type 1 alpha (RIα) is involved in the protein kinase A (PKA) activity. PKA is the main mediator of cAMP signaling in mammals (29). PKA is a serine-threonine kinase and under hormone stimuli it promotes phosphorylation and plays a role in various cellular functions as DNA replication, cell division and cellular metabolism (30-32). There are four different regulatory subunits with tissue-specificity for 2 of them; RI? s the most abundant one with little, if any, tissue specificity. The PKA tetramer is, in fact, composed of two homodimers, one that contains two regulatory subunits and another that contains two catalytic subunits (29) (figure 1). The four genes that code for the various PKA regulatory subunits are grouped into two types, type I and type II. These define the two main isoforms of PKA, which were identified and named after their order of elution in chromatography (23). When cAMP molecule binds to the regulatory subunits, the PKA tetramer is dissolved in the dimmer of the regulatory subunits and the catalytic subunits. The latter phosphorylate nuclear and cytoplasmatic targets following their release from the tetramer (34). Type I PKA enzymes contain either regulatory subunit Ia or Ib; type II PKA enzymes contain either subunit IIa or IIb (35). Heterodimers may also form, but infrequently. *In vitro*, and in basal states, the catalytic subunits bind preferentially to regulatory subunits type II; type I subunits are favored in stimulated states (36).

The PRKAR1A gene has been considered a potential oncogene due to the observation of its high expression in several tumors and tumor-derived cell lines (37,38). In addition, downregulation of the PRKAR1A gene by antisense oligonucleotides leads to growth inhibition in human cancer cells (39). Over-expression of the PKA type I isoform interacts with activated epidermal growth factor receptor (EGF-R) and stimulates growth and proliferation through the mitogen activated protein kinase (MAPK) (40). It is known that under normal growth, the cAMP/PKA pathway is required for phosphorylation of the tyrosine kinase Src that, after activating Rap1 and blocking the activation of Raf-1 by Ras, inhibits cell proliferation (41). The PKA pathway activation by forskolin in CD10+B cell induces apoptosis and also decreases the expression of Mcl-1, an anti-apoptotic Bcl family member (42). Interestingly, the PRKAR1A gene was seen to be acting as a tumor suppressor gene in CNC: loss of heterozygosity is seen for the polymorphic markers within and surrounding the PRKAR1A gene in lesions from CNC patients (2).

The PRKAR1A gene is composed of 12 exons (1A, 1B, 2, 3, 4A, 4B, 5, 6, 7, 8, 9 and 10) and the start codon is located at the exon 2. Mutations in CNC patients are observed throughout the gene. There is high *de novo* incidence (27.3% of kindreds) of the 578delTG mutation (in exon 4B); followed by mutations in exons 2 and 6 (26). The "hot spot" in exon 4B may be explained by the presence of a TG repeat, which may lead to DNA polymerase "stuttering". Almost all mutations observed in CNC patients, lead to a premature stop codon and, consequently, result in a truncated regulatory 1 alpha subunit (R1A) protein (2,26). This abnormal R1a protein is not detected in CNC mutant patients (26), because the non-sense mRNA is degraded by the mechanism of nonsense mRNA decay (43). Thus, all mutations are functionally equivalent. They result in increased PKA activity (2); heterozygosity in CNC patients supports the notion that mutations of the regulatory subunits act in a dominant fashion (44).

Further studies have shown a shift between PKA type I and type II in Epstein Barr virus immortalized-transformed cells from CNC lymphocytes and also in a PPNAD from a CNC patient with a PRKAR1A mutation (45). Cell lines from CNC patients containing the PRKAR1A gene mutation showed increased ERK1/2 phosphorylation that was associated with increased cell proliferation. Thus, reversal of the usual PKA-mediated inhibition of a MAPK pathway in CNC cells may contribute to tumorigenesis (46).

In a PPNAD tissue (with PRKAR1A mutation) a higher level of CREB phosphorylation was seen (47). Immunostaining of PPNAD tissue with the PKA subunits revealed that in the mutated tissue, there is an irregular distribution of the subunits and lower amount of subunit R1A in the nodules (47). It was suggested that PRKAR1A might behave not as a classic tumor suppressor gene; over-expression of PRKAR1A and the other PKA subunits may be present in non-nodular tissue of mutated tumors and, thus, perhaps, precede nodular formation, underlining the complex way with which cAMP controls growth.

PRKAR1A gene mutations and 17q LOH were also observed in sporadic adrenocortical tumors; LOH was more frequently observed in adrenal malignancies than adenomas (48). Other abnormalities in the PKA pathway, as the loss of cAMP response element binding protein (CREB) expression has also been observed in adrenal carcinomas (49). The same pattern of higher LOH of 17q markers in malignant lesions was observed among thyroid tumors (50), but this feature was not seen with high frequency in pituitary adenomas (51). Higher activity of the PKA has also been shown to promote malignancy in other tissues, including breast tissue (52).

The second *locus* at 2p16 is currently under study. However, this specific region is not completely sequenced by the Human Genome Project. Other *loci* are also being investigated; however, 2p16 appears to be a significant *locus* for sporadic adrenal tumors, too (53).

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