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Carney complex and other conditions associated with micronodular adrenal hyperplasias

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Abstract

Carney complex (CNC) is a multiple neoplasia syndrome that is inherited in an autosomal dominant manner and is characterized by skin tumors and pigmented lesions, myxomas, schwannomas, and various endocrine tumors. Inactivating mutations of the *PRKARIA* gene coding for the regulatory type I- α (RI α) subunit of protein kinase A (PKA) are responsible for the disease in most CNC patients. The overall penetrance of CNC among *PRKARIA* mutation carriers is near 98%. Most *PRKARIA* mutations result in premature stop codon generation and lead to nonsense-mediated mRNA decay. CNC is genetically and clinically heterogeneous, with specific mutations providing some genotype-phenotype correlation. Phosphodiesterase-11A (the *PDE11A* gene) and -8B (the *PDE8B* gene) mutations were found in patients with isolated adrenal hyperplasia and Cushing syndrome, as well in patients with PPNAD. Recent evidences demonstrated that dysregulation of cAMP/PKA pathway can modulate other signaling pathways and contributes to adrenocortical tumorigenesis.

Keywords

Carney complex; PPNAD; *PRKARIA*; phosphodiesterase

Epidemiology

The complex of “spotty skin pigmentation, myxomas, endocrine overactivity, and schwannomas”, a disorder that is now known as Carney complex (CNC), is an autosomal dominant and multiple neoplasia syndrome, which was first described in 1985 (1–3). Isolated patients with some components of CNC had been previously diagnosed as NAME (nevi, atrial myxomas, and ephelides) and LAMB (lentigines, atrial myxoma and blue nevi) (4,5). Most of these cases, if not all, were most likely CNC which had not been described as such at the time.

To date, a total of 353 patients with CNC from 185 families have been described, including patients from all ethnicities and with a wide spectrum of clinical manifestations (6,7). Most

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of the patients (68%) had a family history consistent with CNC, whereas 113 cases (32%) had no known affected relatives and were classified as sporadic. A total of 221 patients (63%) were female. The median age at the detection is 20 years, although at least 5 patients were diagnosed at birth (3,8).

Clinical manifestations and diagnosis

A definite diagnosis of CNC is given if two or more major manifestations are present. A number of related clinical components may suggest the diagnosis of CNC, but are not considered diagnostic of the disease (Table 1) (7,9). Alternatively, the diagnosis may be made if one major criterion is present and a first-degree relative has CNC or an inactivating mutation of the gene encoding protein kinase A regulatory subunit 1 α (*PRKARIA*) (10).

Spotty skin pigmentation is the most common clinical manifestation (above 80%) of CNC (Table 2). Cutaneous manifestations constitute three of the major disease criteria: lentigines, cutaneous or mucosal myxomas; and blue nevi (multiple) or epithelioid blue nevus (9,11). Lentiginosis is one of the CNC features that can occur early, but usually acquire their typical intensity and distribution during the peripubertal period. They typically involve the lips, conjunctiva and inner or outer canthi, vaginal and penile mucosa (2).

Cardiac myxomas occurred in 32% of the patients (6). The median age of the first heart tumor was 50 yr, but tumors were detected as early as 3 yr of age and as late as 67 yr. Cardiac myxomas were frequently multicentric and may affect any or all cardiac chambers. Skin and breast myxomas occurred in 20% of the total, and in 20% of the female patients, respectively (6,7,12). Classic sites for skin myxomas included the eyelid, external ear canal, and nipple.

Primary pigmented nodular adrenocortical disease (PPNAD) is the most common endocrine tumor associated with CNC, occurring in 60% of the CNC patients (6). Isolated PPNAD is the only manifestation in 12% of the CNC patients. The median age at diagnosis is 34 yr and it is significantly more frequent in females (sex ratio 2.4:1). PPNAD has a bimodal distribution in CNC patients, a minority presenting during the first 2–3 yr, whereas most of patients are diagnosed at the second and third decades of life. Biochemical screening by a dexamethasone-stimulation test may detect additional patients with PPNAD-associated subclinical, atypical, or periodic Cushing's syndrome (13). Patients with PPNAD exhibit a paradoxical increase in cortisol secretion in response to dexamethasone through a glucocorticoid receptor-mediated effect on protein kinase A (PKA) catalytic subunits (14).

GH-producing pituitary adenomas affect 12% of the CNC patients. Although clinically evident acromegaly is not very frequent, asymptomatic elevation of GH and IGF-1 levels may be present in up to 75% of the patients (15,16). In the CNC consortium, thyroid tumors were diagnosed in 25% of the patients, with thyroid cancer (papillary or follicular or both) being found in nine cases (2.5%) (6). However, up to 75% of CNC patients may have multiple thyroid small hypoechoic lesions detected by ultra-sonography (17). Multicentric and bilateral testicular tumors [large-cell calcifying Sertoli cell tumor (LCCSCT)] are diagnosed in around 40% of the male patients. Testicular ultra-sonography identifies testicular microcalcifications in most of the affected patients with CNC (18).

Psammomatous melanotic schwannoma (PMS) is found in 8% of the patients and occurs mainly in the gastrointestinal tract and paraspinal sympathetic chain. Schwannomas in CNC are distinct from those that occur in the context of the Neurofibromatosis and familial schwannomatosis syndromes because of their pigmentation, calcification, and multicentricity (19).

Molecular genetics of CNC

Genetic linkage analysis revealed two different loci for CNC on chromosome 2p16 and 17q22–24 (20,21). Inactivating mutations of *PRKARIA* gene were identified in patients mapping to chromosome 17 (10,22). The affected gene on chromosome 2 has not been identified. To date, at least 117 pathogenic variants in *PRKARIA* have been identified (6,10,23). Cyclic AMP (cAMP) dependent PKA is the major mediator of the cAMP effects in the eukaryotic cells. In its inactive state, the PKA holoenzyme is a tetramer comprised of dimer of two regulatory subunits bound to two catalytic subunits (24,25). Upon elevated cellular cAMP concentrations, two molecules of cAMP bind each of the regulatory subunits, which leads to the dissociation of the tetramer and, thus, two free catalytic subunits (25). The best known function of the regulatory subunits *in vitro* is the inhibition of the catalytic subunit kinase activity. The free catalytic subunits can phosphorylate several cellular targets and cAMP-response element-binding protein, resulting in activation of transcription of cAMP-responsive element-containing genes (26).

As discussed above, the CNC consortium screened 353 patients with CNC; *PRKARIA* defects were found in 73% of these patients (6). The overall penetrance of CNC among *PRKARIA* mutation carriers was near 98% (7). The mutations in *PRKARIA* are spread along the whole coding sequence and most of them are unique. To date, only 3 mutations have been found in more than three unrelated families: c.82C>T, c.491_492delTG and c.709-7del6 (10,23,27). These three mutations were found in kindreds with different ethnic backgrounds. The majority of the mutations result in premature stop codon generation caused by nonsense and frameshift changes. Mutant mRNAs containing a premature stop codon are unstable, as a result of nonsense-mediated mRNA decay (NMD) (23). Thus, at the molecular level, most of mutations have the same effect: absence of detectable mutant protein and a reduction of RI α protein levels by 50% (23,28).

Germ-line *PRKARIA* deletions were found in two patients with sporadic CNC. In the first patient, the deletion was expected to lead to *PRKARIA* haploinsufficiency, consistent with the majority of *PRKARIA* mutations causing CNC. In the second patient, the deletion led to in-frame elimination of exon 3 and the expression of a shorter protein, lacking the primary site for interaction with the catalytic PKA subunit. *In vitro* transfection studies of this *PRKARIA* mutation showed impaired ability to bind cyclic AMP and a high PKA activity. The patient bearing this expressed *PRKARIA* mutation presented a more severe CNC-phenotype (29).

Recently, the pathogenic effects of seven missense substitutions in *PRKARIA* were investigated (30–32). These missense *PRKARIA* defects (c.26G>A, c.178_348del171, c.220C>T, c.438A>T, c.547G>T, c.638C>A, c.865G>T), whose mRNAs escaped NMD and lead to the expression of a mutant RI α protein, increase PKA activity and PKA-specific activation due to decreased binding of RI α to cAMP or the C subunit, as well as conformational changes that prevent normal enzymatic functioning (30).

Genotype-phenotype correlation

In general, the CNC consortium study showed that the patients with CNC can be separated in three main groups (Table 3). The first group includes patients who are *PRKARIA*-mutation carriers and who have at least two of the manifestations of the originally described triad of “myxomas, spotty skin pigmentation, and endocrine overactivity”. CNC patients with *PRKARIA* mutation presented with clinically disease at a younger age and had more myxomas, schwannomas, and thyroid and gonadal tumors than patients without *PRKARIA* mutations (6). In this group, the c.491–492delTG defect was more frequently associated with patients with cardiac myxomas, lentiginos, and thyroid tumors. Although a small

number of *PRKARIA* missense mutations were identified so far, the clinical follow-up of this subgroup suggests that expressed RI α mutant proteins are associated with a more severe and aggressive CNC phenotype (30,31).

The second group of CNC patients had no abnormalities of the *PRKARIA* gene and presented clinical manifestations later in life. Although most of these patients had sporadic disease, this group included families that collectively were mapped to chromosome 2, the CNC2 locus on 2p16 (20,23,33). There were no differences in the prevalence of lentigines and other pigmented skin lesions between this group and the patients with *PRKARIA* mutations (6).

The third group includes patients with isolated PPNAD, in some cases accompanied with lentiginosis. These patients were diagnosed before 8 yr of age and most of them were carriers of either the c.709-7del6 mutation or the c.1A>G / p.M1V substitution in the *PRKARIA* gene (33,34). Therefore, the association of these two *PRKARIA* mutations with PPNAD and mostly mild Cushing syndrome has important implications for genetic counseling.

Micronodular adrenocortical disease not associated with CNC

Most of the cases of PPNAD, inherited or sporadic, are associated with CNC. Among the remaining kindreds with micronodular adrenocortical disease and no evidence of *PRKARIA* mutation, clinical and histopathological criteria were applied to identify subgroups of patients (Table 4) (35). The genetic basis of isolated corticotropin-independent micronodular adrenocortical disease (MAD) is supported by its invariably bilateral appearance and very early disease onset. Recently, a genome-wide screen of 10 kindreds with Cushing syndrome and adrenocortical hyperplasia, who did not have *PRKARIA* mutations, identified a strong association between the disease and inactivating mutations in phosphodiesterase 11A (*PDE11A*). In most of these individuals, the adrenal glands had an overall normal size and multiple small yellow-to-dark brown nodules surrounded by a cortex with a uniform appearance. Microscopically, there was moderate diffuse cortical hyperplasia with mostly nonpigmented nodules. Although no pigmentation was detected by regular microscopy, electron microscopy did show granules of lipofuscin and features of a cortisol-producing adrenocortical hyperplasia. In other cases, histology was indistinguishable from that in PPNAD (36).

Five different *PDE11A* mutations were identified so far in the patients with isolated MAD or PPNAD; three of them resulted in premature stop codon generation and the other two were single base substitutions in the catalytic domain of the protein, significantly affecting the ability of *PDE11A* to degrade cAMP *in vitro* (36,37). Cyclic AMP and cGMP levels in adrenocortical tumors from individuals with inactivating *PDE11A* mutations were significantly elevated. *PDE11A* catalyzes the hydrolysis of both cAMP and cyclic GMP (cGMP) and is expressed in several endocrine tissues, including the adrenal cortex (38).

The chromosomal locus harboring the gene encoding phosphodiesterase 8B (*PDE8B*) was the second most likely region to be associated with a predisposition to MAD (36). In addition, *PDE8B* is also expressed in the adrenal gland, as compared with other genes of phosphodiesterases that degrade cAMP, including *PDE1A*, *PDE4A*, *PDE4B*, *PDE4C*, *PDE4D*, *PDE7A*, and *PDE9A* (36). Sequencing of the *PDE8B*-coding regions identified a single base substitution (c.914A>T, p.H305P) in a young girl with Cushing syndrome. The patient inherited the mutation from her father, who presented with a very mild-to-indistinguishable adrenocortical phenotype (39). *In vitro* studies indicated an impaired ability of the mutant protein to degrade cAMP.

Interaction between cAMP/PKA signaling and other pathways

RI α haploinsufficiency in human lymphocytes and mouse models causes an increase in total cAMP-stimulated kinase activity and enhances MAPK activity (31,40–42). In mutant-*PRKARIA*-containing tissues, any inhibition conferred by Akt is absent or decreased due to inactivation by a PKA complex with greater catalytic activity, which also stimulates B-raf kinase activity (42). Complete RI α deficiency causes constitutive PKA activation and immortalization of MEFs through up-regulation of cyclin D1 (43). Furthermore, E2F1 mediates proliferative effects of defective RI α in a human cell line harboring a *PRKARIA*-inactivating mutation (44). Recently, it was demonstrated that inactivating mutations of *PRKARIA* observed in adrenocortical tumors affect SMAD3 function, leading to resistance to TGF β -induced apoptosis (45).

Expression studies in massive macronodular adrenocortical disease and primary pigmented nodular adrenocortical disease (PPNAD) indicated the over-expression of genes involved in the Wnt pathway such as *WISP2*, β -catenin (*CTNNB1*), and glycogen synthase kinase-3 β (*GSK3B*) (46,47). Somatic mutations of the *CTNNB1* gene have been found in adrenal tumors from patients with PPNAD, Carney complex and germline *PRKARIA*-inactivating mutations (48,49). Recently, a microRNA profile analysis showed that PKA, via microRNA regulation, affects the Wnt signaling pathway in PPNAD (50). Our previous study demonstrated that mouse *Prkar1a* haploinsufficiency leads to an increase in tumors in the *Trp53*^{+/-} or *Rb1*^{+/-} backgrounds and chemically-induced skin papillomas by dysregulation of the cell cycle and Wnt signaling (51).

Conclusion

CNC is genetically and clinically heterogeneous. Although the most of cases are caused by *PRKARIA*-inactivating mutations, a genetic etiology has not been identified in 27% of the patients with CNC. Recently, an international CNC consortium studied the correlations between genotype and phenotype, providing important clinical data for genetic counseling of CNC patients and their families. Phosphodiesterase-11A (*PDE11A*) and -8B (*PDE8B*) mutations were found in patients with isolated adrenal hyperplasia and Cushing syndrome, as well in patients with PPNAD. PDE11A and PDE8B catalyze the hydrolysis of cAMP and are expressed in several endocrine tissues, including the adrenal cortex. These recent findings support the essential role of the cAMP/PKA pathway for adrenocortical physiology. Furthermore, dysregulation of this pathway can modulate other signaling pathways and contributes to adrenocortical tumorigenesis.

Practice points

- *PRKARIA* defects are present in 73% of CNC patients. The overall penetrance of CNC among *PRKARIA* mutation carriers is near 98%.
- The majority of the mutations result in premature stop codon generation caused by nonsense and frameshift changes.
- Patients with isolated PPNAD were diagnosed before 8 yr of age and most of them were carriers of either the c.709-7del6 mutation or the c.1A>G / p.M1V substitution in the *PRKARIA* gene.
- Genotype-phenotype correlation provides valuable clinical data for genetic counseling of CNC patients and their families.
- PDE11A mutations are associated with isolated MAD or PPNAD and significantly affect the ability of PDE11A to degrade cAMP *in vitro*.

- PDE8B-inactivating mutations are present in MAD and similarly affect cAMP handling by the enzyme.

Research agenda

- Genetic etiology remains to be determined in approximately one third of the patients with CNC.
- Since most of the *PRKARIA* mutations lead to NMD and produce no mutant protein, additional studies are needed to understand what molecular mechanisms may explain phenotypic differences.
- *PRKARIA* and mutations in PDE genes (*PDE11A* and *PDE8B*) represent a unique model that can be used to understand the function and complex interactions of the cAMP/PKA signaling pathway and may be exploited pharmacologically for the benefit of patients with CNC, PPNAD, MAD and related disorders.

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Table 1**Diagnostic criteria for CNC**

Major Diagnostic criteria
1. Spotty skin pigmentation with typical distribution (lips, conjunctiva and inner or outer canthi, vaginal and penile mucosa)
2. Myxoma [*] (cutaneous and mucosal)
3. Cardiac myxoma [*]
4. Breast myxomatosis [*] or fat-suppressed magnetic resonance imaging findings suggestive of this diagnosis
5. PPNAD [*] or paradoxical positive response of urinary glucocorticoid excretion to dexamethasone administration during Liddle's test
6. Acromegaly due to GH-producing adenoma [*]
7. LCCSCT [*] or characteristic calcification on testicular ultrasound
8. Thyroid carcinoma [*] or multiple, hypoechoic nodules on thyroid ultrasound in a young patient
9. Psammomatous melanotic schwannomas [*]
10. Blue nevus, epithelioid blue nevus [*]
11. Breast ductal adenoma [*]
12. Osteochondromyxoma [*]
Supplemental criteria
1. Affected first-degree relative
2. Inactivating mutation of the <i>PRKARIA</i> gene
Findings suggestive of or possibly associated with CNC, but not diagnostic for the disease
1. Intense freckling (without darkly pigmented spots or typical distribution)
2. Blue nevus, common type (if multiple)
3. Café-au-lait spots or other "birthmarks"
4. Elevated IGF-I levels, abnormal GTT, or paradoxical GH response to TRH testing in the absence of clinical acromegaly
5. Cardiomyopathy
6. Pilonidal sinus
7. History of Cushing's syndrome, acromegaly, or sudden death in extended family
8. Multiple skin tags or other skin lesions; lipomas
9. Colonic polyps (usually in association with acromegaly)
10. Hyperprolactinemia (usually mild and almost always combined with clinical or subclinical acromegaly)
11. Single, benign thyroid nodule in a young patient; multiple thyroid nodules in an older patient (detected on ultrasound)
12. Family history of carcinoma, in particular of the thyroid, colon, pancreas, and ovary; other multiple benign or malignant tumors.
[*] After histological confirmation

CNC, Carney complex; PPNAD, primary pigmented nodular adrenocortical disease; LCCSCT, large-cell calcifying Sertoli cell tumor; *PRKARIA* = protein kinase A regulatory subunit 1 α .

Table 2

Clinical manifestations of CNC in 353 patients

Manifestation	No. of patients	%
Spotty skin pigmentation	248	70
Cardiac myxoma	112	32
Skin myxoma	71	20
PPNAD	212	60
LCCSCT	54	41% of the males
Ovarian lesion	31	14% of the females
Acromegaly	42	12
Thyroid tumor	88	25
PMS	28	8

Carney complex; PPNAD, primary pigmented nodular adrenocortical disease; LCCSCT, large-cell calcifying Sertoli cell tumor; PMS, psammomatous melanotic schwannoma.

Table 3

Correlation between genotype and phenotype in CNC patients

CNC-patient groups	Phenotype	Correlation
<i>PRKARIA</i> mutation carriers (CNC1 patients)	Disease at a younger age and a higher frequency of myxomas, schwannomas, and thyroid and gonadal tumors than patients without <i>PRKARIA</i> mutations	c.491–492delTG mutation (cardiac myxomas, lentigines and, thyroid tumors) Expressed RI α mutant protein (more severe and aggressive CNC-phenotype)
Absence of <i>PRKARIA</i> mutation (CNC2 patients)	Sporadic disease later in life with a lower frequency of myxomas, schwannomas, thyroid and LCCSCT	CNC2 locus on 2p16, others
Isolated PPNAD associated with <i>PRKARIA</i> mutation	Isolated PPNAD (lentiginosis in few cases) early in life (before 8 yr)	c.709-7del6 mutation c.1A>G / p.M1V substitution

CNC, Carney complex; PPNAD, primary pigmented nodular adrenocortical disease; *PRKARIA*, protein kinase A regulatory subunit 1 α .

Table 4

Micronodular adrenocortical disease (multiple nodules, most < 1cm diameter) associated or not with CNC

Disease	Epidemiology	Description	Genes
iPPNAD	Children and young adults	Microadenomatous hyperplasia with at least some pigment	<i>PRKARIA</i> , <i>PDE11A</i> , <i>PDE8B</i> , 2p16, others
C-PPNAD	Children, young and middle aged adults	Microadenomatous hyperplasia with (mostly) internodular atrophy and pigment	<i>PRKARIA</i> , 2p16, others
MAD	Mostly children and young adults	Microadenomatous hyperplasia with internodular hyperplasia and limited or absent pigment	<i>PDE11A</i> , 2p12-p16, 5q, others

CNC, Carney complex; iPPNAD, isolated primary pigmented nodular adrenocortical disease; C-PPNAD, primary pigmented nodular adrenocortical disease associated with Carney complex; MAD, adrenocortical disease; PDE = phosphodiesterase; *PDE11A* = the gene for PDE11A; *PDE8B* = the gene for PDE8B, *PRKARIA* = protein kinase A regulatory subunit 1 α .