Chapter 21 – Carney Complex

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TAKE HOME POINTS

- Carney complex (CNC) is a dominantly inherited syndrome of multiple neoplasias combined with cardiocutaneous manifestations.
- The *CNC1* gene, located on 17q22-24 is coding the regulatory subunit (R1a) of the protein kinase A (*PRKAR1A*) and is responsible for 2/3 of CNC cases.
- The putative "CNC2" gene at the 2p16 locus has not been identified as yet
- Clinical manifestations show significant variability between patients, even among members of the same family.
- Cutaneous lesions, although with minimal clinical impact, are very common and occasionally specific (myxomas)
- PPNAD is the endocrine tumor most frequently observed
- Complications due to cardiac myxomas comprise the major factor of mortality
- The diagnosis is based on 12 major clinical criteria and 2 supplemental criteria
- PRKR1A testing is available with a detection rate of ~ 60%. If a mutation is detected, genetic screening is recommended for first degree relatives
- Clinical work-up for all the manifestations of CNC should be performed at least once a year in all patients and should start in infancy.

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 (testicular, adrenal, pituitary or thyroid tumours) and non-endocrine (myxomas, schwannomas). The skin lesions are divided in two major types: pigmented lentigines and blue nevi that can be observed on the face, neck and trunk [1](Figure 1).

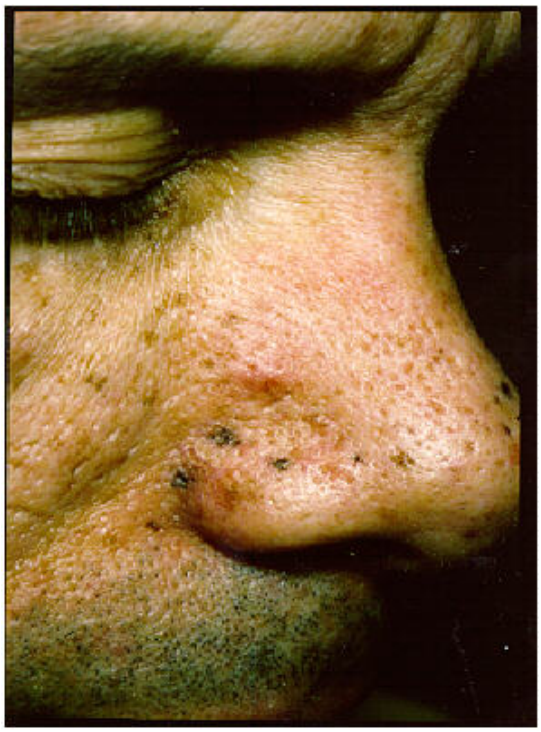

Figure 1. Spotty pigmentation of the face. With permission http://ugen.nichd.nih.gov

This syndrome was first described by J. Carney in 1985 [2], 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, that 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 a 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, ephelide)[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]. Nowadays, diagnosis of the syndrome is feasible in clinically asymptomatic patients by commercially available molecular genetic assays. 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 - Heritance

Carney's complex is a rare disease. Up-to-date, more than 500 patients have been registered by the NIH-Mayo Clinic (USA) and the Cochin centre (Paris, France) consortium and 160 index cases of CNC are presently known [12]. Approximately 70% of individuals diagnosed with CNC have a familial history, while the remaining 30% present a *de novo* germline mutation. Incidence is similar in both sexes (43% males Vs 57% females) and there is no apparent predilection concerning ethnicity. [13].

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 harbour Large Cell Calcified Sertoli Cell Tumors (LCCSCT), that may cause infertility [14]. Recent data from animal models correlate haplo-insufficiency at the PRKR1A gene locus with infertility, without the presence of LCCSCT [15]. The median age of diagnosis is 20 years; however the penetrance of CNC is 70%-80% by the age of 40 years, as clinical manifestations

accumulate during lifespan. The maximum number of affected generations reported in a kindred is 5 [9].

Diagnosis

The diagnosis of CNC is set by clinical criteria and can be confirmed by molecular testing that has a mutation detection rate of approximately 60% [12]:

The following clinical criteria were initially proposed in 1998 and revised in 2001 and have 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 confirm the diagnosis of CNC. In the presence of one supplemental criterion, a single clinical manifestation is sufficient to set the diagnosis [1].

MAJOR CRITERIA

Skin pigmentation disorders

1. Spotty skin pigmentation with a typical distribution (vermilion 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
11. Psammomatous Melanotic Schwannoma*
12. Breast ductal adenoma*

SUPPLEMENTAL CRITERIA:

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

*Histologically confirmed

Molecular Genetics - Pathophysiology

Hitherto, two loci have been 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 (*PRKAR1A*), a protein of 384 aminoacids [16]. Heterozygous inactivating mutations of *PRKAR1A* have been detected in about 65% of affected individuals. Interestingly, in patients presenting with Cushing's syndrome (CS) this frequency rises to about 80%. [17]. Protein Kinase A (PKA), is a second messenger-dependent enzyme involved in G protein-coupled intracellular pathways and serves as a mediator of c-AMP actions on cell metabolism, proliferation and apoptosis. Its quaternary structure consists of 4 peptide chains that form a homotetramer of two regulatory (R), R-I and R-II, and two catalytic (C) subunits [18]. Two genes are responsible for each R subunit (PRKR1A, PRKR1B and PRKR2A and PRKR2B respectively) and four genes for each C subunit (PRKACA, PRKACB, PRKACG and PRKX) [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 (see Fig. 2).

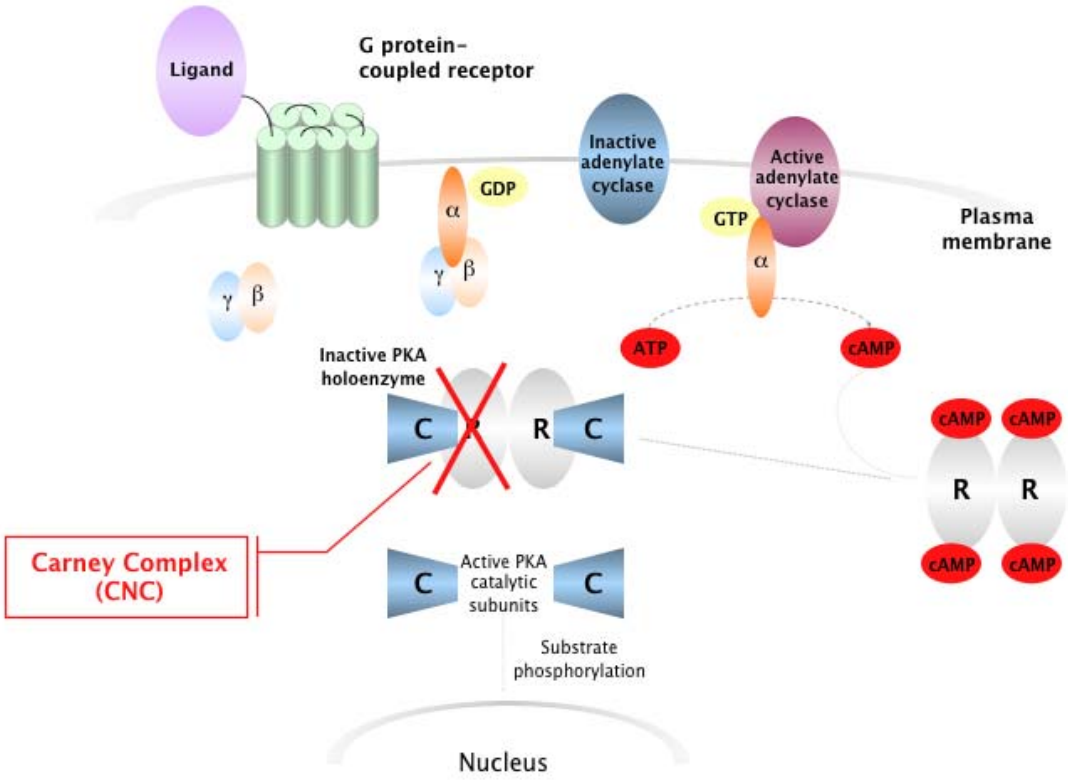

Figure 2. The G protein-coupled intracellular pathways and the defect in CNC patients: PRKAR1A mutations result in deficienct / ineficient regulatory subunits, resulting in constitutional activation of C subunits (http://prkar1a.nichd.nih.gov).

Up to date more than 120 different PRKAR1A mutations have been identified; 117 of them are registered at the CNC consortium database (http://prkar1a.nichd.nih.gov). They are evenly distributed among the PRKAR1A gene exons and most of them are family or patient specific as only 3 mutations have been found in more than three unrelated families. 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 (non-sense) and thus, short mutant mRNAs that are eliminated by selective degradation, a phenomenon known as nonsense-mediated mRNA decay (NMD) [16]. The result is lack of detectable mutant protein and reduction of RIα protein levels by 50%. The rest of the mutations (17%)

result in the expression of an altered protein (mis-sense) that may be associated with more advanced disease [20]. Large PRKAR1A deletions have also been demonstrated in few patients that also expressed a more severe phenotype [21]. Structure of the PRKR1A gene and location of detected mutations are shown in Figure 3.

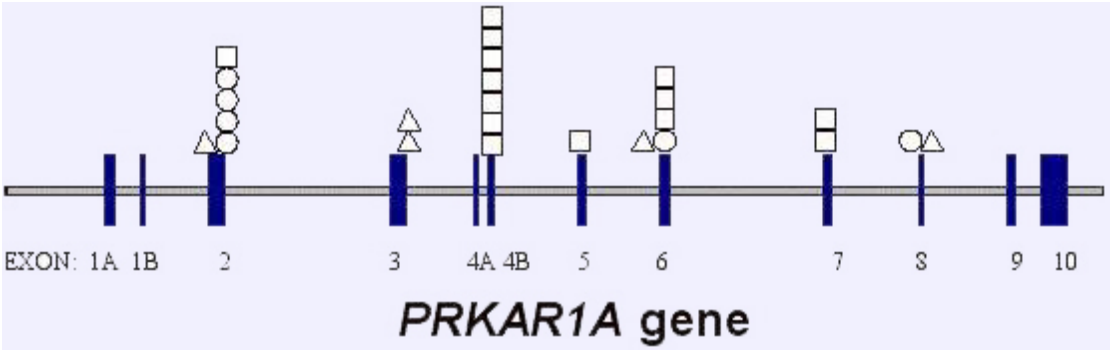

Figure 3. Schematic presentation of the PRKR1A gene and detected mutations in relation to their exon location.

Germline haplo-insufficiency of PRKAR1A leads to a deficiency of the R1a subunits, which in turn results in enhanced intracellular signalling by PKA, as evidenced by an almost 2-fold greater response to c-AMP in CNC tumors [22]. This PKA overactivity may trigger pathways that favour cell proliferation as the upregulation of D-type cyclins [23] or activation of the mTOR pathway [24]. Moreover, loss of heterozygosity (LOH) at 17q22-24 may be observed in the tumors of CNC patients, consistent with the Knudson two-hit model of hereditary tumorigenesis [25]. Interestingly, tumors which do not present inactivation of the remaining wild type allele have also been described, implying that co-existence of PRKAR1A haplo-insufficiency with defects of other known tumor suppressor genes may act synergistic for tumorigenesis.

These findingns were supported by experiments on Prkar1a +/− knockout mice, the genotypic animal model of Carney's complex. These mice developed phenotypes with many of the manifestations of CNC (nonpigmented schwannomas, bone lesions and thyroid neoplasias) [26, 27]; lacking however, several characteristic lesions, like PPNAD, cardiac or skin myxomas, and pituitary adenomas [15]. On the contrary, mice with complete loss of Prkar1a were not viable as this genotype leads to early embryonic demise [28]. Eventually, the development of pituitary cell tumors as well as heart myxomas was achieved by inducing tissue specific complete ablation of Prkar1a, [29].

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 [30].

Other Loci

Approximately 20% 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 been identified as yet. 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. These data suggested that the gene located at 2p16 is a potential oncogene [31] that may code a PKA catalytic subunit. However, the genes of the C catalytic PKA subunits have been identified elsewhere than 2p16. Recently, inactivating mutations of the phosphodiesterase 11A (PDE11A) gene (located at 2q31.2) has been demonstrated in isolated PPNAD patients [32], while, a high frequency of PDE11A sequence variants has been detected among CNC patients [33].

Genotype - Phenotype Correlations

Efforts have been made to relate specific phenotypes to corresponding genotypes. A recent 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. [17]

As most of the identified PRKAR1A mutations lead to a lack of detectable mutant protein, no genotype-phenotype correlations are expected to be seen. However, specific hot-spot mutations show some genotype-phenotype correlation. Especially those few that lead to the expression of a mutant protein are related with more severe forms of CNC syndrome, suggesting that NMD may play a protective role against the deleterious effects of mutant products [17]. No statistically significant phenotypic differences have been observed between individuals with CNC1 and CNC2.

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 [34]. 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. These data are summarised in Table 1.

Table 1 Clinical manifestations of CNC *at the time of presentation* among 338 patients (Stratakis et al, JCEM 2001).

Manifestation	**Percentage (%)**
Spotty skin pigmentation	77
Heart myxoma	53
Skin myxoma	33
PPNAD	26
LCCSCT	33 (of male patients)
Acromegaly	10
PMS	10
Thyroid nodules or cancer	5
Breast ductal adenoma	3 (of female patients)

Most often clinical signs appear in the teen years and early adulthood, with a median age of diagnosis at 20 years of age; however, evidence of the disease, especially cutaneous lesions, can be found even at newborns. During infancy, the most common tumors encountered are cardiac and cutaneous myxomas, as well as PPNAD, while LCCSCT 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 [35]. The average life expectancy of CNC patients is limited to 50 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. Other less important factors are metastatic or intracranial PMS, thyroid carcinomas, and metastatic pancreatic and testicular tumors [1, 34].

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 [36].

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 vermilion border of the lips, on the eyelids, ears and the genital area (Figure 1). Macroscopically, lentigines are flat, poorly circumcised 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). Histologically, the hyperpigmentaion of CNC lesions is associated with melanocytic hyperplasia and hypertrophy, rather than increased melanin production observed in solar lentigines [36].

Blue nevi

These are larger lesions (up to 8 mm), blue to black, and dome-shaped. They are less common and may be multiple with a variable distribution. Histologically, they may present features of epithelioid, junctional or even compound nevi. Occasionally café au lait spots and depigmented lesions may also be observed [37].

Myxomas

Cutaneous Myxomas

The skin myxomas present as non-pigmented subcutaneous nodules with a smooth surface and may look white, flesh-coloured, opalescent, or pink (see 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. Interestingly, hands and feet are preserved [36]. 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 localised to the upper dermis and subcutis and consist of polygonal to stellate cells scattered singly or in clusters against an abundant basophlilic myxoid matrix [2]. Although cutaneous myxomas have minimal impact in the clinical course of CNC, their recognition is crucial since they may herald the presence of a potentially fatal cardiac myxoma [38].

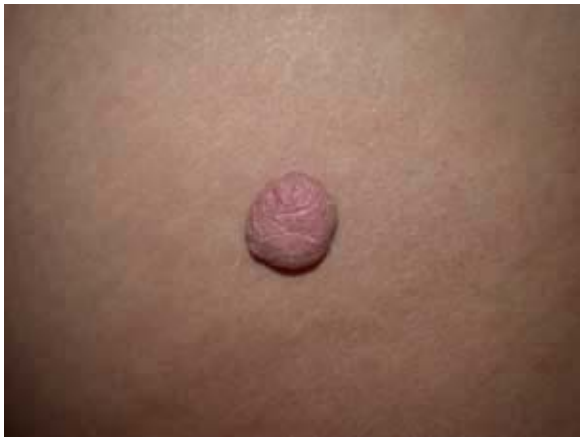

Figure 4. Cutaneous myxoma on the right flank of a CNC patient. With permission from Dermatology Online Journal 2004; 10 (3): 11

Cardiac myxomas

Although these tumours are benign, they are responsible for the majority of deaths (>50%) related to CNC mainly due to cardiovascular complications. Their sporadic counterparts are rare tumors that emerge most commonly in middle aged women and are localised to the left atrial aspect of the interatrial septum at the fossa ovalis. Most of them are cured by surgical resection and do not recur. On the contrary, cardiac myxomas in CNC demonstrate unusual features as they present at younger age (as early as 3 year of age) and can develop in any cardiac chamber. In addition, they may be multiple and recurrent, therefore, their resection cannot guarantee permanent cure [39].

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 tumour [35].

Their size range from a few millimetres 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 [40] Histologically, the tumors have a gelatinous or hemorrhagic appearance and arise from a population of multipotent subendocardial mesenchymal precursor cells [41].

Breast myxomas (myxoid fibroadenomas)

These lesions are observed in about a fifth of women with Carney complex and are generally considered as benign breast tumors. They usually occur in females after puberty and can be multicentric as well as bilateral (see 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 for diffuse nodularity without dominant masses. Nipple discharge, breast skin abnormalities or sentinel lymphadenopathy have not been observed as yet [42].

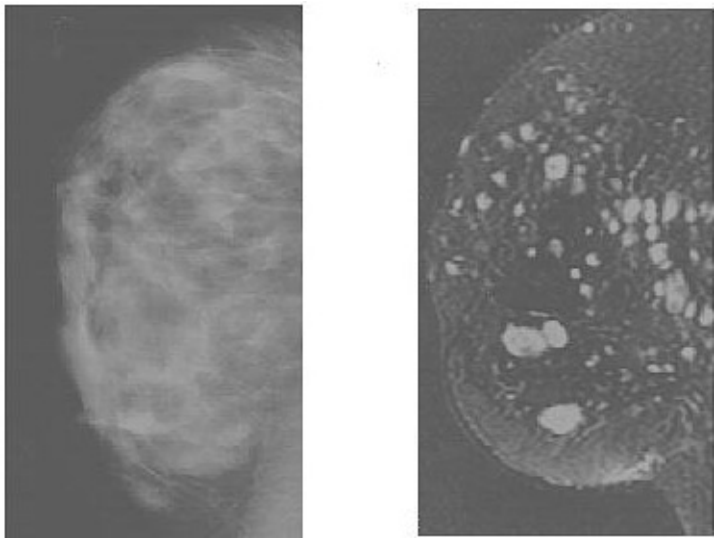

Figure 5. Breast multiple myxomas in a patient with Carney complex. Mammogram (A), showing typical dense breasts in a younger woman with no evidence of tumor. However, in the fat-suppressed magnetic resonance image (B) shown on the right, the presence of multiple small myxomas is clearly seen. With permission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

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]. 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 sonography or conventional mammography. The number of myxoid lesions depicted with this technique are usually numerous (more than 58 per breast in a case) and show homogeneous increase of the signal intensity, a situation characteristic of CNC, also referred to as “breast myxomatosis” [40].

Osteochondromyxomas

Osteochondromyxomas or Carney bone tumors are myxomatous tumors of the bone that principally affect nasal sinuses and long bones. They have been described in few cases until now and exhibit benign behaviour, however they cause bone erosion and can extent to soft tissues. 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 [40]. Complete resection of the tumor is usually curative. Experiments in rodents demonstrated the osteoblastic origin of the tumor. [43, 44]. Less common sites of myxoma formation include the oropharynx (tongue, hard palate and pharynx) and the female genital tract (uterus, cervix and vagina).

Endocrine tumors / overactivity

PPNAD

PPNAD is the endocrine tumor most frequently observed in individuals with CNC. It affects bilaterally the adrenal glands and can cause clinically overt CS in approximately 25 to 30% of patients with CNC. However, histological evidence of PPNAD is present in almost every CNC individual as it has been demonstrated by autopsy studies. 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) [1].

Histologically, the adrenal cortex is dominated by small pigmented micronodules with an average size less than 10mm (see Figure 6). Although unencapsulated, 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 colour of the lesions. Interestingly, tumor cells stain positively for neuroendocrine markers (e.g Synaptophysin), while normal cortical cells don't [45].

Internodular cortical atrophy is typical, thus the overall weight of the adrenal gland remains more or less normal [2].

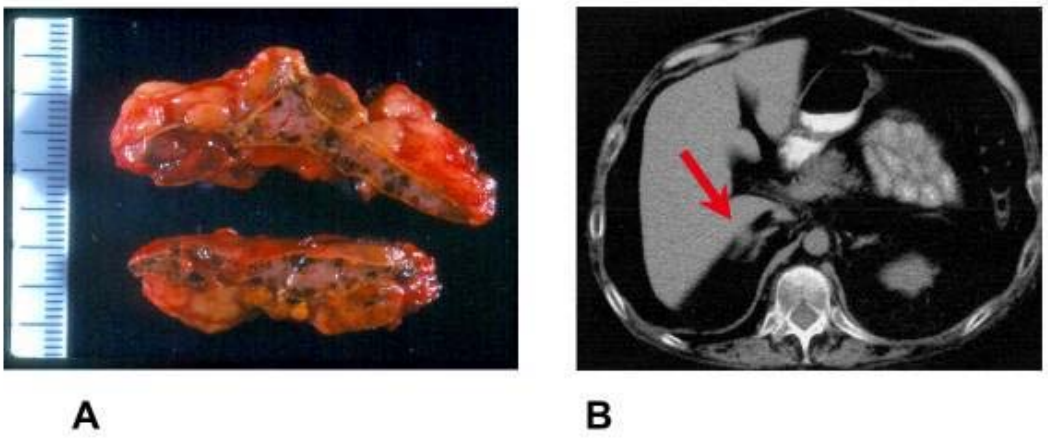

Figure 6. Macroscopic and CT-scan findings in primary pigmented nodular adrenocortical disease (PPNAD). A: Macroscopic appearance of the adrenal gland where multiple pigmented micronodules are evident at the cut surface. B: Adrenal CT-scan revealed a micronodule on the external limb of the left adrenal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

Radiological and scintigraphic findings are not specific, since the adrenals may appear bilaterally or unilaterally enlarged but in most cases they appear normal. Computed Tomography (CT) is the most appropriate examination for depicting adrenal lesions in PPNAD. Particularly, images obtained with 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” [40].

The type of hypercortisolism observed in this disorder is that of ACTH-independent adrenal hyperfunction. However, demonstrating cortisol overproduction can be difficult because it can develop progressively over years. Moreover, cyclic forms of hypercortisolism have been reported [46, 47]. Clinical manifestations are no-specific and similar to those observed in patients with CS of other aetiology (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. A paradoxical increase of UFC and 17-hydroxysteroids the second day after high dose dexamethasone administration is indicative of PPNAD [48].

Recently, the development of adrenocortical cancer (ACC) in the context of CNC has been reported [49, 50]. In both cases the patients carried PRKAR1A mutations and ACC developed in 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. A possible explanation is that mutant PRKAR1A activation of the cAMP pathway favors the development of benign endocrine tumors, whereas defects in other tumor suppressor genes play a role in the susceptibility to develop malignancy [51].

Growth hormone (GH)-secreting pituitary adenomas (acromegaly)

Clinically evident acromegaly due to a pituitary GH-secreting tumor occurs in approximately 10% of patients with CNC, whereas, gigantism, resulting from excessive GH secretion prior to puberty, is quite rare. Pituitary adenomas usually stain positively for GH and PRL and are occasionially associated by mild hyperprolactinemia. However, most patients with CNC (~75%) present with moderate increase in GH, even if not accompanied by abnormal pituitary MRI findings [52]., Somatomammotroph hyperplasia that may represent a precursor of GH-producing adenomas has been demonstrated in CNC patients as well as in studies using pituitary specific Prkr1a knockout mice [53].

Thyroid nodules

Seventy five percent of CNC patients present thyroid nodules, most of them being benign, non-toxic adenomas of follicular type. Some patients (~5%) present with papillary or follicular carcinoma usually after a long history of multiple thyroid adenomas. 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 [26, 54].

Testicular tumors

These tumors are of three types: A) Large Cell Calcifying Sertoli Cell Tumors (LCCSCT), B) Leydig cell and C) adrenocortical rest tumors. Up to date the two latter types have been observed only in patients in whom LCCSCT had already been diagnosed.

LCCSCT are observed at one-third of affected CNC males at the time of presentation, however most males will develop such tumors in their adult life. These tumors are rarely observed in sporadic forms, but in CNC patients they are often multicentric and bilateral. They are almost always benign; malignancy has been reported only once, and occasionally may be hormone producing and demonstrate increased P-450 aromatase expression [14]. LCCSCT are commonly non-palpable, discovered by ultrasonography as bilateral microcalcifications [55]. Otherwise they are described as rock-hard and non-tender masses. 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.

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 behaviour, thus radical resection is recommended. On the contrary, adrenal rests are benign lesions which do not require resection, but can lead to recurrent CS after adrenalectomy. 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 in Leydig cell tumors. However, these crystalloids are not a constant finding [2]. Helpful in this case can be testicular vein sampling which may demonstrate cortisol gradient between peripheral and testicular venous blood [56].

Psammomatous Melanotic Schwannomas

Psammomatous Melanotic Schwannomas (PMS) are observed in less than 10% of individuals with CNC, which is the only hereditary syndrome that may present with PMS, other than NF and isolated familial schwannomatosis. Schwannomas in CNC are heavy pigmented and present frequently calcifications and multicentricity. They are

encapsulated tumors of peripheral nerve sheath and their dark 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 haemorrhage and necrosis [57]. 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 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 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 the brain. Unfortunately, there is practically no specific effective medical or surgical treatment for metastatic PMS [58].

Other Manifestations

Breast ductal adenomas, benign tumors of the mammary gland ducts may also develop in the context of CNC and can be multiple and bilateral as well. Coexistence with breast myxomas can be observed [59]. They are palpable, painless masses that usually appear near the areola and can produce blood nipple discharge. Radiologically their appearance varies from well delineated and spherical to completely irregular lesions and they always contain calcifications. These calcifications may be coarse (typically benign) or microcalcifications, which are often encountered in anocarcinomas. Consequently, the differential diagnosis is difficult, and FNA is always recommended [40].

Apart from the 12 major clinical manifestations there are many other features suggestive of CNC, however they are not present in a constant manner to set the diagnosis [1]. 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 the ovary; other multiple benign or malignant tumors.

Management

Clinical work-up for all the manifestations of CNC should be performed at least once a year in all patients and should start in infancy.

Surveillance

Prepubertal children should be screened as follows:

- Cardiac ultrasound should start during the first 6 months and be performed at least once a year thereafter. In patients with a history of cardiac myxoma, screening should be more frequent, optimally every 6 months.
- Screening for the other manifestations (only by clinical examination) should be performed in patients under 5 years-old. Especially for males, testicular ultrasonography is recommended at the initial evaluation and if microcalcifications are present it should be repeated on a yearly basis. Moreover, pubertal staging and growth rate should be monitored if LCCSCT is discovered.

In postpubertal children and adults, annual screening should be performed for:

- *PPNAD* by measurement of urinary free cortisol and overnight suppression with 1 mg Dexamethasone, followed by a formal Low Dose Dexamethasone Test if abnormal. If this suggests cortisol hypersecretion, a 6-day Liddle's test and an adrenal CT scan is performed.
- *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 oral glucose suppression test (OGTT) and imaging of the pituitary region with MRI is suggested.
- *Thyroid nodules* by ultrasonography as needed. FNA might be helpful in diagnosis.
- *LCCSCT* in males by testicular ultrasound, especially when small sized calcifications are found
- *PMS* with spine MRI once as 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. In case of findings, MRI of the breast maybe more sensitive in mapping the lesions [59].
- *Ovarian lesions* by transabdominal ultrasonography during the first evaluation. The test should be repeated due to the low risk of ovarian malignancy [31].

Treatment

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

- A cardiac myxoma requires surgical removal. However, due to high recurrence rate re-operation might be needed [39].
- Cutaneous and mammary myxomas should be surgically removed.
- Regarding PPNAD, bilateral adrenalectomy although amputative seems the more reasonable approach if CS is evident. Some institutions have reported treatment with O,p'-dichlorodiphenyldichloroethane (Mitotane) [60] with long term effects, however the significant adverse events of such an approach should be balanced.
- LCCSCT has been traditionally treated with orchiectomy especially in prepubertal boys in order to cope with the secondary effects of excess hormone production. 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. In limited cases, testicular-sparing surgery was performed followed by strict monitoring of growth and pubertal staging and administration of antiestrogen drugs in case of recurrence [61]. Treatment of choice for Leydig cell tumor, neoplasias with malignant potential is inguinal orchiectomy,
- Pituitary adenomas according to their size and extension should be removed by transsphenoidal or transcranial approach 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 should be recommended to all CNC index cases taking into consideration the fact that mutation detection rate of PRKR1A testing is at present approximately 60%. Therefore a negative test does not exclude CNC in an individual who meets clinical criteria.

If a mutation is detected, genetic screening 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 counselling 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.
- Most tumors of CNC are in general benign with the exception of thyroid nodules and schwannomas.

Prenatal testing is available by chorionic villous sampling (CVS) at approximately ten to 12 weeks of gestation or amnioparacentesis at 15-18 weeks of gestation.

Preimplantation genetic diagnosis (PGD) is now available with the new advances in fertilization technology and allows the selection of disease free embryos for implantation.

Future Perspectives

Although remarkable progress has been made during the 25 years since CNC was first described there are several issues that need to be answered. There are still CNC families that do not carry a PRKAR1A gene mutation and cannot be assigned to CNC2 either. The CNC2 gene located at the 2p16 locus is still to be determined.

Novel therapeutic strategies are evolving in the search of a more specific therapy for CNC. A c-AMP analogue: 8-Cl-adenosine (8-Cl-ADO) that has been used *in vitro,* inhibited proliferation induced by G protein-coupled receptors [62]. Advances in

genomics and pharmaceutical technologies are promising for timely diagnosis and “etiologic” cure of this syndrome.

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