Carney complex: an update

Ricardo Correa, Paraskevi Salpea and Constantine A Stratakis

Section on Endocrinology and Genetics, Program on Developmental Endocrinology and Genetics, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, 10 Center Drive, Building 10, NIH-Clinical Research Center, Room 1-3330, MSC1103, Bethesda, Maryland 20892, USA

Abstract

Carney complex (CNC) is a rare autosomal dominant syndrome, characterized by pigmented lesions of the skin and mucosa, cardiac, cutaneous and other myxomas and multiple endocrine tumors. The disease is caused by inactivating mutations or large deletions of the PRKAR1A gene located at 17q22–24 coding for the regulatory subunit type I alpha of protein kinase A (PKA) gene. Most recently, components of the complex have been associated with defects of other PKA subunits, such as the catalytic subunits PRKACA (adrenal hyperplasia) and PRKACB (pigmented spots, myxomas, pituitary adenomas). In this report, we review CNC, its clinical features, diagnosis, treatment and molecular etiology, including PRKAR1A mutations and the newest on PRKACA and PRKACB defects especially as they pertain to adrenal tumors and Cushing’s syndrome.

Introduction

Carney complex (CNC) is a rare multiple neoplasia syndrome, inherited in an autosomal-dominant manner or occurring sporadically as a result of a de novo genetic defect. It is characterized by pigmented lesions of the skin and mucosae, cardiac, cutaneous and other myxomatous tumors, and multiple other endocrine and non-endocrine neoplasms (1, 2). It was first described by J Aidan Carney as ‘the complex of myxomas, spotting pigmentation and endocrine over-reactivity’ (3, 4). It was designated as CNC by Bain (5) and in 1994 as Carney syndrome by Mendelian Inheritance in Man (6).

More than half of the cases are familial (2, 7). Most of the patients who in the past had been diagnosed with lentigines, atrial myxomas, myxoid neurofibromas and ephelide or nevi, atrial myxoma, blue nevi should be reclassified today as CNC (1, 3, 8, 9). CNC is in essence a multiple endocrine neoplasia syndrome, but one that affects a number of other tissues (10). This unique condition has similarities to other syndromes/diseases, such as the McCune-Albright, Peutz-Jeghers, Cowden, Bannayan-Zonana and Birt-Hogg-Dube syndromes, neurofibromatosis and other phacomatoses and hamartomatoses (10).

Epidemiology

CNC is a rare disease (4) with an unknown prevalence (11, 12). In the largest genotyped series of patients, 63% were females and 37% were males (12). The NIH-Mayo clinic, and other centers in the USA and the Cochin Hospital in France, have collectively reported more than 750 cases including Caucasians, African-Americans and Asians from three continents (North and South America, Europe, Asia (Japan, China, India)) (2, 11, 13). Approximately 70% of
CNC cases had an affected parent (67 families), whereas the remaining had no known affected relatives and carried de novo germline mutations (2). In all inherited cases, CNC was passed on as an autosomal-dominant trait with an almost 100% penetrance.

### Clinical features

The clinical manifestations of CNC are quite variable and the full spectrum of the disease develops usually over a span of many years. Although the diagnosis is rarely made at birth, cases diagnosed as early as in the second year of life and as late as in the fifth decade of life are known with a median age at detection of 20 years old (2, 13). Table 1 summarizes all the clinical manifestations found in CNC patients.

### Cutaneous manifestations

Skin lesions are the most definitive indication of CNC (1) and more than 80% of the patients report pigmented spots or skin ‘growths’ that are easily recognizable typically early in life. They can vary from lentigines and blue nevi (in particular epithelioid blue nevi, small bluish domed papules with a smooth surface) (14) to cutaneous myxomas. Café-au-lait spots, irregular depigmented areas, many compound and, rarely, Spitz nevi have also been reported (12, 15, 16, 17).

Lentigines (flat small brown to black macules) usually appear before puberty, increase in number and pigment intensity during and after adolescence. They may be located everywhere on the body but a rather typical distribution exists on the face, the lips, genital area and mucosa (Fig. 1A, B, C, and D). Although fading is common in old age, they can still be seen even in the very elderly (8, 18, 19, 20). African Americans may manifest with slightly raised dark papules (21, 22).

Epithelioid blue nevus (EBN) is an interesting subtype of blue nevus that is very rare in the general population, but is relatively commonly seen in patients with CNC. EBN presents with intensive pigmentation and poorly circumscribed proliferative regions with associated dermal fibrosis (16, 23). EBN is not pathognomonic for CNC but is frequently associated with the disease, and its presence should alert the clinician to the possible diagnosis of the complex (15).

Cutaneous myxomas (Fig. 1D, E, and F) are found in 30–55% of CNC patients and usually appear in the eyelid, external ear canal, nipples and the genitalia (12, 15). These lesion can be localized in the dermis or the subcutaneous layer, and usually they are symptomless and <1 cm in diameter (24, 25). Rarely, a sharply circumscribed angiomyxoid nodule may be found (26).

### Ophthalmologic manifestations

The most common ophthalmologic manifestations are facial and palpebral lentigines, pigmented lesions of the caruncle or conjunctival semilunar fold and eyelid myxomas (27, 28). There are some reports of pigmented

<table>
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<th>Table 1 Summary of clinical manifestations of CNC.</th>
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schwannomas of the uvea (29). The differential diagnosis of pigmented lesions of the conjunctiva includes melanocytic nevus, melanosis (congenital or acquired), malignant melanoma or drug-induced secondary pigmentation (28, 30).

**Cardiac manifestations**

The most common noncutaneous lesions found in CNC are cardiac myxomas (in 20–40% of the patients) (11) which can appear early in infancy (17) but the median age at detection is at 20 years (31). Cardiac myxomas in CNC occur anywhere in the heart and are equally present in males and females (Fig. 1G and H). Their epidemiology should be contrasted with that of sporadic cardiac myxomas that develop almost exclusively in the left atrium and are far more frequent in older female patients. Cardiac myxomas present with symptoms related to intracardiac obstruction of blood flow or embolic phenomena (into the systemic circulation) like strokes and/or heart failure. They are responsible for more than 50% of the mortality of the disease (1, 2, 12, 13), myxomas can completely occlude a valvular orifice and may cause sudden death (14). This is why early detection and regular screening with echocardiography are essential (1, 32, 33); cardiac CT or magnetic resonance imaging (MRI) may also be used for the detection of these tumors, especially in post-operative hearts with altered anatomy (34).
Pituitary tumors
Up to 75% of CNC patients exhibit asymptomatic elevation of growth hormone (GH), insulin-like growth factor 1 (IGF1) or prolactin in the serum, abnormal response of GH to oral glucose tolerance test (OGTT) and ‘paradoxical’ response to thyrotropin (TSH)-releasing hormone without tumors detected on imaging studies (31, 35). The incidence of acromegaly due to pituitary tumors is around 10–12% in these patients (11, 12, 13, 36, 37). The adenomas usually appear during or after the third decade of life. Histological investigation of these tumors revealed somatomammotrophic hyperplasia (SH). SH is a putative precursor of GH-producing adenoma and may explain the protracted period of the onset of clinical acromegaly in individuals with CNC (12, 14). Patients can be followed by measuring GH and IGF1 levels and/or performing OGTT (11).

Rare prolactinomas have been also described (11). The tumors in operated CNC patients with acromegaly, frequently immunostained for both prolactin and alpha subunit in addition to GH. Interestingly, TSH, luteinizing hormone and follicle-stimulating hormone staining have also been seen in the foci of normal pituitary cells entrapped within the tumor or hyperplasia (38).

Adrenocortical tumors
The most common endocrine tumor in CNC is primary pigmented nodular adrenocortical disease (PPNAD), a cause of (adrenocorticotropic)-independent overproduction of cortisol (14). It affects between 25 and 60% of the individuals with CNC (70–71% female and 21% males) (12, 17, 31).

Few CNC patients present with PPNAD during the first 2–3 years of life (11); most present in the first 30 years of life (the observed ‘peak’ of diagnosis is during the second and third decades of life) (2, 14). Histologic evidence of PPNAD has been found in almost every individual with CNC who underwent autopsy, showing that a number of patients have asymptomatic PPNAD.

In PPNAD (Fig. 1J), the adrenal cortex is peppered with small pigmented nodules (<1 cm) surrounded by usually atrophic cortex (4, 39). Cortisol production can be variable, often cyclical or periodic, is characterized by a paradoxical rise in response to dexamethasone administration (2, 17, 40).

PPNAD continues to be a diagnostic challenge to the radiologist due to the small overall size of the adrenals and the small size of the pigmented nodules. To observe numerous pigmented nodules, CT slice thickness has to be 3 mm or less; if the slice thickness is 5 mm or more, the radiological report will be normal adrenals. The pigmented nodules are small, round, well delineated and hypodense compared to the rest of the adrenal parenchyma (4, 31).

Symptomatic individuals with PPNAD develop Cushing’s syndrome. The hypercortisolism of PPNAD is usually insidious at onset (14). Cyclical and other forms of atypical Cushing’s syndrome are also common among CNC patients (41).

Adrenocortical cancer has also been recently described in CNC patients. In those cases, co-secretion of androgen and cortisol and rapid occurrence of metastasis were observed (42).

Thyroid neoplasms
CNC patients may have thyroid nodules (up to 60% of all patients and two-thirds among children and adolescents) (12, 31, 43); their histology varies from benign thyroid adenomas (mostly follicular) in 25% of the cases (1, 12) to otherwise nonspecific cystic disease in 75% of the cases (14), to the rare thyroid cancer (papillary or follicular type) in up to 10% of the cases (2, 44). Thyroid nodules often appear during the first 10 years of life in CNC patients (17). Despite thyroid nodularity, CNC patients are clinically and biochemically euthyroid (43, 45).

Psammomatous melanotic schwannomas
Psammomatous melanotic schwannomas (PMS) is a rare tumor of the nerve sheath that has been reported in patients with CNC (up to 10%) (12, 14). PMS has frequent calcification and multi-centricity with heavy pigmentation and may be located anywhere in the central or peripheral nervous system (46, 47). The most frequent sites are in the gastrointestinal tract (esophagus, stomach, liver and rectum) and in the paraspinal sympathetic chain (28%) (11, 48). The chest wall, with involvement of adjacent ribs, is the third most common site (31). The spinal tumors present as pain and radiculopathy in adults (at a mean age of 32 years). Malignant degeneration occurs in ~10% of PMS associated with CNC (49, 50).

Testicular tumors
More than three-quarters of males with CNC may have large cell-calcifying Sertoli cell tumors (LCCSCT). Lesions may be bilateral, palpable and multifocal, with some risk
of malignancy and are associated with reduced fertility (22, 24, 51). LCCSCT are detected by ultrasonography as multiple and bilateral testicular microcalcifications that distinguish them from germ cell and other testicular tumors (2, 26). Leydig-cell tumors or hyperplasia and adrenocortical rest tumors (affected by PPNAD) have also been reported in CNC patients and should be considered especially in male patients with CNC and sexual precocity (24).

Breast tumors
Breast myxomas, often bilateral, occur in females with CNC after puberty. Both males and females may develop nipple myxomas at any age (11). In addition, ductal adenomas and myxoid fibroadenomas have been reported (12, 52, 53).

Ovarian lesions
Ovarian cysts and tumors of the ovarian surface epithelium, including serous cystadenomas and cystic teratomas have been reported in females with CNC. These are typically hypoechoic lesions by sonographic examination. They can grow or progress (rarely) to ovarian carcinoma (11, 12, 54).

Bone lesions
Osteochondromyxoma is a rare component of CNC characterized by a myxomatous tumor of the bone that may affects any bone but has been seen most frequently in the nasal sinuses and the long bones of the upper and lower extremities (14). They usually appear early in life (before the age of 2) (2). These bone lesions are benign, but both local invasiveness and recurrence have been reported (11).

Other lesions
Hepatocellular adenoma was first reported in a 19-year-old female patient (55). Uterine myxoid tumors were also reported in a single patient (56). Pancreatic neoplasms including acinar cell carcinoma, adenocarcinoma and intraductal pancreatic mucinous neoplasia, were seen in as many as 2.5% of CNC patients (12, 57). Other tumors like parathyroid mixed tumor, bronchogenic cysts, colonic, gastric carcinoma and peritoneal fibrous histiocytomas have also been described in rare cases (2, 11).

Diagnosis
CNC should be suspected in patients with a suggestive phenotype (31). Table 2 lists the criteria for the diagnosis of CNC (1, 2, 5, 11, 15, 17). A number of clinical and biochemical manifestations may also suggestive but they are not clearly diagnostic of CNC (sometimes these are mentioned as minor criteria) (Table 2) (58). The median age of diagnosis is 20 years (13). Depending on the affected organ and the type of lesion, there are other syndromes/disorders that will have a similar presentation (Table 3) (8, 14, 59, 60, 61, 62, 63, 64, 65, 66).

Treatment
In CNC, each specific complication/tumor should be addressed separately. Cardiac myxomas should be removed surgically; most patients, however, have two or more open-heart surgeries for recurrent tumors (11, 31, 67, 68). SH and/or GH-producing pituitary adenomas may be treated medically with somastotatin analogues or removed surgically (49). Similarly, for PPNAD, the best treatment is surgical, by bilateral adrenalectomy; however, medical adrenalectomy with inhibitors of steroidogenesis such as ketoconazole or mitotane may also be considered in selected cases (8, 17, 59, 69). Surgical excision of cutaneous and mammary myxomas may be needed, but it is not always necessary as these tumors are completely benign. Fine-needle aspiration for thyroid nodules is recommended in suspicious cases (2, 11) and thyroid cancer may be treated as it is appropriate for the histologic subtype. Boys with LCCSCT may develop gynecomastia, premature epiphyseal fusion and induction of central precocious puberty, and for them surgery and/or treatment with aromatase inhibitors may be needed (14, 19, 70). PMS is more difficult to treat because many of these tumors are located around nerve roots, a location that typically renders them inoperable (49). There is no effective treatment for metastatic PMS; CNC patients that develop metastatic PMS die of complications of the spread of the tumor usually in the lungs, liver or brain (13, 19).

Surveillance and follow-up of patients with CNC
Any patient with the diagnosis of CNC should be followed closely for clinical manifestations of the disease at least once a year. A study has shown that this type of follow-up improves prognosis (13, 58).
The suggested studies (71, 72, 73) include:

i) Annual echocardiogram, beginning in infancy; if a patient was diagnosed with a cardiac myxoma at least once, cardiac imaging may be done biannually.

ii) Regular skin evaluations.

iii) Blood tests to check serum levels of GH, prolactin and IGF1 beginning in adolescence, as appropriate for the detection of GH and prolactin excess; urinary free cortisol and other testing for screening of Cushing’s syndrome, as appropriate.

iv) Thyroid gland (neck) clinical examinations and with ultrasound, if needed.

v) Imaging may include adrenal computed tomography for the detection of PPNAD; pituitary MRI, and MRI of brain, spine, chest, abdomen, retroperitoneum and pelvis for the detection of PMS (14).

vi) In males, testicular examinations with ultrasound may be done annually for the detection and follow-up of LCCSCT.

vii) In females, transabdominal ultrasound of the ovaries (baseline examination; it may be repeated, as needed) (10).

viii) In pre-pubertal children: close monitoring of linear growth rate and annual pubertal staging (14).

Prognosis

The historic adjusted average life span for patients with CNC is 50–55 years, but with careful surveillance, life expectancy may be normal. The most common causes of death are related to complications of heart myxomas, such as emboli (strokes), post-operative cardiomyopathy and cardiac arrhythmias, as well as metastatic PMS, pancreatic and other cancers (14, 62, 74).

Genetics

CNC is caused by mutations in the PRKAR1A gene (OMIM 188830) coding for the regulatory subunit type I alpha of Table 2

<table>
<thead>
<tr>
<th>Diagnostic criteria for CNC a.</th>
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<tr>
<td>Major criteria</td>
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<tr>
<td>1. Spotty skin pigmentation with typical distribution (lips, conjunctiva and inner or outer canthi, vaginal and penile mucosal)</td>
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<tr>
<td>2. Myxoma b (cutaneous and mucosal) or cardiac myxoma a</td>
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<tr>
<td>3. Breast myxomatosis b or fat-suppressed magnetic resonance imaging findings suggestive of this diagnosis</td>
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<tr>
<td>4. PPNAD b or paradoxical positive response of urinary glucocorticosteroid excretion to dexamethasone administration during Liddle’s test</td>
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<tr>
<td>5. Acromegaly as a result of growth hormone (GH)-producing adenoma a</td>
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<tr>
<td>6. LCCSCT b or characteristic calcification on testicular ultrasound</td>
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<tr>
<td>7. Thyroid carcinoma a (at any age) or multiple hypoechoic nodules on thyroid ultrasound in prepubertal child</td>
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<tr>
<td>8. Psammomatous melanotic schwannomas (PMS) b</td>
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<tr>
<td>9. Blue nevus, epithelioid blue nevus (multiple) b</td>
</tr>
<tr>
<td>10. Breast ductal adenoma (multiple) b</td>
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<tr>
<td>11. Osteochondromyxoma b</td>
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<tr>
<td>Supplemental criteria</td>
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<tr>
<td>1. Affected first-degree relative</td>
</tr>
<tr>
<td>2. Activating pathogenic variants of PRKACA (single base substitutions and copy number variation) and PRKACB (83, 84)</td>
</tr>
<tr>
<td>3. Inactivating mutation of the PRKAR1A gene (44)</td>
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Findings suggestive of or possibly associated with CNC, but not diagnostic for the disease (minor criteria)

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 IGF1 levels, abnormal glucose tolerance test (GTT), or paradoxical GH response to thyrotropin-releasing hormone (TRH) testing in the absence of clinical acromegaly
5. Cardiomyopathy
6. History of Cushing’s syndrome, acromegaly or sudden death in extended family
7. Pilonidal sinus
8. Colonic polyps (usually in association with acromegaly)
9. Multiple skin tags or other skin lesions; lipomas
10. Hyperprolactinemia (usually mild and almost always combined with clinical or subclinical acromegaly)
11. Single, benign thyroid nodule in a child younger than age 18 years; multiple thyroid nodules in an individual older than age 18 years (detected on ultrasound examination)
12. Family history of carcinoma, in particular of the thyroid, colon, pancreas and ovary; other multiple benign or malignant tumors

*To make the diagnosis of CNC, a patient must either (1) exhibit two of the major criteria confirmed by histology, imaging or biochemical testing or meet (2) one major criterion and one supplemental one.

*With histologic confirmation.
the protein kinase A (PKA, cAMP-dependent protein kinase) enzyme. PRKAR1A is situated at the 24.2–24.3 locus of the long arm of chromosome 17 and has 11 exons, of which exons 2–11 are protein-coding (75). More than 70% of the patients diagnosed with CNC carry mutations on the PRKAR1A gene (CNC1 locus) and this percentage increases to 80% for those with Cushing’s syndrome due to PPNAD (12, 76). Some families mapped to the CNC2 locus; the majority of these cases presented later in life (10, 77). PRKACA copy number gain (CNG) is seen in patients with adrenal hyperplasias but not in CNC. PRKACB CNG has only been seen in one patient with CNC. For the majority of the PRKAR1A-negative CNC cases the genetic cause remains unknown.

To date, more than 125 PRKAR1A pathogenic mutations have been reported (http://prkar1a.nichd.nih.gov/hmdb/intro.html) in 401 unrelated families of diverse ethnic origin (Table 4) (12, 61, 78, 79). The PRKAR1A pathogenic mutations include single-base substitutions, small (<15 bp) deletions/insertions, combined rearrangements that are spread along the whole open reading frame of the gene and large deletions that cover most of the exons and in some cases the whole gene locus (70, 78, 80). Most of these mutations are unique (presented in a single kindred), and only three pathogenic variants (c.82C>T, c.491_492delTG and c.709-2_709-7 delATTTTT) have been identified in more than three unrelated pedigrees (10, 12, 81).

A second genetic locus is associated with CNC and it is referred to as the ‘CNC2’ locus (CNC1 being the PRKAR1A gene 17q locus) (Table 4). CNC2 is a 10 Mb region in the 2p16 locus that has been detected through linkage analysis in PRKAR1A-negative patients with CNC and further delineated by copy number changes in CNC tumors (12, 78, 82). To date, the gene(s) residing in the 2p16 region that may be responsible for a CNC phenotype or progression of its tumors remain(s) unknown.

Two recent studies associated elements of the CNC phenotype with PRKACA and PRKACB gene defects (Table 4). Comparative genomic hybridization (CGH) in 35 patients with cortisol-producing bilateral adrenal hyperplasia and overt Cushing’s syndrome identified five patients with CNGs of the genomic region that included the PRKACA gene on chromosome 19 (83). In a single individual with CNC, developmental delay and skeletal defects were identified; in her, the disease was caused by somatic PRKACB gene locus CNGs on chromosome 1 (84). The precise roles of PRKACA and PRKACB in the CNC phenotype remain to be elucidated.

### Molecular pathogenesis of CNC: PKA and its subunits

PRKAR1A gene encodes for the most widely expressed regulatory subunit of the PKA enzyme. The PKA heterotetramer consists of two regulatory (R) and two catalytic (C) subunits. Stimulation of adenyl cyclases through G protein subunit (Gs) activation leads to cAMP synthesis (Fig. 2). cAMP, binds to the regulatory subunits and leads to their dissociation from the catalytic subunits.
<table>
<thead>
<tr>
<th>Locus/gene</th>
<th>Chromosomal locus</th>
<th>Mutation</th>
<th>Mutation type</th>
<th>Expression/protein</th>
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<tr>
<td>PRKAR1A (CNC1)</td>
<td>17q24, (12, 70, 78)</td>
<td>c.26G &gt; A, c.206A &gt; G, c.439A &gt; G,c.440G &gt; A, c.502G &gt; A, c.547G &gt; T</td>
<td>Missense</td>
<td>Altered protein</td>
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<tr>
<td>c.550G &gt; A, c.638C &gt; A, c.865G &gt; T</td>
<td>Missense</td>
<td>NMD</td>
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<tr>
<td>c.220C &gt; T, c.438A &gt; T, c.545 C &gt; G</td>
<td>Non-sense</td>
<td>NMD</td>
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<tr>
<td>c.592G &gt; A, c.627G &gt; A, c.632G &gt; T</td>
<td>Missense</td>
<td>NMD</td>
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<tr>
<td>c.258G &gt; A, c.377G &gt; A, c.382G &gt; T</td>
<td>Missense</td>
<td>NMD</td>
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<tr>
<td>c.708G &gt; A, c.710G &gt; T</td>
<td>Missense</td>
<td>NMD</td>
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<tr>
<td>c.709(-107 K) del103</td>
<td>Frameshift</td>
<td>Elongated protein</td>
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<tr>
<td>c.440 +1G &gt; A, c.440 +1_440 +2insG</td>
<td>Splice site</td>
<td>NMD/shorter protein/skip exon</td>
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<td>Large deletions (one exon-whole gene)</td>
<td>NMD/allele depletion</td>
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The catalytic subunits after their dissociation from the PKA complex phosphorylate many downstream factors such as the CREB (Fig. 2). PRKAR1A defects associated with CNC lead to PRKAR1A haploinsufficiency and, thus, to the loss of this regulatory subunit’s function: ‘unrestrained’ catalytic subunit activity leads to increased cell proliferation in cAMP-responsive tissues and tumor formation in tissues affected by CNC (10, 85).

Almost all PRKAR1A nonsense substitutions, small insertions/deletions, variations of splicing sites lead to frame shifts and/or premature stop codons that result in shorter or otherwise defective mRNAs, which are not encoded to protein because they are degraded by the nonsense-mediated mRNA decay (NMD) surveillance mechanism (10, 79, 86). Large deletions that include the whole gene also lead to PRKAR1A haploinsufficiency and almost half the protein levels compared to normal cells (70, 80).

Rarely, missense mutations of the PRKAR1A gene, short in-frame insertions/deletions and splice variants that are expressed at the protein level (because NMD is not activated) lead to the disease, not due to haploinsufficiency but to a defective protein that fails to respond appropriately to cAMP or does not bind effectively to the PKA catalytic subunits (87, 88). Mutations that give rise to alternate splice sites may also be pathogenic by either leading to mRNA that is subject to NMD or a protein that does not bind effectively to cAMP or the catalytic subunits (81). Some PRKAR1A mutations lead to a longer protein due to the loss of the stop codon normally situated in the last coding exon that is not subject to NMD. These longer PRKAR1A proteins undergo proteosomal degradation resulting again in PRKAR1A haploinsufficiency (89).

There are groups of CNC patients who show specific genotype-phenotype correlation, and this also explains the CNC heterogeneity (12, 79). For example, mutation c.709–7del6 is present in most patients with isolated PPNAD (81), and most of the remaining were c.1A>G carriers (79). It is difficult to conceive what molecular mechanisms underlie these phenotypic differences, since all these mutations lead to NMD. One suggestion is that small amounts of mutant RIα protein are in fact...
produced at least in certain tissues. These phenotype-genotype correlations can be due to their high frequency, and that additional phenotypic differences between the various PRKAR1A mutations leading to NMD will emerge as the number of observations increases. On the other hand, mutations that lead to an alternate PRKAR1A protein and not NMD (see Table 1) are associated with an overall higher total number of CNC manifestations and this could be due to a dominant-negative effect of the expressed mutant protein (5, 12, 78).

Mouse models have been created in order to study the role of PRKAR1A in CNC and the ability to cause CNC phenotypes. Prkar1a+/− mice developed non-pigmented schwannomas and fibro-osseous bone lesions beginning around 6 months of age. Later in life, 10% of the mice also developed thyroid tumors (90). Another mouse model that had significantly higher Prkar1a down-regulation and significantly higher cAMP signaling produced a more severe CNC phenotype (91).

In the cases where PRKACA and PRKACB amplification was found, the mechanism of disease is presumed to be increased free PKA catalytic subunit activity (83). Although the molecular stoichiometry of how this occurs remains to be studied in vitro and in animal studies, tissues from patients with PRKACA and PRKACB CNG both had increased PKA activity (83). This is less clearly understood in the single patient with PRKACB gene amplification, since based on what is known about PKA, two additional copies of the gene (and modestly higher Cβ protein levels) should have had minimal effects on total PKA activity; nevertheless, the defect led to an unquestionable phenotype of CNC with pigmented spots, myxomas and a growth-hormone (GH)-producing pituitary tumor (84).

Genetic counseling
The identification of a pathogenic variant in the PRKAR1A gene can be used for diagnosing CNC. Molecular testing may be suggested then for relatives, or for new patients with two or more diagnostic criteria. If sequencing of the PRKAR1A gene does not show a defect, copy number variant analysis by CGH and/or deletion PRKAR1A gene deletion testing may be needed to rule out a PRKAR1A defect. If all testing is negative for PRKAR1A defects, other candidate genes or loci may be screened including the PRKACA, PRKACB and the phosphodiesterase genes. However, the latter are mostly limited to research at this point.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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