

## CARNEY COMPLEX

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### ABSTRACT

Carney complex (CNC) is a rare dominantly inherited syndrome of multiple neoplasias combined with cardio-cutaneous manifestations. Approximately 70% of index cases have a familial history, while the remaining 30% have a *de novo* germline mutation. Hitherto, two loci have been principally involved in the genetics of CNC: the CNC1 gene, located on 17q22-24, which is coding the regulatory subunit (R1a) of the protein kinase A (PRKAR1A) and is responsible for 2/3 of cases, whereas the putative “CNC2” gene at the 2p16 locus has not been identified as yet. As most of the identified PRKAR1A mutations are nonsense and lead to a lack of detectable mutant protein, no genotype-phenotype correlations are generally observed. Cutaneous lesions (lentigines, nevi, and myxomas), although with minimal clinical impact, are the most common and occasionally specific findings, assisting early diagnosis. Cardiac myxomas show an atypical presentation and contribute substantially to mortality. Among several associated endocrine neoplasias, Primary Pigmented Nodular Adrenal Dysplasia is the one most frequently observed, followed by thyroid nodules, somatomammotrope adenomas, and testicular tumors. The diagnosis is principally set by 12 major clinical criteria and 2

supplemental criteria, including molecular testing and family history. Molecular testing, which has a mutation detection rate of approximately 60%, cannot currently be recommended for all patients. If testing is performed and a mutation is detected, genetic screening is recommended for first-degree relatives. Surveillance for all the manifestations of CNC should be performed at least annually, starting in infancy. As CNC is generated by a constitutional genetic defect, no etiologic therapy is available yet. The therapeutic approach should target each clinical manifestation and treat accordingly.

### INTRODUCTION - HISTORICAL OVERVIEW

Carney complex (CNC - Online Mendelian Inheritance in Man 160980, 608837) is a dominantly inherited syndrome of multiple neoplasias combined with cardiocutaneous manifestations. The neoplastic lesions are both endocrine (adrenal, pituitary, thyroid, testicular tumors) and non-endocrine (myxomas, schwannomas). The skin lesions are divided into two major types: a) pigmented such as lentigines and blue nevi that can be observed on the face, neck, and trunk and b) not pigmented such as cutaneous myxomas (1) (Figure 1).



**Figure 1. Spotty pigmentation of the face. With permission <http://ugen.nichd.nih.gov>.**

This syndrome was first described by J. Carney in 1985, as “the complex of myxomas, spotty pigmentation, and endocrine overactivity”. In the original study, 40 patients were included, and a familial distribution was reported in 10 of them. Additional evidence for unifying this coexistence of otherwise rare conditions in an inherited clinical entity was the young age at presentation and the unusual type of involvement of most affected sites, which tended to be multicentric (heart and skin) and bilateral in paired organs (adrenal, breast, and testis) (2). One year later Carney reported observations consistent with Mendelian dominant inheritance of the syndrome (3) that in the meanwhile was designated as “Carney complex” (CNC) by Bain (4). This new entity included patients manifesting cardiocutaneous lesions, previously diagnosed as LAMB (lentigines, atrial myxoma, mucocutaneous myxoma, blue nevi) (5) and

NAME (nevi, atrial myxoma, myxoid neurofibroma, ephelides) (6).

In 1996, linkage analysis studies by Stratakis et al. (7) demonstrated a locus potentially linked to CNC on chromosome 2p16, in proximity to the gene encoding proopiomelanocortin and the DNA-mismatch repair genes *hMSH2* and *hMSH6*. However, the syndrome was later shown to be genetically heterogeneous (8), and in 1998, a second possible locus located on chromosome 17q2 was detected (9). In 2000, two different research teams demonstrated that germline mutations in the gene coding the alpha regulatory subunit (R1a) of protein kinase A (PKAR1A) located on the locus 17q22-24 were responsible for several phenotypes of CNC (10,11). More recently, mutations of other genes, which encode the catalytic subunit  $\alpha$

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or  $\beta$  of the PKA, and phosphodiesterases 11A and 8B have been reported in CNC patients (1).

Nowadays, diagnosis of the syndrome is feasible in clinically asymptomatic patients by commercially available molecular genetic assays and next-generation sequencing techniques. Notably, Carney's complex should not be confused with Carney's triad, a completely different entity consisting of the triad of gastric leiomyosarcoma, pulmonary chondroma, and extra-adrenal paraganglioma.

## EPIDEMIOLOGY AND INHERITANCE

Carney's complex is a rare disease and the majority of cases have been registered by the NIH-Mayo Clinic (USA) and the Cochin Center (Paris, France) consortium (12,13). Approximately 70% of individuals diagnosed with CNC have a familial history, while the remaining 30% present as a *de novo* germline mutation. In large series, a predilection of female over male gender has been observed (63% vs. 37% respectively), whereas there is no apparent predilection concerning ethnicity (14).

CNC is inherited as a dominant trait, although transmission through a female affected parent is almost 5-fold more frequent than the male. A possible explanation for this discrepancy might be the fact that male patients often harbor Large Cell Calcified Sertoli Cell Tumors (LCCSCT), which may hamper fertility (15). Moreover, data from animal models correlate haploinsufficiency at the *PRKR1A* gene locus with male infertility, independently of LCCSCT (16). The median age of diagnosis is 20 years; however, in many cases, a diagnostic delay of decades is reported. In general, the penetrance of CNC is 70%-80% by the

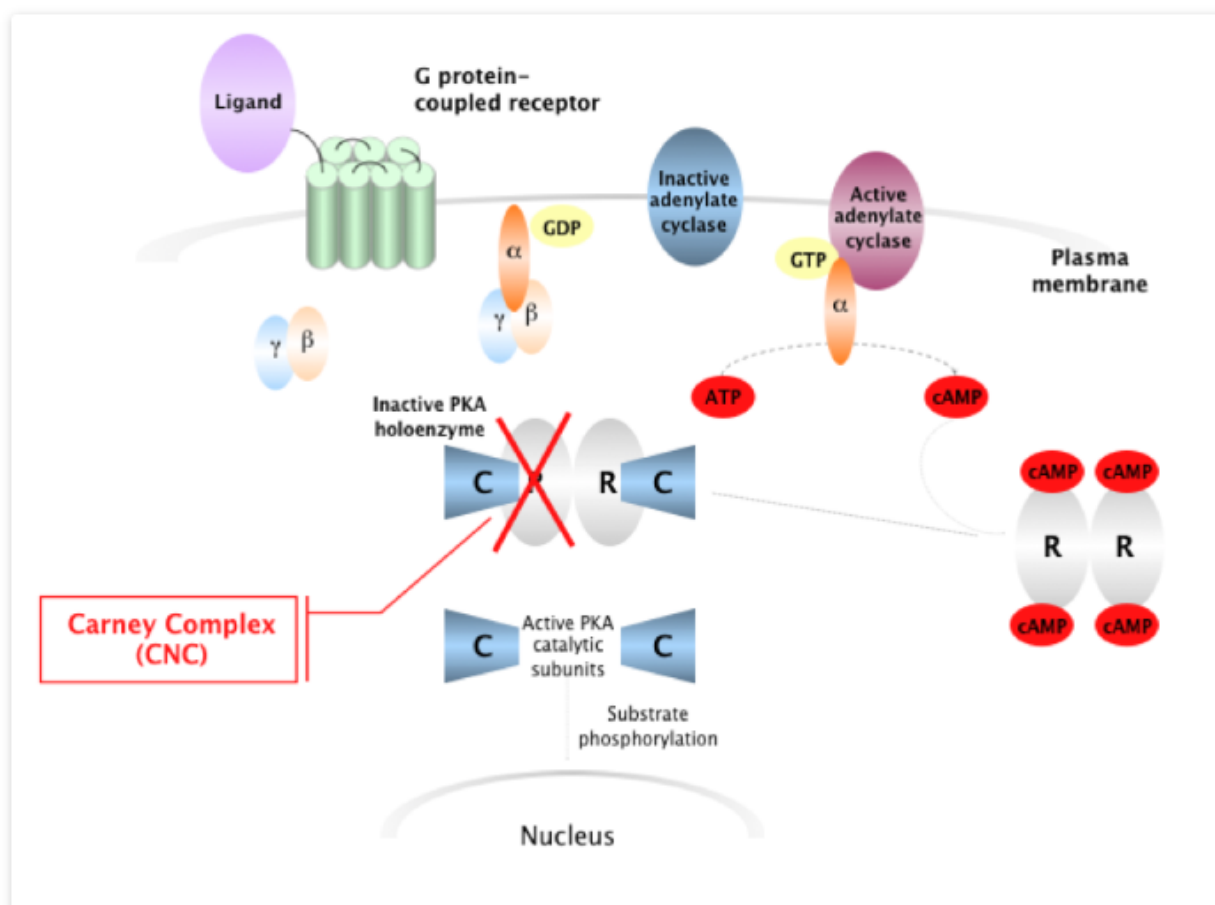
age of 40 years, as clinical manifestations accumulate during the lifespan. The maximum number of affected generations reported in one kindred is 5 (9).

## MOLECULAR GENETICS AND PATHOPHYSIOLOGY

Hitherto, two loci have been principally involved in the genetics of CNC: 17q22-24 and 2p16.

### CNC1 Gene

The *CNC1* gene, located on 17q22-24, is 21 kb long and contains 11 exons, coding the regulatory subunit (R1a) of the protein kinase A (*PRKR1A*), a protein of 384 amino acids (17). Protein Kinase A (PKA) is an enzyme involved in G protein-coupled intracellular pathways and serves as a mediator of c-AMP actions promoting cell metabolism, proliferation, and apoptosis. Its quaternary structure consists of 4 peptide chains that form a tetramer of two regulatory (R) subunits, each bound to one catalytic (C) subunit (18). So far four subtypes of regulatory (RI $\alpha$ , RI $\beta$ , RII $\alpha$ , and RII $\beta$ ) and four subtypes of catalytic subunits (C $\alpha$ , C $\beta$ , C $\gamma$ , and Prkx) have been identified. A corresponding gene is coding each R (*PRKR1A*, *PRKR1B*, *PRKR2A*, *PRKR2B*) and each C subunit (*PRKACA*, *PRKACB*, *PRKACG*, *PRKX*) respectively (19). When c-AMP binds to the regulatory subunits, their conformation is altered, causing the dissociation of each active C subunit from the dimer with the corresponding R subunit. The free catalytic subunits then phosphorylate serine and threonine residues of proteins critical to the activation of downstream processes, such as cAMP response-binding protein (CREB) (Figure 2).



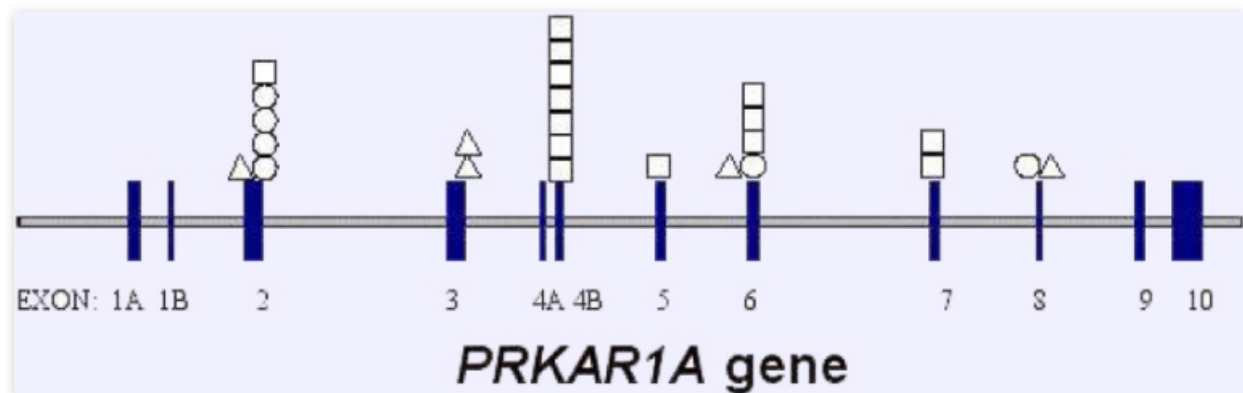
**Figure 2. The G protein-coupled intracellular pathways and the defect in CNC patients: PRKAR1A mutations result in deficient / inefficient regulatory subunits, resulting in constitutional activation of C subunits (<http://prkar1a.nichd.nih.gov>).**

Heterozygous inactivating mutations of PRKAR1A have been detected in more than 70% of affected individuals. Interestingly, in patients presenting with Cushing's syndrome (CS) this frequency rises to about 80%. (20). Up to date, 140 different PRKAR1A mutations have been registered at the CNC consortium database (<http://prkar1a.nichd.nih.gov>), and they are distributed among the 11 exons of the PRKAR1A gene, showing a predilection for exons 2, 3, 5 and 7, which are more often mutated, whereas exon 1, is non-coding and rarely mutated. Most of them are family or patient-specific; however, certain hot-spot mutations have been identified such as the c.709-7del6 in intron 7, c.491-492delTG in exon 5, and c.82C > T in exon 2 (21).

The penetrance for CNC due to PRKAR1A mutations is higher than that encountered in CNC due to other genetic defects, reaching 98% (12). The vast majority of mutations (83%) lead to a premature stop codon (nonsense) and thus, short mutant mRNAs that are eliminated by selective degradation, a phenomenon known as nonsense-mediated mRNA decay (NMD) (17). The result is a lack of detectable mutant protein and a reduction of R1α protein levels by 50%. The rest of the mutations (17%) result in the expression of an altered protein (missense) that may be associated with more severe phenotypes (22). Large PRKAR1A deletions have also been detected in a proportion of CNC patients, who also express a more severe phenotype with unusual features. These deletions are more prominent (21.6%) among patients negative by

conventional Sanger sequencing, rendering array-based studies necessary for diagnostic confirmation of such cases (23). The structure of the PRKR1A gene

and the location of detected mutations are shown in Figure 3.



**Figure 3. Schematic presentation of the PRKR1A gene and detected mutations in relation to their exon location.**

Germline haploinsufficiency of PRKR1A leads to a deficiency of the R1a subunits, which in turn results in enhanced intracellular signaling by PKA due to unhindered activation of the catalytic (C) subunits, as evidenced by an almost 2-fold greater response to c-AMP in CNC tumors and cell lines (24,25). How this PKA overactivity leads to tumor development has not been fully elucidated. According to previous studies, PKA-enhanced activity may trigger pathways that favor cell proliferation as the upregulation of D-type cyclins (26) or activation of the mTOR pathway (27). Recent studies in adrenocortical cell lines have confirmed the accumulation of cyclin D1 and further suggest Bcl-xL upregulation, which is associated with resistance to apoptosis (28). Consistent with the Knudson two-hit model of hereditary tumorigenesis, PRKR1A haploinsufficiency (first hit) has been considered as a predisposition for tumorigenesis, which when combined with loss of heterozygosity (LOH) at 17q22-24 (second hit), may lead to the development of tumors in CNC patients (29). Interestingly, tumors that do not present inactivation of the remaining wild-type allele have also been described, implying that the coexistence of PRKR1A haploinsufficiency with defects of other tumor suppressor genes or proto-oncogenes may act synergistic for tumorigenesis (30). Accordingly, activating somatic mutations of the beta-catenin gene (CTNNB1) have been detected in adrenocortical tumors of CNC patients, carriers of a PRKR1A mutation (31).

These findings were supported by experiments on Prkar1a +/- knockout mice, the genotypic animal model of Carney's complex. These mice were developed by inserting an antisense transgene for Prkar1a exon 2 and present with many of the manifestations of CNC, such as adrenocortical hyperplasia with cortisol hypersecretion, thyroid follicular neoplasia non-pigmented schwannomas and bone lesions (32). On the contrary, mice with complete loss of Prkar1a were not viable as this genotype leads to early embryonic demise due to failure of heart tube development (33). Eventually, the development of thyroid and pituitary cell tumors as well as heart myxomas was achieved by inducing tissue-specific complete ablation of Prkar1a (34,35). Moreover, mice double heterozygote for Prkar1a and Trp53 or Rb1 developed more sarcomas and grew more, and larger pituitary and thyroid tumors compared to the single Prkar1a heterozygotes (36).



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## Other Loci

Approximately 30% of the families affected with CNC are not related to defective PRKAR1A. The putative “CNC2” gene located at the 2p16 locus is linked to the majority of them; nevertheless, it has not yet been identified. These patients present with a milder phenotype, they are diagnosed later in life and are usually sporadic cases. Initial studies demonstrated amplification of a 10 Mb region at the 2p16–23 locus in PRKAR1A-negative CNC patients. Moreover, somatic alterations of the 2p16 region have been reported in CNC tumors which are usually gene amplifications, whereas, tumor-specific LOH has not been a consistent feature of CNC2 (37). These data suggested that the gene located at 2p16 is a potential oncogene that may code a PKA catalytic subunit.

Recently, alterations of PRKACA and PRKACB resulting in a gain of function of the catalytic subunits  $\alpha$  and  $\beta$  of PKA respectively, have been associated with components of CNC (38,39). Similarly, inactivating mutations of the phosphodiesterase 11A (PDE11A) gene (located at 2q31.2) and PDE8B, which result in augmented cAMP signaling, have been demonstrated in isolated PPNAD patients (40,41), while CNC patients present a high frequency of PDE11A sequence variants (42).

## GENOTYPE AND PHENOTYPE CORRELATIONS

Efforts have been made to relate specific phenotypes to corresponding genotypes. A study analyzing 353 patients and 80 different genotypes demonstrated that individuals carrying a PRKAR1A mutation tended to present manifestations earlier and were more likely to have pigmentary disorders, myxomas, and thyroid as well as gonadal tumors. Mutations located in exons were more often associated with acromegaly, myxomas, lentigines, and schwannomas, while intronic mutations had a less serious phenotype (21). As most of the identified PRKAR1A mutations are nonsense and lead to a lack of detectable mutant protein due to NMD, no genotype-phenotype correlations are expected to be seen. However, specific hot-spot mutations show some genotype-phenotype correlation, such as c.709-7del6, which is associated with the development of PPNAD and c.491-492del, which is frequently associated with cardiac myxomas, lentigines, and thyroid tumors. Regarding those few missense mutations that lead to the expression of a mutant protein, they are related to more severe forms of CNC syndrome, suggesting that NMD may play a protective role against the deleterious effects of mutant products (43).

## CLINICAL MANIFESTATIONS

Carney's complex is a constellation of clinical manifestations that shows significant variability between patients, even among members of the same family. Some of these features are quite specific, like PPNAD, while others are not, such as thyroid nodules or blue nevi (1). The maximum number of conditions reported to be present together in a single patient is five. Skin disorders are the most common, followed by cardiac myxomas and PPNAD (44,45). These data are summarized in Table 1.

<b>Table 1. Clinical Manifestations of CNC</b>			
	<b>A</b>	<b>B1</b>	<b>B2</b>
Adrenal			
PPNAD	26.0	54.3	57.1
Possible PPNAD			11.4
Cardiac myxoma	53.0	18.6	22.9
Skin		37.1	60.0
Lentigines	77.0	30.0	55.7
Skin myxomas	33.0	14.3	20.0
Blue nevi		11.4	17.1
Pituitary			
Certain hypersomatotropism	10.0	8.6	18.6
Possible hypersomatotropism		-	30.0
Multiple thyroid nodules or carcinoma	5.0	7.1	11.4
Testicular calcifications/LCCST	33.0	20.0	35.0
Breast			
Benign lesions	3.0		42.0
Myxomatosis		2.0	0
Adenoma		4.0	0
ACR 2-3		n/a	0
Carcinoma		6.0	10.0
Schwannomas	10.0		
Confirmed by histology		4.3	4.3
Suspected		0	5.7
Osteochondromyxomas			
Confirmed by histology		2.9	2.9
Suspected		0	2.9

A) at the time of presentation among 338 patients from an older study (44) and B) from a recent prospective study including 70 patients at the time of presentation (B1), and after three years of follow-up (B2); (45)

Most often clinical signs appear in the teen years and early adulthood, with a median age of diagnosis at 20 years of age, while, evidence of the disease, especially cutaneous lesions, can be found even in newborns. During infancy, the most common tumors encountered are cardiac and cutaneous myxomas, as well as PPNAD, while LCCST and thyroid nodules appear somewhat later. Acromegaly is clinically evident during the third and fourth decade of life, while cardiac myxomas are equally distributed during the life span (46).

The average historic adjusted life expectancy of CNC patients has been reported to be 50-55 years, principally due to individuals who succumb from early cardiovascular sudden death: complications due to cardiac myxoma (myxoma emboli, cardiomyopathy, cardiac arrhythmia, surgical intervention) comprise the major factor of mortality for CNC patients (43). Other less important factors are metastatic or intracranial PMS, thyroid carcinomas, and metastatic pancreatic and testicular tumors (1,44).

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## Cutaneous Pigmentary Disorders

These lesions may appear either as multiple lentigines or as blue nevi. They may be present at birth; however, they acquire their typical intensity and distribution around puberty when they increase in number and appear anywhere on the body. Typically, they fade after the fourth decade, although they have been reported in individuals as old as 70 years. Occasionally café au lait spots and depigmented lesions may also be observed (47).

### LENTIGINES

They are the most common cutaneous manifestation of CNC patients (70-75%) and usually present as multiple small (0.2 to 2 mm) brown to black macules that can practically appear on any part of the body with areas of confluence and foci of deeper pigmentation. They are typically located around the orifices of the body, such as on the vermilion border of the lips, on the eyelids, ears, and the genital area (Figure 1). Macroscopically, lentigines are flat, poorly circumscribed macules, though in African-Americans, they may be slightly raised, similar to nevi. They may look like solar lentigines; however, they differ as they develop predominantly in areas that have not been exposed to sunlight (e.g., genitalia) and do not change with sun exposure. Histologically, the hyperpigmentation of CNC lesions is associated with melanocytic hyperplasia and hypertrophy, rather than increased melanin production as observed in solar lentigines (47).

### BLUE NEVI

These are larger lesions (up to 8 mm), blue to black, and dome-shaped. They are less common than

lentigines but still represent the second most frequent skin manifestation in CNC. They may be multiple with a variable distribution. Histologically, they may present features of epithelioid, junctional, or even compound nevi. Epithelioid blue nevi, currently known as Pigmented Epithelioid Melanocytomas, comprise a class of uncommon melanocytic tumors of intermediate malignancy, which may frequently present lymph nodes metastasis but rarely disseminate to distant organs (48).

## Myxomas

### CUTANEOUS MYXOMAS

The skin myxomas present as non-pigmented subcutaneous nodules with a smooth surface and may look white, flesh-colored, opalescent, or pink (Figure 4). They are generally asymptomatic and appear up to the fourth decade. Myxomas can emerge on the face and trunk, while typical sights in CNC are the eyelids (the most common site), external ear canal, and nipples. Less common sites of myxoma formation include the oropharynx (tongue, hard palate, and pharynx) and the female genital tract (uterus, cervix, and vagina). Interestingly, hands and feet are preserved (47). Clinical diagnosis is quite difficult as they are often confused with common “skin tags” and other overgrowths, thus histological confirmation is usually required. Lesions can be localized to the upper dermis and subcutis and consist of polygonal to stellate dermal fibroblasts scattered singly or in non-encapsulated clusters against an abundant basophilic myxoid matrix (2). Although cutaneous myxomas have minimal impact on the clinical course of CNC, their recognition is crucial since they are the most specific manifestation of CNC and may herald the presence of a potentially fatal cardiac myxoma (49)





**Figure 4. Cutaneous myxoma on the right flank of a CNC patient. With permission from Dermatology Online Journal 2004; 10 (3): 11.**

## CARDIAC MYXOMAS

Although these tumors are benign, they are responsible for the majority of deaths (>50%) related to CNC mainly due to cardiovascular complications. In a recent prospective study of 319 CNC patients, 42.6% developed cardiac myxomas and the mean age at diagnosis was in the 3<sup>rd</sup> decade of life, occasionally presenting as early as at 4 years. The risk of developing cardiac myxomas was elevated among patients already presenting thyroid lesions or breast myxomas. They can develop in any cardiac chamber, with a predilection in the left atrial septum, while they may be multiple and recurrent, therefore, their resection cannot guarantee a permanent cure. Almost half of the patients harboring a cardiac myxoma will

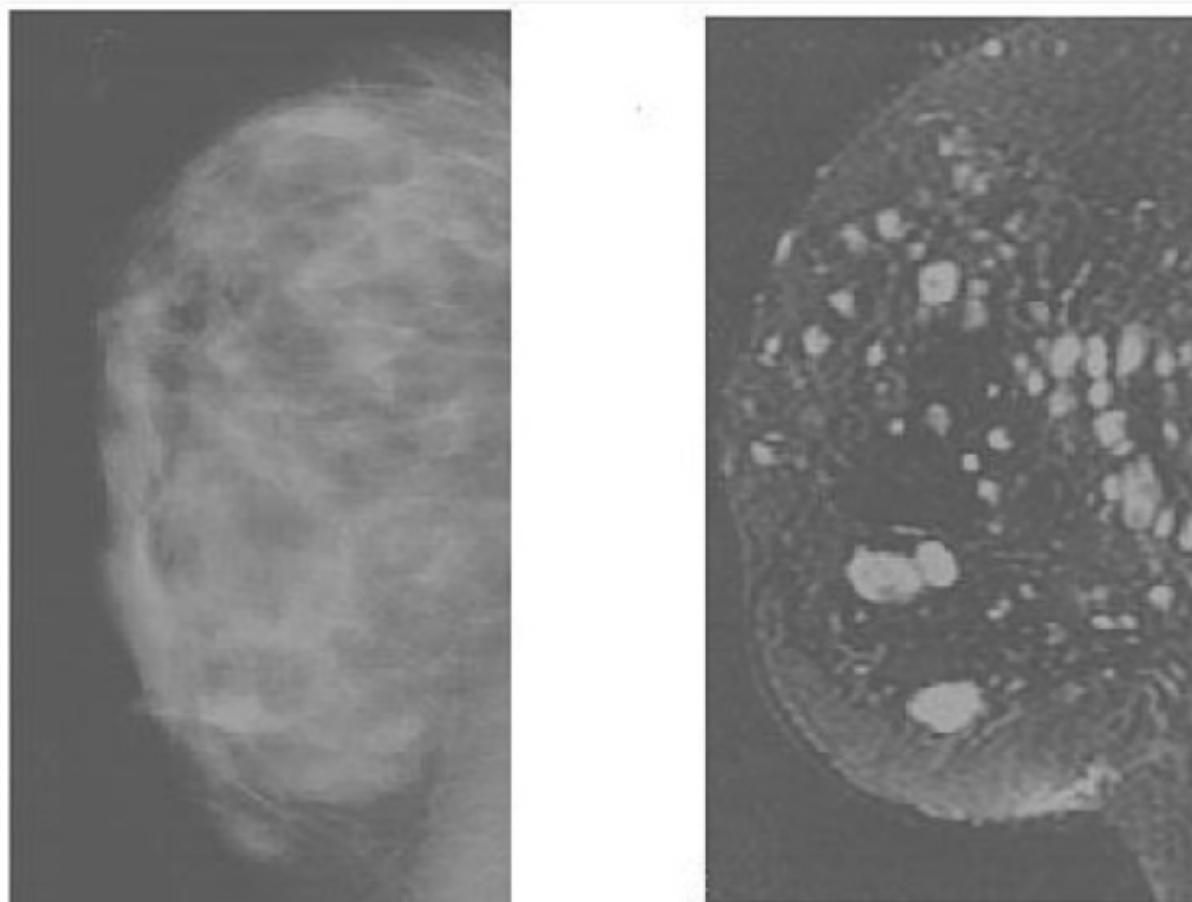
experience recurrence, and the risk is increased among women, lasting up to 20 years after the initial detection (49). Of notice, the detection of an apparently sporadic cardiac myxoma should alert the physician, as CNC-associated myxomas represent a significant proportion (7%) of these rare tumors. However, there are significant differences between cardiac myxomas in CNC and their sporadic counterparts regarding their epidemiology, distribution, and biological behavior. Sporadic myxomas emerge most commonly in middle-aged women and are almost exclusively localized at the left atrial aspect of the interatrial septum, at the fossa ovalis. In addition, most of them are cured by surgical resection and do not recur (50).

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Heart myxomas typically present with a triad of symptoms: a) Symptoms related to myxoma embolization (e.g., stroke, peripheral artery occlusions), b) Heart failure due to reduced cardiac output (complete occlusion of a valvular orifice can lead to sudden death) c) Constitutional symptoms (emaciation, recurrent fevers) probably related to production of cytokines [e.g., interleukin (IL-6)], by the tumor (51). Their size ranges from a few millimeters to 8 cm in diameter and can be partially calcified. They can be depicted sonographically as isoechoic (compared with the heart wall) masses inside the cardiac chambers. They can be studied further with magnetic resonance imaging (MRI), where they appear as hyperintense lesions on T2-weighted images (52). Histologically, the tumors have a gelatinous or hemorrhagic appearance and arise from a population of multipotent subendocardial mesenchymal precursor cells (53)

## BREAST MYXOMAS (MYXOID FIBROADENOMAS)

These lesions are observed in about a fifth of women with Carney complex and are generally considered benign breast tumors. They usually occur in females after puberty and can be multicentric as well as bilateral (Figure 5). Their size ranges from 2mm to 2cm in diameter and may be pink or white with a mucoid appearance. Physical examination of the breast is indicative of diffuse nodularity without dominant masses. Nipple discharge, breast skin abnormalities, or sentinel lymphadenopathy are not features of breast myxomas (54). Histologically, breast myxomas appear as lobulated mesenchymal lesions, characterized by accumulations of large amounts of ground substance in the lobules, as well as in the interlobular stroma. The tumors may or may not be encapsulated (2).



**Figure 5. Breast multiple myxomas in a patient with Carney complex. Mammogram (A), shows typical dense breasts in a younger woman with no evidence of tumor. However, in the fat-suppressed magnetic resonance image (B) shown on the right, the presence of multiple small myxomas is clearly seen. With permission <http://ugen.nichd.nih.gov>.**

When detected in mammography, they appear as well-defined, non-calcified, isodense, or hypodense lesions. Occasionally, they may have an irregular contour, a worrisome finding that warrants fine-needle aspiration (FNA), even in proven CNC patients. However, the imaging modality of choice is MR mammography as it has greater sensitivity compared to ultrasonography or conventional mammography. The number of myxoid lesions depicted with this technique is usually numerous (more than 58 per breast in a case) and shows a homogeneous increase of the signal intensity, a situation characteristic of CNC, also referred to as “breast myxomatosis” (52).

## OSTEOCHONDROMYXOMAS

Osteochondromyxomas or Carney bone tumors are rare myxomatous tumors of the bone that principally affect nasal sinuses and long bones. They have been described in a few cases (1-5%) and exhibit benign behavior; however, they can occasionally cause bone erosion and extend into soft tissues (55). Radiologically they can present as osteolytic lesions with aggressive periosteal new bone formation or as an expansive bone area with mixed sclerotic and lucent regions). These lesions often get the characteristic appearance of “ring sign”, which is

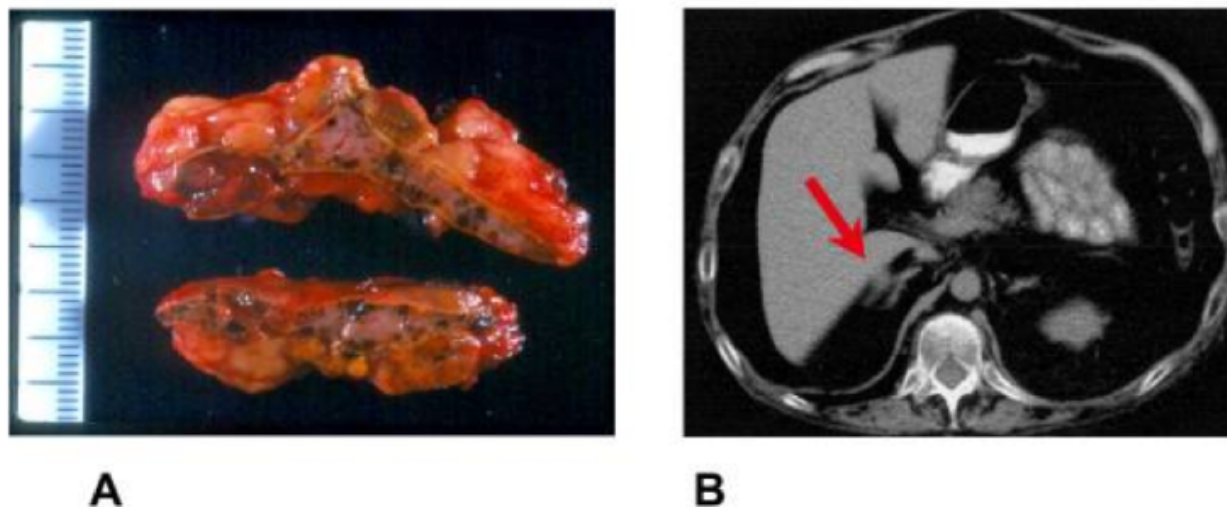
evident in plain radiographs, computed tomography, and MRI and are quite specific for CNC (56). Complete resection of the tumor is usually curative. Experiments in rodents demonstrated the osteoblastic origin of the lesion and that the knockdown of PRKAR1A disrupts the differentiation of osteoblasts (56,57).

### Endocrine Tumors and Overactivity

#### PPNAD (PRIMARY PIGMENTED NODULAR ADRENAL DYSPLASIA) PPNAD

This is the endocrine disorder most frequently observed in individuals with CNC. It bilaterally affects the adrenal glands and, according to recent data, can cause clinically overt Cushing's Syndrome in more than 50% of patients with CNC (45). However, autopsy studies have provided histological evidence that PPNAD is present in almost every CNC individual (2).

In 12% of the CNC patients, isolated PPNAD is the only manifestation. A bimodal age distribution is observed: a first peak occurs during infancy, while a second one that includes the majority of cases takes place between the second and third decade of life. The median age at diagnosis is 34 years and it is predominantly observed in females (sex ratio 2.4:1) (44). Histologically, the adrenal cortex is dominated by small pigmented micronodules with an average size of less than 10mm (Figure 6). Although not encapsulated, the nodules are sharply demarcated from the remainder of the cortex and most of them appear to originate deep in the cortex almost at the level of the medulla. A brown-pigmented substance, lipofuscin, is contained in many of the tumor cells and is responsible for the characteristic color of the lesions. Interestingly, tumor cells stain positively for neuroendocrine markers (e.g., Synaptophysin), while normal cortical cells don't (58). Internodular cortical atrophy is typical, thus the overall weight of the adrenal gland remains more or less normal (2).



**Figure 6. Macroscopic and CT-scan findings in primary pigmented nodular adrenocortical disease (PPNAD). A: Macroscopic appearance of the adrenal gland where multiple pigmented micronodules are evident at the cut surface. B: Adrenal CT scan revealed a micronodule on the external limb of the left adrenal (see red arrow). Copyright © 2006 Bertherat; licensee BioMed Central Ltd.**

Radiological and scintigraphic findings are not specific, since the adrenals may appear bilaterally or unilaterally enlarged but in most cases, they appear

normal (59). Computed Tomography (CT) is the most appropriate examination for depicting adrenal lesions in PPNAD. Particularly, images obtained with a slice

thickness of 3 mm or less, before and after intravenous (IV) injection of contrast are preferable as they might reveal subtle contour irregularities and the presence of hypodense spots that correspond to small, pigmented nodules. The characteristic picture is that of “beads on a string” (52). The type of hypercortisolism observed in this disorder is that of ACTH-independent adrenal hyperfunction. However, demonstrating cortisol overproduction can be challenging because it may develop progressively over the years. Moreover, intermittent, or cyclic forms of hypercortisolism have been reported (60). Clinical manifestations are non-specific and similar to those observed in patients with Cushing syndrome (CS) of other etiologies (central obesity, hypertension, myopathy), with a predisposition to osteoporosis. A 6-day Liddle’s test (low dose dexamethasone for 2 days followed by high dose dexamethasone for 2 days) has been used for the distinction of PPNAD from CS caused by other primary adrenal disorders (61). A paradoxical increase of UFC and/or 17-hydroxysteroids of more than 50% on the second day after high-dose dexamethasone administration is indicative of PPNAD. However, recent reports have argued against the utility of Liddle’s test by demonstrating low sensitivity (39%) and specificity (45). Initial screening with overnight dexamethasone suppression test and urine free cortisol is suggested instead.

Notably, reports from independent groups describe the development of adrenocortical cancer (ACC) in the context of CNC (62,63). In both reports, the patients carried PRKAR1A mutations and ACC developed on the background of PPNAD. This observation together with previous reports of benign macronodules (between 1 and 3.5 cm) in adrenal glands affected with PPNAD implies a continuum of tumorigenesis from adrenal hyperplasia to benign nodules, and then cancer, associated with alterations in other tumor suppressor genes apart from PRKAR1A (64).

#### GROWTH HORMONE (GH)-SECRETING PITUITARY ADENOMAS (ACROMEGALY)

Clinically evident acromegaly due to a pituitary GH-secreting tumor has been observed in approximately 10-12% of patients with CNC, whereas, gigantism, resulting from excessive GH secretion prior to puberty, is quite rare (44). Data from a recent prospective study, raise this figure to 18%, with a median age at diagnosis 34.5 years (45).

The usual underlying pathology is a solitary pituitary adenoma, while cases of multiple adenomas or even diffuse somatomammotrope hyperplasia, a possible precursor of GH-producing adenomas, have been demonstrated in CNC patients (65) as well as in specific Prkr1a knockout mice (66). Pituitary adenomas usually stain positively for both GH and PRL and are occasionally accompanied by mild hyperprolactinemia (67). However, almost a third of CNC patients present asymptomatic disturbances of the somatotroph axis, without meeting the diagnostic criteria of acromegaly, even without pituitary MRI findings (45).

#### THYROID NODULES

Seventy-five percent of CNC patients present with thyroid nodules, most of them being benign, non-toxic follicular adenomas. Thyroid nodules usually appear during the first ten years of life in CNC patients. Occasionally, patients (~3%) present with papillary or follicular carcinoma, particularly after a long history of multiple thyroid adenomas (21). In contrast to experimental data and what is observed in CNC patients with adrenal and pituitary tumors, thyroid nodules do not appear to have a predilection for hyperfunction (68).

#### TESTICULAR TUMORS

These tumors are of three types: A) Large Cell Calcifying Sertoli Cell Tumors (LCCSCT), B) Leydig cell tumors, and C) adrenocortical rest tumors. So far, the two latter types have been observed only in



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patients in whom LCCSCT had already been diagnosed.

LCCSCTs are observed in 20-50% of affected CNC males at the time of presentation, however, most males will develop such tumors in their adult life (21,45). These tumors are rarely observed in sporadic forms (<1% of testicular tumors), however, they are common in syndromes such as CNC and Peutz-Jeghers, where they are often multicentric and bilateral. They are almost always benign; malignancy has been rarely reported, particularly in tumors exceeding 6 cm in diameter. Nevertheless, their local expansion results in the replacement and compression of the normal testicular tissue (69). Occasionally (25%), LCCSCT may be hormone-producing and demonstrate increased P-450 aromatase expression (15). LCCSCT often presents as rock-hard and non-tender testicular masses and in ultrasonography, they appear as heterogeneous lesions of increased echogenicity with large areas of calcification (70). Macroscopically they are well-demarcated, yellow, and calcified tumors. Clinically, these hormone-producing tumors may cause sexual precocity in young males with low gonadotropin levels, as well as gynecomastia that may result from aromatase overactivity. Typically, fertility is impaired due to obstruction of the seminiferous tubules (16).

Leydig cell tumors and adrenocortical rests are both steroid-producing tumors and macroscopically are quite similar, characterized by a brownish hue and relatively soft texture. Leydig cell tumors may show malignant behavior, thus radical resection has been typically recommended. On the contrary, adrenal rests are benign lesions that do not require resection but can lead to recurrent Cushing's syndrome after adrenalectomy (71). The histological distinction between these two types of tumors can be difficult and a useful feature is the detection of crystalloids of Reinke that are present solely in Leydig cell tumors. However, these crystalloids are not a constant finding. In such case, testicular vein sampling can be helpful,

as it may demonstrate cortisol gradient between peripheral and testicular venous blood.

## OVARIAN LESIONS

Eight to 14% of female patients with CNC may present with ovarian lesions, either cystic or solid tumors of the ovarian surface epithelium, such as serous cystadenomas and teratomas. The percentage of ovarian lesions rises up to 60% in autopsy series (72). Ovarian cysts are usually clinically insignificant, whereas, tumors may progress, occasionally, to ovarian carcinoma, particularly in the elderly.

### **Psammomatous Melanotic Schwannomas**

Psammomatous Melanotic Schwannomas (PMS) are encapsulated tumors of the peripheral nerve sheath and are observed in less than 10% of individuals with CNC, usually in the fourth decade of life (21). Other hereditary syndromes that may present with PMS are neurofibromatosis and isolated familial schwannomatosis. Schwannomas in CNC are heavily pigmented and present frequently with calcifications and multicentricity. Their dark, brownish pigmentation is attributed to elongated spindle-shaped Schwann cells with melanogenic potential. Calcifications are encountered in a laminated form called psammomas and may be accompanied by hemorrhage and necrosis (73). PMS can develop anywhere in the central and peripheral nervous system; however, the most frequent locations are the nerves of the gastrointestinal tract and the paraspinal sympathetic chain (28% of cases). Other sights involved are the chest wall with involvement of the adjacent ribs and the trigeminal ganglion.

The initial presentation is usually characterized by local compression; whenever located in the gastrointestinal tract or within soft tissues they may evoke pain and discomfort. If they develop in the spine they may present as radiculopathy. Schwannomas are

among the most difficult tumors to treat, especially when they emerge around nerve roots along the spine, a location that makes excision not feasible. In addition, in rare cases (10%), they can be malignant and then often metastasize to the lungs, liver, or brain (74). Unfortunately, there is a paucity of effective treatments for metastatic PMS. Promising results have been recently published for the combination of the checkpoint inhibitor Nivolumab along with concurrent external beam radiotherapy (75).

## Other Manifestations

Apart from the major clinical manifestations, there are many other features suggestive of CNC, however, they are not present in a constant manner to set the diagnosis (44). These features are listed in Table 2.

**Table 2. Findings Suggestive or Possibly Associated with CNC, but Not Diagnostic for the Disease.**

1. Intense freckling (without darkly pigmented spots or typical distribution).
2. Blue nevus, usual type (if multiple).
3. Café-au-lait spots or other "birthmarks".
4. Elevated IGF-I levels, abnormal OGTT, or paradoxical GH responses 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 and other skin lesions; lipomas.
9. Colonic polyps (usually in association with acromegaly).
10. Hyperprolactinemia (usually mild and almost always in association with clinical or subclinical acromegaly).
11. Single, benign thyroid nodule in a young patient; multiple thyroid nodules in an older patient (detected by ultrasonography).
12. Family history of carcinoma, in particular of the thyroid, colon, pancreas, and ovary; other multiple benign or malignant tumors.

Breast ductal adenomas are benign tumors of the mammary gland ducts that may also develop in the context of CNC and can be multiple and bilateral as well. Coexistence with breast myxomas can be observed (54). They are palpable, painless masses that usually appear near the areola and can produce bloody nipple discharge. Radiologically their appearance varies from well-delineated and spherical to completely irregular lesions and they always contain calcifications (52). These calcifications may be coarse (typically benign) or microcalcifications, which are

often encountered in adenocarcinomas. Moreover, a possible association between CNC and breast cancer has been demonstrated in the most recent prospective study, affecting up to 13.5% of female patients at an unusually young age (<50 years) (45). Consequently, the differential diagnosis is difficult, and FNA is always recommended.

Other tumors reported in around 2,5-4,5% of CNC patients are pancreatic neoplasms including acinar cell carcinoma, adenocarcinoma, and intraductal

pancreatic mucinous neoplasia (45,76). In addition, CNC is associated with increased detection of liver lesions, including hepatocellular adenomas, carcinomas, and fibrolamellar carcinomas (77,78).

## DIAGNOSIS

The diagnosis of CNC is principally set by clinical criteria and can be confirmed by molecular testing, which has a mutation detection rate of approximately 60-70%. Genetic testing currently can only be recommended either as an adjunctive test for individuals who meet the clinical criteria or for the detection of affected members of families where the index case harbors a known mutation, in order to avoid unnecessary medical surveillance of non-carriers (1).

The following clinical criteria were initially proposed in 1998, were revised in 2001, and have not been modified since then. They yield a sensitivity of nearly 98%. They include 12 clinical manifestations that set the major criteria for diagnosis, as well as 2 supplemental criteria regarding molecular testing and family history. At least two major criteria need to be present to establish the diagnosis of CNC and their occurrence has to be confirmed either biochemically, histologically, or by imaging as indicated. In the presence of one supplemental criterion, a single clinical manifestation is sufficient to establish the diagnosis (44).

### Major Criteria

#### Skin pigmentation disorders

1. Spotty skin pigmentation with a typical distribution (vermillion border of the lips, conjunctiva, and inner or outer canthi, vaginal and penile mucosa)
2. Blue nevus, epithelioid blue nevus (multiple)\*

#### Myxomas

3. Cutaneous and mucosal myxomas\*
4. Cardiac myxomas\*

5. Breast myxomatosis\* or fat-suppressed magnetic resonance imaging findings suggestive of this diagnosis

6. Osteochondromyxoma\*

#### Endocrine tumors / Overactivity

7. Primary pigmented nodular adrenal dysplasia (PPNAD)\* or a paradoxical positive response of urinary glucocorticosteroids to dexamethasone administration during Liddle's test

8. Acromegaly due to GH-producing adenoma or evidence of excess GH production

9. Large-Cell Calcifying Sertoli Cell Tumor (LCCSCT)\* or characteristic calcification on testicular ultrasonography

10. Thyroid carcinoma\* or multiple, hypoechoic nodules on thyroid ultrasonography, in a young patient

#### Miscellaneous

11. Psammomatous Melanotic Schwannoma\*

12. Breast ductal adenoma\*

\* histologically confirmed

#### Supplemental Criteria

1. Affected first-degree relative
2. Inactivating mutation of the *PRKAR1A* gene

## MANAGEMENT

So far, no evidence-based monitoring schedule has been established for CNC; however, clinical work-up for all the manifestations of CNC should be performed at least once a year in all patients, and asymptomatic known mutation carriers, should start in infancy.

### Surveillance

Prepubertal children should be screened as follows:

- Cardiac ultrasound should start as soon as the diagnosis is made, based either on clinical or genetic grounds, and be performed at least once a year thereafter.

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In patients with a history of cardiac myxoma, screening should be more frequent, optimally every 6 months, due to the increased risk of recurrence (49).

- Patients should undergo an initial thyroid ultrasound within the first decade of age, and then repeated according to findings.
- Screening for the other manifestations should be performed in patients under 5 years of age only by clinical examination. Especially for males, testicular ultrasonography is recommended at the initial evaluation and if microcalcifications are present it should be repeated yearly. Regarding ovarian and breast imaging of female patients, these may be deferred until after puberty (1).
- Pubertal staging and growth rate should be monitored as pediatric patients with CNC may present with failure to thrive, a possible outcome of various CNC components, such as Cushing's syndrome due to PPAD or hepatic involvement (79). On the other hand, the presence of a functional LCCST may be associated with growth and maturation acceleration.

In post-pubertal adolescents and adults, the following investigations should be performed at initial diagnosis and annually thereafter, including screening for:

- Cardiac myxomas by echocardiography, which if positive, should be repeated bi-annually. The possibility of first occurrence decreases with age and is exceptional after the age of 50 (49).
- PPAD by measurement of urinary free cortisol, ACTH, and overnight suppression with 1 mg Dexamethasone, followed by a formal Low Dose Dexamethasone Test if abnormal. If this suggests cortisol hypersecretion, the diagnosis may be supported by a 6-day Liddle's test and an adrenal CT scan.
- Acromegaly by measurement of serum GH, PRL, and Insulin-Like-Growth-Factor I (IGF I). In case of abnormal findings, confirmation of GH hypersecretion with an oral glucose suppression test (OGTT) and imaging of the pituitary region with MRI is suggested.

- Thyroid nodules by ultrasonography and further evaluation with FNA as needed, according to the relevant guidelines for the general population.
- LCCST in males by testicular ultrasound, especially when small-sized calcifications are found. Follow-up may be less frequent than annual due to the slow progression of these tumors (45)
- PMS with spine MRI once at baseline and thereafter when clinical signs suggest the presence of this tumor.
- *Breast myxomas* as well as ductal adenomas in females should be screened and followed up in the context of screening for breast cancer including self-examination, clinical evaluation, mammography, and ultrasound, starting earlier than in the general population, maybe earlier than the age of 40 (45). In the case of findings, MRI of the breast is more sensitive in mapping the lesions (52).
- *Ovarian lesions* by transabdominal ultrasonography during the first evaluation. The test should be repeated due to the low risk of ovarian malignancy (72).

## Treatment

As CNC is generated by a constitutional genetic defect, no etiologic therapy is available yet. The therapeutic approach should target each clinical manifestation and treat accordingly.

- Cardiac myxomas require surgical removal. However, due to the high recurrence rate, re-operation is usually indicated (49)
- Cutaneous and mammary myxomas may be surgically removed, mainly for cosmetic and/or diagnostic purposes.
- Regarding PPAD, bilateral adrenalectomy has been typically suggested if overt Cushing's syndrome is evident. Some institutions though have reported treatment with a low dose regimen (0,5-4 g daily) of O,p'-dichlorodiphenyldichloroethane (Mitotane) (80,81) with long term effects; however, the possible significant adverse events of such an approach should be considered.

- LCCSCT has been traditionally treated with orchiectomy; however, the fact that these tumors often occur bilaterally and are grossly benign has raised an issue to consider treatment options that might preserve fertility. Such an approach is testicular-sparing surgery, followed by strict monitoring of growth and pubertal staging and administration of anti-estrogen drugs in case of recurrence (82). Alternatively, successful treatment of prepubertal gynecomastia and growth acceleration by exclusively using aromatase inhibitors has been reported; however long-term efficacy and safety data are still lacking (83). Similarly, management of Leydig tumors, which often present as small non-palpable testicular lesions, tends to change with the implementation of advanced imaging modalities (magnetic resonance, contrast-enhanced ultrasonography, strain elastography) which may allow a more conservative approach, including surveillance and testis-sparing surgery (84)
- Pituitary adenomas should be removed by transsphenoidal or transcranial approach, according to their size and extension as in sporadic tumors. Alternatively, long-term medical treatment can be offered.
- Thyroid nodules should be evaluated and treated surgically according to current guidelines.
- PMS: surgery to remove primary and/or metastatic lesions.

## Genetic Counseling

Genetic analysis may be suggested for CNC index cases, taking into consideration the fact that the mutation detection rate of PRKR1A testing with standard sequencing is at present approximately 60%. Therefore, a negative test does not exclude CNC in an individual who meets clinical criteria. In such cases, copy number variant (CNV) analysis by comparative genomic hybridization (CGH) and/or PRKR1A gene deletion testing may be suggested to rule out a PRKR1A defect. Currently, genetic diagnosis may be assisted by NGS techniques. If all testing for PRKR1A defects is negative, screening for other candidate genes or loci, including the PRKACA,

PRKACB and the phosphodiesterase 8 and 11 genes has been suggested.

In those cases where a mutation is detected, genetic screening (specific sequencing) is recommended for first-degree relatives (parents, siblings, and offspring). In case of a positive test, mutation carriers should undergo the same follow-up and management as that suggested for CNC patients. The first cardiac ultrasound should be performed at the same time as the molecular testing.

Genetic counseling should include the following general information:

- If a parent of the index case is affected, the risk to his siblings is 50%. On the contrary, in case of a *de novo* mutation, this risk falls to approximately 1%.
- Each child of an individual with CNC has a 50% chance of being affected.
- Fertility may be impaired in males with CNC. Contrary to male patients, CNC is not specifically associated with female infertility and successful pregnancies and deliveries of female CNC patients have been reported (85).
- Most tumors of CNC are in general benign except for thyroid nodules and schwannomas; however, they are associated with significant morbidity. Prenatal testing is available by chorionic villous sampling (CVS) at approximately ten to 12 weeks of gestation or amniocentesis at 15-18 weeks of gestation. Pre-implantation genetic diagnosis (PGD) is available for PRKR1A mutation carriers and in conjunction with *in-vitro* fertilization allows the selection of disease-free embryos for implantation.

## FUTURE PERSPECTIVES

Although remarkable progress has been made since CNC was first described, several issues need to be answered. There are still CNC families that do not carry a PRKR1A gene mutation and cannot be



assigned to CNC2 either. The CNC2 gene located at the 2p16 locus remains to be determined.

Researchers put efforts in the search for a more specific therapy for CNC. An older study demonstrated that 8-Cl-adenosine (8-Cl-ADO), a cAMP analog was able to, inhibit *in vitro* the proliferation induced by G protein-coupled receptors (86); however, no further research has been published on this substance. Moreover, PRKAR1A haploinsufficiency has been shown to induce cyclooxygenase-2 (COX2) activation and prostaglandin E2 (PGE2) overproduction, a disorder that has been associated with the abnormal

proliferation of adult bone stromal cells (ABSCs) seen in osteochondromyxomas of CNC patients (87). Experimental administration of celecoxib, a COX2 inhibitor, in mice with PKA defects decreased PGE2 and associated proliferation of ABSCs, resulting in a substantial reduction of bone tumor growth and improved organization of cortical bone that was adjacent to the tumor. Based on the same principle, experiments with celecoxib on adrenocortical cell lines and in a mouse model of PPNAD demonstrated *in vitro* and *in vivo* reduction of steroid secretion and cell proliferation (88). Recent advances in genomics and pharmaceutical technologies are promising for timely diagnosis and “etiologic” cure of this syndrome.

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