Genetics of adrenocortical tumors: Carney complex

C.A. Stratakis

Unit on Genetics and Endocrinology, DEB, NICHD, NIH, Building 10, Room 10N262, 10 Center Dr. MSC1862, Bethesda, Maryland 20892-1862, USA.

Reprint Requests: C.A. Stratakis, address above
e-mail: stratak@c1.nichd.nih.gov

Introduction on Adrenocortical Cancer

Benign adrenal tumors are common [1-12]. Malignant neoplasias of the adrenal cortex account for 0.05-0.2 % of all cancers, with an approximate prevalence of two new cases per million of population per year [1-4]. Adrenal cancer occurs at all ages, from early infancy to the eighth decade of life. A bimodal age distribution has been reported, with the first peak occurring before age of 5 years, and the second in the fourth to fifth decade. In all published series, females predominate, accounting for 65-90 % of the reported cases.

In some areas of the world, higher incidence of adrenal cancer, especially in children, has been documented. This is particularly true for Southern Brazil, where environmental mutagens have been postulated as the relevant pathogenic event [13]. Although the incidence of adrenal incidentalomas appears to be higher in some familial neoplasia syndromes like multiple endocrine neoplasia type-1 (MEN 1) and familial adenomatous polyposis (FAP), it is unclear whether this finding is accompanied by a higher predisposition to adrenal cancer.

Molecular Genetics of Adrenocortical Cancer

The genetic background of adrenocortical cancer remains poorly cha-
we have identified two genetic loci harboring genes for PPNAD and/or CNC on chromosomal loci 2p16 and 17q22-24. The chromosome 17 gene, PRKAR1A, was recently cloned and the identification of other responsible genes is currently under way in our, and collaborating laboratories. The present report reviews the genetics of adrenocortical cancer first, followed by what is known today about the genetics of PPNAD and/or CNC.

Key words: Adrenal cortex, genetics, tumor, primary pigmented adrenocortical disease (PPNAD), Carney complex.

racterized despite recent advances in the molecular understanding of adrenal function. Adrenocortical hyperplasia is a polyclonal process but carcinomas are monocular lesions [6, 7], indicating that genetic changes at specific loci in the genome are needed for adrenal tumorigenesis [4]. Investigations have focused on obvious candidates, such as the corticotropin (ACTH) receptor (the MC2R gene) and molecules that participate in its signalling pathway, including the guanine-nucleotide binding protein (G-proteins) subunits Gsa and Gia2. Although mutations have not been found, loss-of-heterozygosity (LOH) of the MC2R gene locus on the short arm of chromosome 18 (18p11.2) was frequent in carcinomas but not in adenomas, suggesting that, perhaps, LOH of this gene participates in the dedifferentiation process leading to adrenocortical carcinogenesis. The Gsa gene (the Gsp proto-oncogene) has not been found mutated in adrenal cancer, but patients with McCune-Albright syndrome who bear somatic mutations of this gene, do develop benign adrenal lesions. A fraction of adrenal carcinomas harbor mutations of the gene that codes for Gia2. Other candidate genes that have been investigated in adrenocortical carcinomas include those coding for aldosterone synthase (the CYP11B2 gene) and 21-hydroxylase (the CYP21B gene), and for aldosterone-producing carcinomas, the angiotensin-II type-1 (AT-1) receptor gene. The MEN 1 gene, menin, and the FAP gene, APC, have also been investigated as possible candidates because patients with MEN1 and FAP do get adrenal tumors, which, however, are mostly benign and non-functional.

Cytokines and growth factors and their receptors, which may be expressed eutopically or ectopically in adrenocortical tissue, have been implicated in carcinogenesis [5, 8, 9]. Expression of the major histocompatibility class-II (MHC-II) antigens in adrenocortical tissue correlates with adrenocortical cell differentiation. The expression of both transforming growth factor-α (TGFα) and epidermal growth factor receptor (EGFR) is markedly elevated in carcinomas (unlike adenomas) and naptophysin and other neuroendocrine markers are « inappropriately » expressed in adrenocortical cancer. The unexpected presence of proteins with neuroendocrine and other functions in adrenal cancer follows a pattern similar to that observed in benign adrenocortical hyperplasias [8, 9], although in cancer it seems to occur in a wider scale. It is also worth noting that cortisol-producing adrenocortical carcinomas often respond to dexamethasone administration with a « paradoxical » rise of their glucocorticoid production, a feature that is almost universally present in primary pigmented adrenocortical disease (PPNAD), a benign, bilateral hyperplasia of the adrenal cortex [10] (see below).

Molecular cytogenetic cloning approaches have successfully been employed in the investigation of the genetics of ACT. Thus, a number of chromosomal abnormalities have been implicated in adrenocortical tumorigenesis, including genomic loci on chromosomes 11 and 17. These include the genes coding for p53 (TP53) (on 17p13.1), p57 (on 11p15.5) (KIP2), and the insulin-like growth factor type-II (IGF-II) (on 11p15.5) [4,7]. LOH of the chromosome 17 locus of the gene that codes for p53 in tumors from patients with Li-Fraumeni syndrome (LFS), led to the identification of germline TP53 mutations in this genetic condition. However, LFS patients develop adrenal cancer rarely. In sporadic cancer, TP53 mutations may be present in approximately 30 % to 50 % of all lesions but p53 expression does not correlate with prognosis and it is rarely seen in monolclonal but highly differentiated tumors. The latter finding suggests that TP53 mutations in sporadic cancer are a late event in the process of carcinogenesis, suggesting that other genetic events precede and may even predispose to TP53 mutations in adrenal cancer.

Comparative genomic hybridization (CGH) is a molecular cytogenetic technique, which allows a genome-wide screening of tumor DNA to identify chromosomal gains and losses. Regions of gains may contain dominantly acting oncogenes, while tumor suppressor genes may map to deleted regions [4, 13]. One important advantage of CGH is that frozen or paraffin-embedded samples can be evaluated because only tumor DNA — not cells in culture — is required for the analysis. In a recent CGH study, 8 adrenal carcinomas and 14 adenomas from adult patients were investigated [12]. The most common genetic aberrations in carcinomas were gains of chromosomes 4 and 5 and losses of chromosomes 11 and 17. CGH was also used to investigate genetic events leading to adrenal tumor formation in children from a region in southern Brazil (Curitiba), which, along with the state of Sao Paulo, has the highest incidence of these tumors worldwide.
INTRODUCTION ON PRIMARY PIGMENTED ADRENOCORTICAL DISEASE (PPNAD)

In recent years, two primary adrenal disorders affecting the adrenal cortex have been implicated in the pathogenesis of corticotrophin (ACTH)-independent Cushing syndrome (CS) [9]. Primary pigmented adrenocortical disease (PPNAD), also known as « micronodular adrenal disease », is a congenital disorder, which, in the majority of the reported cases, is associated with Carney complex. The complex is a multiple endocrine neoplasia (MEN) syndrome that affects the adrenal cortex and other endocrine glands, and is associated with abnormal pigmentation of the skin and mucosae, myxomas, and other neoplasms [14]. Massive macronodular adrenocortical disease, is another form of bilateral adrenal hyperplasia, which leads to CS but is not associated with any other clinical findings. Macronodular disease should be contrasted with PPNAD: It is not congenital, almost always occurs in older patients, and its etiology is unclear. Other forms of bilateral adrenocortical hyperplasia distinct from PPNAD and not always associated with hypercortisolism, include the lesions of the adrenal glands described in patients with the McCune-Albright and MEN type-1 syndromes. In the following text, we will focus on PPNAD and Carney complex.

Features of PPNAD

In case reports dating back as early as 1949, children and young adults were described with pituitary-independent CS and a unique type of adrenal pathology: a bilateral form of adrenal hyperplasia, characterized by multiple, small, pigmented, adrenocortical nodules that were surrounded by internodular cortical atrophy [10]. Various names were given to this peculiar lesion, the most common of which was « micronodular adrenal disease »; this was later replaced by « primary pigmented nodular adrenal disease » (PPNAD), a term that was first coined by Dr. J. Aidan Carney in 1984.

PPNAD may occur independently or, more commonly, as part of the complex of « spotty skin pigmentation, myxomas and endocrine overactivity » or Carney complex, which was described in 1985. This syndrome also encompasses several familial cases of cutaneous and cardiac myxomas associated with lentigines and blue nevi of the skin and mucosae, which have been described under the acronym NAME (for nevi, atrial myxoma, myxoid neurofibromata, and ephelides) and LAMB (for lentigines, atrial myxoma, mucocutaneous myxoma, blue nevi) syndromes.

Patients with PPNAD often present with a variant CS called “atypical” (ACS) [10], which is characterized by an asthenic, rather than obese, body habitus. This phenotype is caused by severe osteoporosis, short stature, and severe muscle and skin wasting. Patients with ACS tend to have normal or near-normal 24-hour urinary free cortisol (UFC) production, but this is characterized by the absence of the normal circadian rhythm of cortisol. Occasionally, normal cortisol production is interrupted by days or weeks of hypercortisolism, which gives rise to a yet another variant called « periodic CS » (PCS). PCS is frequently found in children and adolescents with PPNAD. In both ACS and PCS, as well as in classic CS, caused by PPNAD, paradoxical increase of UFC and/or 17-hydroxy-corticosteroids (17-OHS) is seen during the second phase (high dose dexamethasone administration) of the Liddle’s test.

PPNAD only rarely is present isolated. At the National Institutes of Health (NIH), where vigorous screening for Carney complex signs has been instituted under a research protocol, over 25 patients have been treated for PPNAD since 1968. All these patients met the diagnostic criteria for Carney complex that were developed by Stratakis et al. [15], with the exception of a 32-year-old patient, who had PPNAD in her mid-twenties, underwent adrenalectomy and has had no other tumors or
skin pigmentation characteristic of the complex. Thus, we believe that fewer than 10% of the PPNAD cases represent isolated forms of the disease; most of these patients have Carney complex, a syndrome that has a well-defined, but extremely variable phenotype.

**Carney complex**

Among the individual components of Carney complex, the cardiac myxoma is the one most responsible for the significant morbidity and mortality associated with the syndrome. This tumor occurs often at multiple sites (affecting any or all cardiac chambers), at a relatively young age, and is equally distributed between the sexes [14]. The cutaneous myxomas have a predilection for the eyelids and external ear canals, although they may affect any part of the skin. Mammary myxoid tumors may also occur at multiple sites and be bilateral; even the clinically « normal » breast of patients with the complex commonly shows microscopic foci of myxomatous masses, which can be identified by MRI.

The centrofacial spotty pigmentation of Carney complex involves the vermilion border of the lips and the conjunctiva. The pigmented spots may be tan, irregularly shaped and poorly outlined, or small, sharply delineated, and dark brown to black. The conjunctival pigmentation typically affects the lacrimal caruncle and the conjunctival semilunar fold, and may involve the sclera. Five to 10% of patients with Carney complex have one or more intraoral pigmented spots, and the female external genitalia are commonly heavily pigmented. Blue nevi (the usual type, as well as the exceptionally rare epithelioid type) and combined and common junctional, dermal, and compound nevi, as well as café-au-lait spots also occur in the syndrome.

About 10% of patients with Carney complex have a GH-secreting pituitary adenoma that results in acromegaly. Although most of the known patients with this condition had macroadenomas, a number of recently investigated cases show that abnormal 24-hour GH secretion can precede the development of a pituitary tumor in the complex. The disorder, therefore, provides the unusual opportunity for prospective screening of affected patients without clinical acromegaly. In one such case, serial measurements of GH or somatomedin C, or both, became progressively abnormal over several years; recently, a pituitary mass was identified on computed tomography, and partial hypophysectomy revealed minute foci of a GH-producing adenoma.

Endocrine involvement in Carney complex also includes three types of testicular tumors: large-cell calcifying Sertoli cell tumor (among the rarest of testicular neoplasms), adrenocortical rests, and Leydig cell tumor. About one-third of affected male patients have these masses. The large-cell calcifying Sertoli cell tumor, a bilateral, multicentric, and benign neoplasm, may secrete estrogens and cause precocious puberty, gynecomastia, or both. Finally, three new components of the syndrome have been identified: psammomatomatous melanotic schwannoma, epithelioid blue nevus, and ductal adenoma of the breast. Because thyroid follicular neoplasms, both benign and malignant, have been found in a number of patients, thyroid involvement is also considered a component of the syndrome.

**GENETICS OF CARNEY COMPLEX AND PPNAD**

Like the other MEN syndromes, Carney complex is inherited in an autosomal dominant manner. Sporadic cases constitute approximately half of the known patients [14]. A genetic locus was determined for Carney complex by linkage analysis of polymorphic markers from likely areas of the genome [15]. Positive lod scores were obtained for nine markers on the short arm of chromosome 2, identifying an approximately 4 centiMorgan (cM)-long area in the cytogenetic band 2p16 (CNC locus), which is likely to contain at least one of the loci responsible for the complex. This region includes the D2S123 locus, where another genetic syndrome, hereditary nonpolyposis colorectal cancer (HNPPC), was recently mapped.

The mapping of the Carney complex gene at a locus where genes responsible for regulating DNA stability reside is of particular interest, since several lines of evidence indicate that chromosomal instability may be a feature of the tumor cell lines established from patients with the lentiginosis syndromes. Indeed, the formation of telomeric associations and dicentric chromosomes is a frequent feature of fibroblasts derived from the myxoid tumors excised from patients with Carney complex; a recent study found similar features in cultured in vitro adrenocortical cells derived from PPNAD nodules [16]; microsatellite analysis of the tumors excised from patients with Carney complex confirmed the significant genomic instability that accompanies tumorigenesis in this syndrome [16]. Numerous areas of loss or gain of heterozygosity, and/or deletions, involving all 22 autosomal chromosomes were found. These changes did not include the CNC locus on chromosome 2p16, a finding that suggests that alterations of the heterozygosity of the responsible gene(s) may not be necessary for oncogenesis in this condition. Although mutations of the gsp proto-oncogene were not present in Carney complex tumors, and the locations of several genes that code for components of the guanine nucleotide-binding proteins (G-proteins) were excluded by linkage analysis [15], it seems likely that the gene or genes responsible...
for this condition may participate in G-protein-controlled or -related signalling systems.

Recently, a family with Carney complex that does not map to chromosome 2p16 was reported. Indeed, a major locus was identified recently on chromosome 17 (17q22-24) [17]. It remains to be seen what the contribution is of each one of the 2 loci to the genetics of PPNAD/Carney complex*.

CONCLUSION
This is currently an exciting time for clinicians and basic scientists interested in primary diseases of the adrenal glands. As the molecular basis of these conditions becomes better understood, basic researchers will be provided with a detailed insight into adrenocortical function and early differentiation, and clinicians will offer better therapies to patients affected by these disorders.

REFERENCES

* Note added in proof: Since the submission of this paper, the chromosome 17 gene that is responsible for Carney complex was identified in Dr Stratakis’ laboratory [Kirschner et al. Mutations of the gene encoding the protein kinase A type I-alpha regulatory subunit in patients with the Carney complex. Nat Genet 2000; 26 (11): 89-92]. Additional mutations and elucidation of the contribution of the two loci in the disease phenotype were most recently also published from the same group [Kirschner et al. Genetic heterogeneity and spectrum of mutations of the PRKAR1A gene in patients with the carney complex. Hum Mol Genet 2000; 9 (20): 3037-46].