Adrenocortical cancer: pathophysiology and clinical management

Rossella Libè¹,2,3,4, Amato Fratticci¹,5 and Jérôme Bertherat¹,2,3,4,6

¹INSERM U567, Endocrinology, Metabolism and Cancer Department, Institut Cochin, Paris, France
²CNRS UMR8104, Paris, France
³Université Paris-Descartes, Site Cochin-Port-Royal, Paris, France
⁴Assistance Publique Hôpitaux de Paris, Hôpital Cochin, Oncogenetic Unit, Paris, France
⁵Department of Experimental Medicine, University of l’Aquila, l’Aquila, Italy
⁶Department of Endocrinology, Assistance Publique Hôpitaux de Paris, Hôpital Cochin, Reference Center for Rare Adrenal Diseases, Paris, France

(Requests for offprints should be addressed to J Bertherat, Service des Maladies Endocriniennes et Métaboliques, Hôpital Cochin, 27, rue du Faubourg Saint-Jacques, 75014 Paris, France; Email: jerome.bertherat@cch.aphp.fr)

Abstract

Adrenocortical cancer (ACC) is a rare tumor with a poor prognosis. By contrast, benign adrenocortical tumors are frequent, underlying the importance of a correct diagnosis of malignancy of such tumors. ACC can be diagnosed by the investigation of endocrine signs of steroid excess, symptoms due to tumor growth or an adrenal incidentaloma. Hormonal investigations demonstrate in most ACC steroid oversecretion, the dominant characteristics being a co-secretion of cortisol and androgens. Imaging by CT-scan or MRI shows a large heterogeneous tumor with a low fat content. Careful pathological investigation with the assessment of the Weiss score is important for the diagnosis of malignancy. Molecular markers can also be helpful and in the future might be important for prognosis. Tumors localized to the adrenal gland (McFarlane stages 1 and 2) have a better outcome than invasive and metastatic tumors (stages 3 and 4). Tumor removal by a specialized team is crucial for treatment and should always aim at complete removal. In patients with metastatic or progressive disease, medical treatment is started with mitotane that requires a close monitoring of its blood level. Surgery is indicated when possible for local recurrence but also in some cases of metastasis. Local treatment (radiofrequency, chemoembolization, and radiation therapy) can have some indications for metastatic disease. In patients with disease progression cytotoxic chemotherapy can be used. Despite the best care, the overall prognosis of ACC is poor with a 5-year survival rate below 30% in most series. Therefore, progress in the understanding of the pathophysiology of ACC is important. Despite the rarity of ACC, significant advances have been made in the understanding of its pathogenesis in the last decade. These progresses came mainly from the study of the genetics of ACC, both at the germline level in rare familial diseases, and at the somatic level by the study of molecular alterations in sporadic tumors. These advances underline the importance of genetic alterations in ACC development and point-out to various chromosomal regions (2, 11p15, 11q, 17p13) and genes (IGF-II, p53, β-catenin, ACTH receptor). This review will summarize these advances as well as the current clinical management of ACC.

Endocrine-Related Cancer (2007) 14 13–28

Introduction

Adrenocortical tumors, mostly benign adenomas, are frequent in the general population and nowadays most often found incidentally (Grumbach et al. 2003). By contrast, adrenocortical cancer (ACC) is rare, with an estimated prevalence between 4 and 12 per million in adults (Grumbach et al. 2003). Despite this rarity, malignancy is always feared because of its poor prognosis when investigating an adrenocortical mass (Luton et al. 2000). The diagnosis of malignancy of adrenocortical tumors relies on careful investigations of clinical, biological and imaging features before surgery and pathological examination after tumor removal.
Progress in the understanding of the pathophysiology of ACC is important to improve diagnosis, prognosis evaluation, and treatment. This review will summarize the advances in the molecular mechanisms of adrenocortical tumors development that have recently been made and the current clinical management of ACC.

Pathophysiology of adrenocortical cancer

The analysis of tumor clonality is an important step to establish the cellular origin of neoplasms and to identify the mechanisms underlying tumor progression. Monoclonality indicates that tumor progression is the end result of an intrinsic genetic mutation, whereas polyclonality suggests that tumor cells are affected by local or systemic stimuli. Analysis of the pattern of X-chromosome inactivation in heterozygous female tissue has shown that ACC consists of monoclonal populations of cells, whereas benign tumors might be monoclonal as well as polyclonal (Beuschlein et al. 1994, Gicquel et al. 1994).

Monoclonal tumors result from genetic alterations conferring a growth advantage to the cell initially affected. These genetic events can be studied at the scale of the whole genome, as losses or gains of part or all of a chromosome. A large number of molecular techniques, such as comparative genomic hybridization (CGH) and microsatellite analysis, can be used in genome-wide screen for such chromosomal alterations. These approaches have identified alterations affecting various chromosomes and loci. Interestingly, a positive correlation has been observed between tumor size and the number of CGH changes in adrenocortical tumors, suggesting that chromosomal alterations accumulate during tumor progression (Sidhu et al. 2002). It was demonstrated by CGH that chromosomal alterations are observed in 28% of benign adrenocortical tumors (Kjellman et al. 1996). Most of the changes observed concern losses on chromosomes 2, 11q and 17p and gains on chromosomes 4 and 5 (Kjellman et al. 1999, Zhao et al. 1999, Dohna et al. 2000, Sidhu et al. 2002).

In more recent studies, CGH identified changes in 61% of benign tumors and the most common gains observed were on chromosomes 5, 12, 19, and 4 (Sidhu et al. 2002). Losses were observed at 1p, 17p, 22p, 22q, 2q, and 11q in up to 62% of cases of ACC. Studies using microsatellite markers have demonstrated a high percentage of loss of heterozygosity (LOH) or allelic imbalance at 11q13 (≥90%), 17p13 (≥85%), and 2p16 (92%) in ACC (Kjellman et al. 1999, Gicquel et al. 2001).

The genes involved in these molecular alterations could be classified as tumor suppressor genes on one hand, and oncogenes on the other hand. Molecular alterations would lead to inactivation of the tumor suppressor genes and activation of the oncogenes. This simple way to classify the various alterations involved in oncogenesis will be used in this paragraph to review the pathophysiology of ACC.

Oncogenes

IGF-II (insulin-like growth factor II)

The IGF-II gene located at 11p15 encodes an important fetal growth factor, is maternally imprinted and is therefore expressed only from the paternal allele (DeChiara et al. 1991; Fig. 1). The 11p15 region is

![Figure 1](https://www.endocrinology-journals.org)

**Figure 1** Alterations of 11p15 locus and IGF-II overexpression in ACC. The imprinted 11p15 locus contains the CDKN1C (p57kip2), IGF-II, and H19 genes. In normal differentiated tissue (on the left), only the paternal allele of the IGF-II gene is expressed, whereas only the maternal alleles of CDKN1C and H19 are expressed. Paternal isodisomy is usually observed in adrenal cancers (on the right) with loss of the maternal allele at 11p15. This leads to the overexpression of IGF-II and decreased expression of CDKN1C and H19. According to the studies performed in cells lines, IGF-II could act as an autocrine growth factor through binding to the IGF-I receptor on adrenocortical cancer cells.
organized into two different clusters: a telomeric domain including the IGF-II gene (DeChiara et al. 1991), H19 (Hao et al. 1993) and a centromeric domain including CDKNIC (p57kip2; Lee et al. 1995, Matsuoka et al. 1995). The H19 mRNA is not translated and this gene may modulate IGF-II expression. The p57kip2 gene encodes a cyclin-dependent kinase inhibitor involved in the G1/S phase of the cell cycle. The H19 and p57kip2 genes are paternally imprinted and are therefore expressed from the maternal allele only. Genetic or epigenetic changes in the imprinted 11p15 region, resulting in increases in IGF-II expression, and mutations of the p57kip2 gene have been implicated in Beckwith–Wiedemann syndrome (Lam et al. 1999). This overgrowth disorder is characterized by macrosomia, macroglossia, organomegaly, and developmental abnormalities (in particular, abdominal wall defects with exomphalos), embryonal tumors, such as Wilms’ tumor – and ACC (Wiedmann 1983, Hertel et al. 2003), neuroblastoma, and hepatoblastoma.

IGF-II mRNA is efficiently translated and malignant tumors contain large amounts of IGF-II protein, some of which is in the prohormone form. The insulin-like growth factors system is involved in the development of the adrenal cortex and its role has been largely documented in adrenocortical tumors (Mesiano et al. 1993, Gicquel et al. 1995, 2001). Many studies have demonstrated that IGF-II is strongly overexpressed in malignant adrenocortical tumors, with such overexpression observed in approximately 90% of ACC (Ilvesmaki et al. 1993a, Gicquel et al. 1997, Boulle et al. 1998; Fig. 2). Transcriptome analysis of adrenocortical tumors has demonstrated that IGF-II is the gene most overexpressed in ACC by comparison with benign adrenocortical adenomas or normal adrenal glands (Giordano et al. 2003, Bourdeau et al. 2004, de Fraipont et al. 2005) The mechanisms underlying IGF-II overexpression are paternal isodisomy (loss of the maternal allele and duplication of the paternal allele) or, less frequently, loss of imprinting (Ogawa et al. 1993, Rainier et al. 1993); with maintenance of both parental alleles but a paternal-like IGF-II gene expression pattern (Gicquel et al. 1997).

Receptors for IGF-I and IGF-II are present in adrenal tissues and strong overexpression of intact IGF-I receptors has been shown in ACC (Weber et al. 1997a). The mitogenic effect of IGF-II is dependent on the IGF-I receptor, as reported by Logie et al. (1999), who demonstrated that IGF-II is involved in the H295R cell line (derived from an ACC) proliferation and acts through the IGF-I receptor. IGF-II effects are restricted to tumors and plasma IGF-II concentrations are usually in the normal range. The biological effects of IGFs are modulated in vivo by six IGF-binding proteins (IGFBPs), which positively or negatively regulate the effects of IGFs, depending on their abundance and affinity for growth factors. H295R cells and adrenocortical tumors with IGF-II overproduction have been shown to contain large amounts of IGFBP-2 (Boulle et al. 1998), suggesting that IGFBP-2 may regulate IGF-II effects in ACC. Furthermore, IGFBP-2 levels have been shown to correlate with tumor stage in ACC.
In ACC, only the maternal $H19$ allele is expressed, so expression of this gene is abolished in most ACC displaying paternal isodisomy (Gicquel et al. 1997). Methylation of the $H19$ promoter has been shown to be involved in the abnormal expression of both $H19$ and $IGF-II$ in human ACC (Gao et al. 2002). Expression of $p57kip2$ is also abolished in ACC (Liu et al. 1997), but the precise role of the product encoded by this gene in the cell cycle machinery and tumorigenesis requires confirmation. Chromosome region 11p15 LOH is associated with a higher risk of tumor recurrence, is more frequent in ACC than in adrenal adenomas (78.5 vs 9.5%) and correlates with Weiss score (a score of cytopathological alterations used for the diagnosis of malignancy, see below; Gicquel et al. 2001). Thus, 11p15 alterations could be used as a biological marker for predicting ACC malignancy after surgical removal of the tumor (Gicquel et al. 2001). However, 11p15 LOH seems to have a lower predictive value than 17p13 LOH.

**β-Catenin activation in ACC**

Genetic alterations of the Wnt signaling pathway were initially identified in familial adenomatous polyposis coli and have been extended to a variety of cancers (Kikuchi 2003). Adrenocortical tumors have been observed in some case reports of patients with familial adenomatous polyposis coli (Naylor & Gardner 1981). Furthermore, familial adenomatous polyposis coli patients with germline mutations of the $APC$ (Adenomatous Polyposis Coli) gene that lead to an activation of the Wnt signaling pathway, may develop ACTs (Blaker et al. 2004). Molecular studies have suggested that somatic mutations of APC could occur in these tumors in patients already having a germline defect.

The Wnt signaling pathway is normally activated during embryonic development. β-Catenin is a key component of this signaling pathway. It has a structural role in cell–cell adhesion, and is a transcription cofactor with T-cell factor/lymphoid enhancer factor (TCF/LEF) mediating transcriptional activation of target genes of the Wnt signaling pathway (Fig. 3).

Interestingly, gene profiling studies in various types of adrenocortical tumors have shown the frequent activation of Wnt signaling target genes: in ACC, a microarray approach has shown that ectodermal-neural cortex-1 (ECN-1) was up-regulated (Giordano et al. 2003). In both benign and malignant ACT, β-catenin accumulation can be observed. These alterations seem very frequent in ACC, consistent with an abnormal activation of the Wnt-signaling pathway. This is explained in a subset of adrenocortical tumors by somatic mutations of the β-catenin gene altering the glycogen synthase kinase 3-β (GSK3-β) phosphorylation site (Tissier et al. 2005). GSK3-β is implicated in the regulation of β-catenin. In the absence of Wnt signaling, the level of β-catenin is low: β-catenin is phosphorylated at critical NH2-terminal residues by the GSK3-β bound to a scaffolding complex of axin and adenomatous polyposis coli protein (APC) and subsequently the phosphorylated protein is degraded by the ubiquitin–proteasome system. Mutations of β-catenin abolish or reduce GSK3-β phosphorylation of β-catenin, leading to its accumulation, by preventing its degradation by the ubiquitin–proteasome system.

**RAS oncogene**

Ras proteins are membrane associated proteins involved in downstream signaling, once ligand stimulation of growth factor receptor occurs. The three ras proteins (H, N, and K) are one of the most commonly mutated oncogenes in human cancers (Shields et al. 2000). Controversial data are present in the literature: Lin et al. (1998) found K-ras mutations in about 50% of tumor tissues of Conn’s adenomas and no mutations are observed in H-ras, while Moul et al. (1993) and Ocker et al. (2000) did not identify Ras mutations.
Growth factors

Various growth factors and cytokines other than IGFs have been shown to regulate adrenal growth and function in normal adult and fetal adrenals. These include basic fibroblast growth factor (FGF-2), transforming growth factor-α (TGF-α) and transforming growth factor-β1 (TGF-β1), vascular endothelial growth factor (VEGF), and interleukins (Hotta & Baird 1986, Feige et al. 1991, 1998, Ilvesmaki et al. 1993b, Weber et al. 1997a,b, de Fraipont et al. 2000, Turner et al. 2003). Among these factors, FGF-2 may be a prime candidate to evaluate in adrenocortical tumors. This growth factor is highly expressed in adrenal tissues and is one of the most potent mitogens in cell culture of adult and fetal adrenal (Mesiano et al. 1991, Feige et al. 1998). In human fetal adrenal glands, Mesiano et al. (1993) showed a cooperative mitogenic effect of IGF-II and FGF-2. Boule et al. (2000) demonstrated that FGF-2 is mitogenic for the H295R cells, regulates the expression of both IGF-II and IGFBP-2, and modulates the processing of pro-IGF-II.

Angiogenesis plays a major role in cancer growth and metastasis. The angiogenic status of a tumor can be assessed by the study of VEGF expression. In ACC, an overexpression of VEGF by comparison with adrenal adenomas has been observed (de Fraipont et al. 2000, Bernini et al. 2002). However, a dissociation between a high expression of VEGF and a low vascularization was observed in ACC, suggesting a dissociation between the angiogenic status and the neoangiogenic capabilities of these tumors (Bernini et al. 2002). Very interestingly, serum VEGF levels were significantly higher in patients with ACC than in patients with adrenal adenomas and normal subjects (Kolomecki et al. 2001). Although a significant reduction of serum VEGF levels 1 month after surgery of ACC has been reported (Kolomecki et al. 2000), its use as a tumor marker remains to be investigated.

Transforming growth factor-β1 (TGF-β1), another multifunctional growth modulator, inhibits the proliferation of epithelial cells and regulates adult and fetal adrenal growth and functions. Two different studies demonstrated a reduced TGF-β1 mRNA expression in ACC, while no difference in the expression (nor mutation) of TGF-β1 receptor were observed in ACC (Boccuzzi et al. 1999, Arnaldi et al. 2000).

Tumor suppressor genes

TP53

The tumor suppressor gene TP53 is located at 17p13 and involved in the control of cell proliferation. Acquired mutations of the TP53 gene are common tumor-specific alterations in humans, and have been identified in most of the major types of cancer (Caron de Fromentel & Soussi 1992). Germline mutations in TP53 are identified in 70% of families with Li-Fraumeni syndrome (LFS). This syndrome displays dominant inheritance and confers susceptibility to breast carcinoma, soft tissue sarcoma, brain tumors, osteosarcoma, leukemia, and ACC (Hisada et al. 1998). Other possible component tumors include melanoma, gonadal germ cells tumors, and carcinoma of the lung, pancreas, and prostate. These tumors have an early onset, affecting mostly children and young adults. Mutations in checkpoint kinase 2 gene (hCHK2), encoding a kinase that can directly phosphorylate TP53, have been reported in LFS patients (Bell et al. 1999). However, in these few kindreds there is no report of ACC (Bell et al. 1999). Germline mutations in TP53 have been observed in 50–80% of children with apparently sporadic ACC in North America and Europe (Wagner et al. 1994, Varley et al. 1999). The incidence of pediatric ACC is about 10 times higher in Southern Brazil than in the rest of the world, and a specific germline mutation has been identified in exon 10 of the TP53 gene (R337H) in almost all cases (Latronico et al. 2001, Ribeiro et al. 2001). Molecular studies about this mutation have shown that its tissue-specific effects may be pH-dependent, due to the replacement of an arginine by a histidine in the tetramerization domain of TP53 (DiGiammarino et al. 2002).

In sporadic ACC in adults, somatic mutations of TP53 are found in only 25% of cases and are located in four ‘hot spot regions’ within exons 5 and 8, as first demonstrated by Ohgaki et al. (1993) and Reincke et al. (1994) in a small series. An Italian group recently reported a TP53 mutation rate of 70% in 10 ACC (Barzon et al. 2001). Lin et al. (1994) reported TP53 mutations in 73% of adrenocortical adenomas from Taiwanese patients, with 82% of these mutations located in exon 4. Reincke et al. (1996) sequenced exon 4 of TP53 in 19 adrenocortical adenomas from Caucasian patients but found no mutation; they suggested that environmental factors might account for this discrepancy.

LOH at 17p13 has been consistently demonstrated in ACC but not in adrenocortical adenomas (Yano et al. 1989, Gicquel et al. 2001; Fig. 2). LOH at 17p13 was recently reported to occur in 85% of malignant tumors and less than 30% of benign adenomas. LOH at 17p13 is correlated with the Weiss score. It has therefore been suggested that 17p13 LOH could be used as a molecular marker of malignancy in adrenocortical tumors.
In a large prospective study of patients with ACT, 17p13 LOH was demonstrated to be an independent variable predictive of recurrence after complete surgical removal of localized adrenocortical tumors (Gicquel et al. 2001).

The discrepancy between the frequencies of TP53 mutation and 17p13 LOH may be accounted for by the existence of another tumor suppressor gene in this region. The HIC-1 gene (hypermethylated in cancer) is such a candidate. It encodes a transcription factor triggered by TP53 and inactivated by hypermethylation or allelic losses in various cancers (Wales et al. 1995).

**MEN 1 gene**

The MEN 1 gene, located at the 11q13 locus, is thought to be a tumor suppressor gene. A heterozygous inactivating germline mutation of MEN 1 is found in about 90% of families affected by multiple endocrine neoplasia type 1 (MEN 1). The principal clinical features of this autosomal dominant syndrome include parathyroid (95%), endocrine pancreas (45%) and pituitary (45%) tumors, and thymic carcinoids (Thakker 1998). Adrenocortical tumors and/or hyperplasia are observed in 25–40% of MEN 1 patients (Kjellman et al. 1999, Schulte et al. 2000). In most cases, they are non-functional adrenocortical adenomas. Hyperplasia is typically found in MEN 1 patients presenting ACTH hypersecretion (Cushing’s disease), whereas ACC has rarely been reported in MEN 1 patients. Somatic mutation of the MEN 1 gene is very rare in adrenocortical tumors (Heppner et al. 1999, Schulte et al. 2000). By contrast, LOH at 11q13 are observed in more than 90% of informative ACC and only 20% of adrenocortical adenomas (Heppner et al. 1999, Kjellman et al. 1999, Schulte et al. 2000). However, LOH in ACC involves almost all the 11q domain, suggesting that an as yet unidentified tumor suppressor gene located on the long arm of the chromosome is involved in ACC formation.

**PRKAR1A**

The regulatory R1A subunit of protein kinase A (PRKAR1A) is a key component of the cAMP signaling pathway that has been implicated in endocrine tumorigenesis (Bertherat 2001, Bossis & Stratakis 2004). This gene, that maps at the 17q22-24 locus, is implicated in a dominantly multiple neoplasia inherited syndrome, the Carney complex (CNC; Kirschner et al. 2000a,b), characterized by spotty skin pigmentation (lentiginosis), endocrine overactivity with primary pigmented nodular adrenocortical disease (PPNAD) and cardiac myxomas (Carney et al. 1985, Groussin et al. 2002a,b, 2006). Heterozygous inactivating germline mutations of PRKAR1A have been demonstrated in about 45 to 65% of CNC families (Kirschner et al. 2000b, Veugelers et al. 2004). Somatic PRKAR1A mutations have been demonstrated in sporadic secreting adrenocortical adenomas, with clinical, hormonal, and pathological characteristics similar to those of PPNAD (Bertherat et al. 2003).

LOH at 17q22-24 has been also observed in sporadic adrenocortical adenomas and seems to be restricted to the PRKAR1A locus, suggesting the possible involvement of this tumor suppressor gene. By contrast, LOH seems to affect a large part of 17q in ACC, suggesting that PRKAR1A alteration may play only a minor role in malignant adrenocortical tumor growth.

**ACTH receptor (ACTH-R)**

ACTH-R belongs to a subgroup of five receptors of the G-protein-coupled receptors superfamily. It is encoded by an intron-less gene on chromosome 18p11.2. ACTH-R LOH has been observed in two of four informative ACC, but not in 15 hypersecreting adrenocortical adenomas, suggesting a role for ACTH-R in cellular differentiation (Reincke et al. 1997). ACTH-R expression studied by Northern blot or in situ hybridization seems up-regulated in functional adrenocortical adenomas. By contrast, a low ACTH-R mRNA level, suggesting down-regulation of the receptor, is observed in non-functional adrenocortical adenomas and ACC (Reincke et al. 1997, 1998). Moreover, Fassnacht et al. (1998) demonstrated that aminoglutethimide, an inhibitor of glucocorticoids synthesis, induces profound ACTH-R down-regulation in the human H295 adrenocortical carcinoma cell line, either by altering the gene expression or by decreasing transcript accumulation through an effect on RNA stability.

**Clinical management of adrenocortical cancer**

**Epidemiology**

ACC is a rare disease with an estimated incidence between 1 and 2 per million and per year in adults in North America and Europe (Soreide et al. 1992, Lindholm et al. 2001). In children, the incidence is considered as ten times lower except in South Brazil where there is a higher incidence of pediatric ACC, recently explained by a specific germline p53 mutation as discussed above (Ribeiro et al. 2001). There is in most series an increased female to male ratio (Hutter & Kayhoe 1966, Luton et al. 2000, Icard et al. 2001),
although not always reported (Venkatesh et al. 1989). The prevalence of ACC in female patients with Cushing syndrome diagnosed during pregnancy is higher than in non-pregnant patients (Guilhaume et al. 1992).

**Diagnosis of adrenocortical cancer**

**Clinical and hormonal investigations**

Symptoms leading to the diagnosis of adrenocortical cancer (ACC) can be due to hormone hypersecretion and/or tumor mass and metastasis (Luton et al. 1990, Abiven et al. 2006). Although ACC are rare among incidentalomas, the diagnosis of ACC is nowadays more often made during the diagnostic work-up of an adrenal incidentaloma (Luton et al. 2000). This circumstance is important since it might be a way to diagnose an ACC at an earlier stage and to improve the prognosis (Abiven et al. 2006). Other specific feature that may be associated with rare genetic diseases, such as the Li-Fraumeni and Wiedemann–Beckwith syndromes, where ACC is part of a more complex syndrome as discussed above.

The proportion of secreting tumors among ACC varies in the literature from one-quarter to three-quarters (Didolkar et al. 1981, Abiven et al. 2006). This could be due to the differences in hormonal investigations and/or recruitment bias. It seems that the majority of ACC are usually secreting tumors when careful hormonal investigations are performed (MacFarlane 1958, Kasperlik-Zaluska et al. 1995, Favia et al. 2001, Abiven et al. 2006). By contrast with benign adrenocortical tumors (that usually secrete a single class of steroid), ACC can secrete various types of steroids (see Table 1). Co-secretion of androgens and cortisol is the most frequent and highly suggestive of a malignant adrenocortical tumor (Luton et al. 1990, Wajchenberg et al. 2000, Allolio et al. 2004, Abiven et al. 2006). Cortisol oversecretion will induce centripetal obesity, protein wasting with skin thinning and striae, muscle atrophy (myopathy), and osteoporosis. Cortisol excess can also cause impaired defense against infection, diabetes, hypertension, psychiatric disturbances, and gonadal dysfunction in men and women. Androgen oversecretion may induce various manifestations in women: hirsutism, menstrual abnormalities, infertility, and eventually frank virilisation (alopecia, deepening of the voice, clitoris hypertrophy). ACC can also secrete mineralocorticoids and steroids precursors. Oversecretion of estrogens can be observed in rare cases. Estrogen excess is responsible for gynecomastia in males. Routine hormonal investigations therefore aim at the characterization of the steroid secretory profile of ACC. Steroid excess diagnosis is useful to establish the adrenocortical origin of the tumor and can be used for follow-up. ACTH-independent cortisol oversecretion is easily demonstrable (Newell-Price et al. 2006); increased urinary cortisol excretion that is not suppressible with high doses of dexamethasone, and undetectable ACTH plasma levels. Plasma 17-OH progesterone is often elevated (baseline and/or after ACTH stimulation), as well as the specific adrenal androgen dehydroepiandrosterone (DHEA)-S which leads to increased plasma testosterone in females. Other steroids as compound S, DOC, Delta 4 androstenedione, and estradiol can be overproduced by the tumor. Secretion of aldosterone by ACC is not frequent and can be detected by plasma aldosterone and renin assays. Probably less than a third of ACCs are ‘non-hypersecretory’ after careful hormonal investigations. In these cases, one should be cautious not to overdiagnose a tumor of the adrenal area as an ACC. These non-hypersecretory ACCs can be diagnosed after investigation of adrenal incidentalomas or discovered by the manifestations of the tumor growth or extension: local symptoms (pain, palpation of a tumor, venous thrombosis, etc.), or distant metastases (liver, lung, and bones). Fever may occur, concomitant to tumor necrosis. However, the general condition of the patient is most often preserved except at a very late stage when the tumor is non-secreting. It explains that non-hypersecretory ACCs may be diagnosed at a late stage.

| Table 1 Hormonal investigations in patients with ACC |
|---------------------------------------------------|
| Glucocorticoid secretion (a minimum of three tests) |
| 24 h urinary free cortisol and urinary creatinine |
| Dexamethasone suppression test (1 mg) |
| Basal ACTH |
| Basal cortisol |
| Sex steroids |
| Testosterone (in female) |
| Estradiol (in male and post-menopausal women) |
| Androstenedione |
| DHEA-S (or DHEA) |
| Precursors |
| 17-OH-progesterone |
| S-compound |
| Desoxycorticosterone |
| Mineralocorticoids |
| Aldosterone/renin ratio (patients with hypertension or hypokalemia) |

These assays are adapted from the recommendation of the ACC working group of the European Network for the Study of Adrenal Tumors (ENSAT), the steroids in italics are not part of the minimal ENSAT work-up. This imply prior exclusion of a pheochromocytoma by the assay of urinary or plasma metanephrine.
Imaging investigations

Imaging is an essential diagnostic step for ACC, especially in cases of incidentaloma. It is important for both the diagnosis of malignancy of an adrenal mass and the extension work-up. Adrenal computed tomography scan (CT-scan) is a very informative imaging procedure for adrenocortical tumors (Boland et al. 1998, Hamrahian et al. 2005). In ACC, it shows a unilateral mass, that is most often large (above 5–6 cm, typically 10 cm and above), lowering the kidney. A part from the size of the tumor, the features suggestive of malignancy are the lack of homogeneity with foci of necrosis and irregular margins; a high spontaneous density observed before contrast media injection during CT-scan (above 10 HU), indicating a low fat density, may give additional information to distinguish between benign and malignant lesions. The wash-out after contrast media injection during CT-scan is typically below 50% in ACC (Pena et al. 2000). CT-scan also participates in the detection of local invasion and distant metastases (liver, lung). Locoregional vessel invasion through the renal veins and the inferior vena cava can proceed up to the right atrium and result in metastatic lung embolism (Icard et al. 2001). Adrenal scintigraphy (Gross et al. 1994) with iodocholesterol is not routinely needed. It might help in some situations since adrenocortical adenomas usually give a positive scintigraphy. By contrast in ACC, especially non-secreting ones, a negative scintigraphic result can be observed (Gross et al. 1994). MRI and/or ultrasound also participate in the diagnosis of liver nodules, and venous invasions. Bone scintigraphy may help to evaluate bone metastases. However, in patients with Cushing’s syndrome, bone remodeling and/or fracture can induce false positive results of bone scintigraphy.

More recently studies have demonstrated that ACCs almost invariably have a high up take of 18-fluorodesoxyglucose ((18)-FDG). Thus, (18)-FDG PET scan appears to distinguish between benign and malignant adrenal tumors (Becherer et al. 2001, Maurea et al. 2001, Tenenbaum et al. 2004). This simple, non-traumatic imaging procedure also participates in the extension work-up (Lebouleux et al. 2006). PET using (11)C-labeled metomidate is a tissue-specific imaging procedure that has been demonstrated to detect both adrenal adenomas and ACC (Hennings et al. 2006). Its use in the extension work-up of ACC needs to be investigated.

Pathology and molecular analysis

As often with endocrine tumors, the diagnosis of malignancy of adrenocortical lesions is not always easy for the pathologist. There is not a single pathological feature which will lead to the diagnosis of a malignant adrenal cortical tumor. Combinations of various histological parameters allowing the calculation of a ‘score’ for a given tumor have been developed. The most widely used is the Weiss score, which is made of nine different items. Each item is given a value of one, when it is present, and zero when it is absent. The score is obtained by summing the values of each individual item. Since the initial paper of Weiss (1984), it is assumed that a score above 3 is most likely to be associated with a malignant tumor. However, there is often a strong doubt for malignancy for scores at 3 and even in some rare cases 2 (Pohlink et al. 2004). Since the Weiss score has limitations and it depends on the experience of the pathologist, there is an effort to develop molecular markers of malignancy. As described previously, IGF-II overexpression and allelic losses at 17p13 have been suggested as useful markers (Gicquel et al. 2000, Libé & Bertherat 2005). Immunohistochemistry of Cyclin E or Ki-67 that are higher in malignant adrenocortical tumors has also been suggested in the literature as potential useful tools (Terzolo et al. 2001, Tissier et al. 2004).

Prognosis of adrenocortical cancer

Among the various clinical parameters that have been shown to impact on ACC prognosis, tumor staging has been demonstrated as one of the most important. The MacFarlane staging (MacFarlane 1958), modified by Sullivan et al. (1978) is the most commonly used and relies on surgical finding and extension work-up. Four different stages are differentiated with this score. Stages 1 and 2 tumors are localized to the adrenal cortex and present a maximum diameter below or above 5 cm respectively. Locally invasive tumors or tumors with regional lymph node metastases are classified as stage 3, whereas stage 4 consists of tumors invading adjacent organs or presenting with distant metastases. The prognosis of stages 1 and 2 tumors is better than that of stages 3 or 4 tumors (Icard et al. 2001, Abiven et al. 2006; Fig. 4). A better survival is usually reported in younger patients (Luton et al. 1990, Abiven et al. 2006). Cortisol secreting tumor is associated with a worse prognosis (Berrutti et al. 2005, Abiven et al. 2006). This could be due to the morbidity associated with Cushing’s syndrome and/or to a different tumor progression. Some pathological features, such as a high mitotic rate or...
atypic mitotic figures have been shown to be associated with a poor prognosis (Stojadinovic et al. 2002).

In the future, it is expected that molecular tools will give a better prediction of the prognosis of ACC. Gene profiling approach can already differentiate malignant from benign tumors (Giordano et al. 2003, de Fraipont et al. 2005). Preliminary results suggest that it might also be of value in determining the prognosis of malignant tumors (de Fraipont et al. 2005).

**Treatment of adrenocortical cancer**

**Surgery and local therapy**

Surgery of the adrenal tumor is the major treatment of stage 1–3 ACC (Fig. 5). It can also be discussed in stage 4 patients. Only complete tumor removal can lead to long-term remission (Icard et al. 2001, Schteingart et al. 2005, Abiven et al. 2006). Open adrenalectomy is currently recommended as laparoscopic removal of malignant adrenocortical tumors could be associated with a high risk of peritoneal dissemination (Cobb et al. 2005). Substitutive glucocorticoid therapy should be started after surgery of cortisol-secreting tumors to avoid adrenal deficiency. In stage 4 patients, with distant metastases, tumor, debulking with removal of the primary adrenal tumor can be discussed in order to improve both prognosis and reduce steroid excess. One should note that if reduction of steroid excess by partial tumor removal is obvious, no prospective trial investigating the effect on survival has been reported. However, tumor debulking might also help to improve the results of other therapeutic options. When the number of metastases is limited their surgical removal can also be discussed. Radiofrequency thermal ablation of liver and lung metastasis below 4–5 cm of maximal diameter can be an alternative to surgical removal (Wood et al. 2003). Chemoembolization has also been used for liver metastasis (de Baere 2006). Surgery of bone metastasis can be indicated to reduce fracture risk, or, in case of spinal localization, neurological symptoms.

**Radiation therapy**

Radiation therapy is usually considered as not very effective to control tumor growth. However, it has been recently suggested that tumor bed radiation therapy could help to prevent local recurrence after surgical removal (Allolio & Fassnacht in press). For bone metastasis, radiotherapy can be used as a palliative treatment to reduce pain and limit the risk of development of local complications (neurological symptoms and/or fracture).

**Medical therapy with mitotane**

When complete tumor removal is not possible, or in case of recurrence, medical treatment with O,p’DDD (ortho, para’, dichloro-, diphenyl-, dichloro ethane, or mitotane) is recommended (Luton et al. 1990, Wooten and King, 1993). It has both an anticortisolic action and inhibits steroid synthesis by an action on steroidogenic enzymes, as 11β-hydroxylase and cholesterol side chain cleavage. It is quite specific of the adrenal cortex. Interestingly, O,p’DDD has also a cytotoxic effect on the adrenocortical cells that is important for its use in ACC. It is usually effective to control steroid excess in patients with secreting ACC. Most series reported in the literature on the efficacy of O,p’DDD in ACC are retrospective analysis with variable results on tumor progression. A recent review suggests that an objective tumor regression could be observed in 25% of the cases (Allolio & Fassnacht 2006). We have recently shown in a retrospective study, that patients with cortisol secreting ACC have a better survival rate when starting treatment with mitotane (O,p’DDD) 3 months following the surgery of the adrenal tumor (Abiven et al. 2006). However, the effect of mitotane after complete removal of MacFarlane stage 1 or 2 tumors has never been studied in prospective trials. Considering the very poor prognosis of ACC it might be discussed in patients with bad prognostic factors as an adjuvant treatment after complete tumor removal. This issue is important and randomized trials are needed for its clarification.

A mitotane blood level of at least 14 mg/l seems to improve the tumor response rate (van Slooten et al. 1984, Baudin et al. 2001). However, the side effects of mitotane (mainly digestive and neurological) often
limit the ability to reach this suggested optimal level. The daily mitotane dose required to achieve this 14 mg/l level varies from patients to patients. Therefore, close monitoring of mitotane blood level is very helpful to remain in the narrow range between 14 and 20 mg/l, considered by most authors as the therapeutic range of mitotane in ACC. Since O,p’DDD can induce adrenal insufficiency, substitutive glucocorticoid and mineralocorticoid therapy should be associated.

Medical treatment with cytotoxic chemotherapy
Several cytotoxic chemotherapy regimens have been used in ACC. They are usually considered in patients with tumor progression under mitotane therapy reaching the plasma blood level of 14 mg/l or presenting severe side effects limiting its use. Various drugs have been used and the experience is still limited. It is currently accepted since the Ann Arbor international conference on ACC (Schteingart et al. 2005), that the

Figure 5 Summary of clinical management of patients with ACC. (a) Except in cases of major contraindication to anesthesia or surgery, is indicated in patients with localized tumors (MacFarlane stage 1, 2, and 3). In patients with distant metastasis (stage 4), surgery should be discussed to reduce tumor mass. When possible, surgery of metastasis should also be discussed (in particular removal of the primary adrenal tumor associated with liver surgery might be indicated in a patient presenting at diagnosis liver metastasis removable by surgery and/or radiofrequency ablation). (b) Local recurrence without distant metastasis usually requires surgery. (c) Local TT denotes local therapy targeted to metastasis (mostly liver and lung, rarely bone). Local TT include radiotherapy (especially for bone metastasis), chemoembolization (mostly for liver metastasis), radiofrequency thermal ablation of lung or liver metastasis as well as surgical removal of limited metastasis (de Baere 2006). (d) The delay between imaging and biochemical work-ups can be prolonged to 3–4 months in patients with complete remission and good prognostic factors, and might be extended up to a 6 months interval after 2 years if there is still no recurrence.
combined treatment with cis-platine, etoposide, doxorubicin (EDP regimen) associated with O,p’DDD (Berruti et al. 2005) and streptozotocin also given with O,p’DDD (Khan et al. 2000) are the better regimens. The EDP regimen consists of 4 days of treatment repeated every 28 days and for each cycle the following dose of each drug is given: doxorubicine: 40 mg/m², etoposide: 300 mg/m², and cisplatin 80 mg/m² (Berruti et al. 2005). An international trial first inter national randomized trial in locally advanced and metastatic adrenocortical carcinoma treatment (FIRM-ACT) is currently done to investigate the results of these two treatments (Allolio & Fassnacht 2006).

Conclusion
Considering the rarity of ACC, significant advances reviewed herein have been made this last decade to understand its pathophysiology. These advances have been also important for a better diagnosis of these tumors and might ultimately lead to a better prediction of prognosis. However, there is need for much more progress, especially in improving therapeutic efficiency (Kirschner 2006). Due to the rarity of ACC, collaborative work performed in national and international networks dedicated to adrenocortical tumors will be important. In Europe, this will be the goal of the European Network for the Study of Adrenal tumors (ENSAT) which has been recently developed on the background of several national networks already working successfully in this field.

Acknowledgements
This work was supported in part by the Plan Hospitalier de Recherche Clinique to the COMETE network (AOM 02068), the Ligue National Contre le Cancer (04-7571) and the GIS-INSERM Institut des Maladies Rares for the European Network for the Study of Adrenal Tumors (ENSAT). The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

References
Abiven G, Coste J, Groussin L, Anract P, Tissier F, Legmann P, Dousset B, Bertagna X & Bertherat J 2006 Bioclinical features in the prognosis of adrenocortical cancer: poor outcome of cortisol-secreting tumors from a series of 202 consecutive patients. Journal of Clinical Endocrinology and Metabolism 91 2650–2655.

Allolio B & Fassnacht M 2006 Adrenocortical carcinoma: clinical update. Journal of Clinical Endocrinology and Metabolism 91 2027–2037.

Allolio B, Hahner S, Weismann D & Fassnacht M 2004 Management of adrenocortical carcinoma. Clinical Endocrinology 60 273–287.

Arnaldi G, Freddi S, Mancini T, Kola B & Mantero F 2000 Transforming growth factor beta1: implications in adrenocortical tumorigenesis. Endocrine Research 26 905–910.

Barzon L, Chilosi M, Fallo F, Martignoni G, Montagna L, Palu G & Boscaro M 2001 Molecular analysis of CDKN1C and TP53 in sporadic adrenal tumors. European Journal of Endocrinology 145 207–212.

Baudin E, Pellegriti G, Bonny M, Penfornis A, Laplanche A, Vassal G & Schlumberger M 2001 Impact of monitoring plasma 1,1-dichlorodiphenyldichloroethane (o,p’DDD) levels on the treatment of patients with adrenocortical carcinoma. Cancer 92 1385–1392.

Becherer A, Vierhapper H, Potzi C, Karanakis G, Kurtaran A, Schmaljohann J, Staudenherz A, Dudczak R & Kletter K 2001 FDG-PET in adrenocortical carcinoma. Cancer Biotherapy and Radiopharmaceuticals 16 289–295.

Bell DW, Varley JM, Szydlo TE, Kang DH, Wahrer DC, Shannon KE, Lubratovich M, Verselis SJ, Isselbacher KJ, Fraumeni JF et al. 1999 Heterozygous germ line hCHK2 mutations in Li-Fraumeni syndrome. Science 286 2528–2531.

Bernini GP, Moretti A, Bonadio AG, Menicagli M, Viacava P, Naccarato AG, Iaconi P, Miccoli P & Salvetti A 2002 Angiogenesis in human normal and pathologic adrenal cortex. Journal of Clinical Endocrinology and Metabolism 87 4961–4965.

Berruti A, Terzolo M, Sperone P, Pia A, Casa SD, Gross DJ, Carnaghi C, Casali P, Porpiglia F, Mantero F et al. 2005 Etoposide, doxorubicin and cisplatin plus mitotane in the treatment of advanced adrenocortical carcinoma: a large prospective phase II trial. Endocrine-Related Cancer 12 657–666.

Bertherat J 2001 Protein kinase A in carney complex: a new example of cAMP pathway alteration in endocrine tumors. European Journal of Endocrinology 144 209–211.

Bertherat J, Groussin L, Sandrini F, Matyahkina L, Bei T, Stergiopoulos S, Papageorgiou T, Bourdeau I, Kirschner LS, Vincent-Dejean C et al. 2003 Molecular and functional analysis of PRKAR1a and its locus (17q22-24) in sporadic adrenocortical tumors: 17q losses, somatic mutations, and protein kinase a expression and activity. Cancer Research 63 5308–5319.

Beuschlein F, Reincke M, Karl M, Travis WD, Jaursch-Hancke C, Abdelhamid S, Chrousos GP & Allolio B 1994 Clonal composition of human adrenocortical neoplasms. Cancer Research 54 4927–4932.

Blaker H, Sutter C, Kadmon M, Otto HF, Von Knebel-Doebereit M, Gebert J & Helmkne BM 2004 Analysis of somatic APC mutations in rare extracolonic tumors of patients with familial adenomatous polyposis coli. Genes, Chromosomes and Cancer 41 93–98.

www.endocrinology-journals.org
Bocuzzi A, Terzolo M, Cappia S, De Giuli P, De Risi C, Leonardo E, Bovio S, Borriero M, Paccotti P & Angeli A 1999 Different immunohistochemical patterns of TGF-beta1 expression in benign and malignant adrenocortical tumors. Clinical Endocrinology 50 801–808.

Boland GW, Lee MJ, Gazelle GS, Halpern EF, McNicholas MM & Mueller PR 1998 Characterization of adrenal masses using unenhanced CT: an analysis of the CT literature. American Journal of Roentgenology 171 201–204.

Bosissi I & Stratakis CA 2004 Minireview: Prkar1a: normal and abnormal functions. Endocrinology 145 5452–5458.

Boule N, Logie A, Gicquel C, Perin L & Le Bouc Y 1998 Increased levels of insulin-like growth factor II (IGF-II) and IGF-binding protein-2 are associated with malignancy in sporadic adrenocortical tumors. Journal of Clinical Endocrinology and Metabolism 83 1713–1720.

Boule N, Gicquel C, Logie A, Christol R, Feige JJ & Le Bouc Y 2000 Fibroblast growth factor-2 inhibits the maturation of pro-insulin-like growth factor-II (pro-IGF-II) and the expression of insulin-like growth factor binding protein-2 (IGFBP-2) in the human adrenocortical tumor cell line NCI-H295R. Endocrinology 141 3127–3136.

Bourdeau I, Antonini SR, Lacroix A, Kirschner LS, Matyakhina L, Lorang D, Libutti SK & Stratakis CA 2004 Gene array analysis of macronodular adrenal hyperplasia confirms clinical heterogeneity and identifies several candidate genes as molecular mediators. Oncogene 23 1575–1585.

Carney JA, Gordon H, Carpenter PC, Shenoy BV & Go VL 1985 The complex of myxomas, spotty pigmentation, and endocrine overactivity. Medicine (Baltimore) 64 270–283.

Caron de Fromentel C & Soussi T 1992 TP53 tumor suppressor gene: a model for investigating human endocrine overactivity. Genes, Chromosomes and Cancer 4 1–15.

Cobb WS, Kercher KW, Sing RF & Heniford BT 2005 Laparoscopic adrenalectomy for malignancy. Journal of Surgery 405–411.

De Baere T 2006 Local treatment of adrenal cortical carcinoma metastases with interventional radiology techniques. In Adrenal Cancer, pp 97–106. Ed X Bertagna. Montrouge, France: John Libbey Eurotext.

DeChiara TM, Robertson EJ & Efstratiadis A 1991 Parental imprinting of the mouse insulin-like growth factor II gene. Cell 64 849–859.

Didolkar MS, Bescher RA, Elias EG & Moore RH 1981 Natural history of adrenal cortical carcinoma: a clinicopathologic study of 42 patients. Cancer 47 2153–2161.

DiGiannarino EL, Lee AS, Cadwell C, Zhang W, Bothner B, Ribeiro RC, Zambetti G & Kriwacki RW 2002 A novel mechanism of tumorigenesis involving ph-dependent destabilization of a mutant p53 tetramer. Nature Structural Biology 9 12–16.

Dohna M, Reinecke M, Mincheva A, Allolio B, Solinas-Toldo S & Lichter P 2000 Adrenocortical carcinoma is characterized by a high frequency of chromosomal gains and high-level amplifications. Genes, Chromosomes and Cancer 28 145–152.

Fassnacht M, Beuschlein F, Vay S, Mora P, Allolio B & Reinicke M 1998 Aminoglutethimide suppresses adrenocorticotropin receptor expression in the NCI-h295 adrenocortical tumor cell line. Journal of Endocrinology 159 35–42.

Favia G, Lumachi F & D’Amico DF 2001 Adrenocortical carcinoma: is prognosis different in nonfunctioning tumors? Results of surgical treatment in 31 patients World Journal of Surgery 25 735–738.

Feige JJ, Cochet C, Savona C, Shi DL, Keramidas M, Defaye G & Chambaz EM 1991 Transforming growth factor beta 1: an autocrine regulator of adrenocortical steroidogenesis. Endocrine Research 17 267–279.

Feige JJ, Vilgrain I, Brand C, Bailly S & Souchelnitskiy S 1998 Fine tuning of adrenocortical functions by locally produced growth factors. Journal of Endocrinology 158 7–19.

de Fraipont F, El Atifi M, Gicquel C, Bertagna X, Chambaz EM & Feige JJ 2000 Expression of the angiogenesis markers vascular endothelial growth factor-A, thrombospondin-1, and platelet-derived endothelial cell growth factor in human sporadic adrenocortical tumors: correlation with genotypic alterations. Journal of Clinical Endocrinology and Metabolism 85 4734–4741.

de Fraipont F, El Atifi M, Cherradi N, Le Moigne G, Defaye G, Houlgatte R, Bertherat J, Bertagna X, Plouin PF, Baudin E et al. 2005 Gene expression profiling of human adrenocortical tumors using cDNA microarrays identifies several candidate genes as markers of malignancy. Journal of Clinical Endocrinology and Metabolism 90 1819–1829.

Gao ZH, Suppola S, Liu J, Heikkila P, Janne J & Voutilainen R 2002 Association of H19 promoter methylation with the malignant phenotype in sporadic adrenocortical tumors: correlation with genotypic alterations. Clinical Endocrinology and Metabolism 87 1170–1176.

Gicquel C, Leblond-Francillard M, Bertagna X, Louvel A, Chapuis Y, Laton JP, Girard F & Le Bouc Y 1994 Clonal analysis of human adrenocortical carcinomas and secreting adenomas. Clinical Endocrinology 40 465–477.

Gicquel C, Bertagna X & Le Bouc Y 1995 Recent advances in the pathogenesis of adrenocortical tumors. European Journal of Endocrinology 133 133–144.

Gicquel C, Raffin-Sanson ML, Gaston V, Bertagna X, Plouin PF, Schlumberger M, Louvel A, Laton JP & Le Bouc Y 1997 Structural and functional abnormalities at 11p15 are associated with the malignant phenotype in sporadic adrenocortical tumors: study on a series of 82 tumors. Journal of Clinical Endocrinology and Metabolism 82 2559–2565.

Gicquel C, Bertherat J, Le Bouc Y & Bertagna X 2000 Pathogenesis of adrenocortical incidentalomas and genetic syndromes associated with adrenocortical neoplasms. Endocrinology and Metabolism Clinics of North America 29 1–13.
Gicquel C, Bertagna X, Gaston V, Coste J, Louvel A, Baudin E, Bertherat J, Chapuis Y, Duclos JM, Schlumberger M et al. 2001 Molecular markers and long-term recurrences in a large cohort of patients with sporadic adrenocortical tumors. *Cancer Research* 61 6762–6767.

Giordano TJ, Thomas DG, Kuick R, Lizyness M, Misek DE, Smith AL, Sanders D, Alaunji RT, Gauger PG, Thompson NW *et al.* 2003 Distinct transcriptional profiles of adrenocortical tumors uncovered by DNA microarray analysis. *American Journal of Pathology* 162 521–531.

Gross MG, Shapiro B & Francis IR 1994 Scintigraphic evaluation of clinically silent adrenal masses. *Journal of Nuclear Medicine* 35 1145–1152.

Groussin L, Kirschner LS, Vincent-Dejean C, Perlemoine K, Jullian E, Delemer B, Zacharieva S, Pignatelli D, Carney JA, Luton JP *et al.* 2002a Molecular analysis of the cyclic AMP-dependent protein kinase a (PKA) regulatory subunit 1a (PRKAR1a) gene in patients with Carney complex and primary pigmented nodular adrenocortical disease (PPNAD) reveals novel mutations and clues for pathophysiology: augmented PKA signaling is associated with adrenal tumorigenesis in PPNAD. *American Journal of Human Genetics* 71 1433–1442.

Groussin L, Jullian E, Perlemoine K, Louvel A, Leheup B, Luton JP, Bertagna X & Bertherat J 2002b Mutations of the PRKAR1a gene in Cushing’s syndrome due to sporadic primary pigmented nodular adrenocortical disease. *Journal of Clinical Endocrinology and Metabolism* 87 4324–4329.

Groussin L, Horvath A, Jullian E, Boikos S, Rene-Corail F, Lefebvre H, Cephise-Velayoudom FL, Vantyghem MC, Chanson P, Conte-Devolx B *et al.* 2006 A PRKAR1A mutation associated with primary pigmented nodular adrenocortical disease in 12 kindreds. *Journal of Clinical Endocrinology and Metabolism* 91 1943–1949.

Grimbach MM, Biller BM, Braunstein GD, Campbell KK, Carney JA, Godley PA, Harris EL, Lee JK, Oertel YC, Posner MC *et al.* 2003 Management of the clinically apparent adrenal mass (‘incidentaloma’). *Annals of Internal Medicine* 138 424–429.

Guilhaume B, Sanson ML, Billaud L, Bertagna X, Laudat MH & Luton JP 1992 Cushing’s syndrome and pregnancy: aetiology and prognosis in twenty-two patients. *European Journal of Medicine* 1 83–89.

Hamrahian AH, Ioachimescu AG, Remer EM, Motta-Ramirez G, Bogabathina H, Levin HS, Reddy S, Gill IS, Siperstein A & Bravo EL 2005 Clinical utility of noncontrast computed tomography attenuation value (hounsfield units) to differentiate adrenal adenomas/hyperplasias from nonadenomas: cleveland clinic experience. *Journal of Clinical Endocrinology and Metabolism* 90 871–877.

Hao Y, Crenshaw T, Moulton T, Newcomb E & Tycko B 1993 Tumour-suppressor activity of H19 RNA. *Nature* 365 764–767.

Hennings J, Lindhe O, Bergstrom M, Langstrom B, Sundin A & Hellman P 2006 11C]metomidate positron emission tomography of adrenocortical tumors in correlation with histopathological findings. *Journal of Clinical Endocrinology and Metabolism* 91 1410–1414.

Heppner C, Reincke M, Agarwal SK, Mora P, Allolio B, Burns AL, Spiegel AM & Marx SJ 1999 MEN1 gene analysis in sporadic adrenocortical neoplasms. *Journal of Clinical Endocrinology and Metabolism* 84 216–219.

Hertel NT, Carlsen N, Kehndrup G, Pedersen IL, Clausen N, Hahnenmann JM & Jacobsen BB 2003 Late relapse of adrenocortical carcinoma in Beckwith-Wiedemann syndrome. Clinical, endocrinological and genetics aspects. *Acta Paediatrica* 92 439–443.

Hisada M, Garber JE, Fung CY, Fraumeni JP & Li FP 1998 Multiple primary cancers in families with Li-Fraumeni syndrome. *Journal of the National Cancer Institute* 90 606–611.

Hotta M & Baird A 1986 Differential effects of transforming growth factor type beta on the growth and function of adrenocortical cells in vitro. *PNAS* 83 7795–7799.

Hutter AM Jr & Kayhoe DE 1966 Adrenal cortical carcinoma. Results of treatment with o,p’DDD in 138 patients. *American Journal of Medicine* 41 581–592.

Icard P, Goudet P, Charpenay C, Andreassian B, Carmaille B, Chapuis Y, Cougard P, Henry JF & Proye C 2001 Adrenocortical carcinomas: surgical trends and results of a 253-patient series from the French Association of Endocrine Surgeons study group. *World Journal of Surgery* 25 891–897.

Ilvesmaki V, Kahri AI, Miettinen PJ & Voutilainen R 1993a Insulin-like growth factors (IGFs) and their receptors in adrenal tumors: high IGF-II expression in functional adrenocortical carcinomas. *Journal of Clinical Endocrinology and Metabolism* 77 852–858.

Ilvesmaki V, Jaattela M, Saksela E & Voutilainen R 1993b Tumor necrosis factor-alpha and interferon-gamma inhibit insulin-like growth factor II gene expression in human fetal adrenal cell cultures. *Molecular and Cellular Endocrinology* 91 59–65.

Kasperlik-Zaluska AA, Migdalbska SM, Zgliczynski S & Makowska AM 1995 Adrenocortical carcinoma. A clinical study and treatment results of 52 patients. *Cancer* 75 2587–2591.

Khan TS, Imam H, Juulh C, Skogseid B, Grondal S, Tibblin S, Wilander E, Oberg K & Eriksson B 2000 Streptozocin and o,p’DDD in the treatment of adrenocortical cancer patients: long-term survival in its adjuvant use. *Annals of Oncology* 11 1281–1287.

Kikuchi A 2003 Tumor formation by genetic mutations in the components of the Wnt signaling pathway. *Cancer Science* 94 225–229.

Kirschner LS 2006 Emerging treatment strategies for adrenocortical carcinoma: a new hope. *Journal of Clinical Endocrinology and Metabolism* 91 14–21.

Kirschner LS, Carney JA, pack SD, Taymns SE, Giatzakis C, Cho YS, Cho-Chung YS & Stratakis CA 2000a Mutations
of the gene encoding the protein kinase a type 1-alpha regulatory subunit in patients with the Carney complex. *Nature Genetics* 26 89–92.

Kirschner LS, Sandrini F, Monbo J, Lin JP, Carney JA & Stratakis CA 2000b Genetic heterogeneity and spectrum of mutations of the PRKAR1a gene in patients with the Carney complex. *Human Molecular Genetics* 9 3037–3046.

Kjellman M, Kallioniemi OP, Karhu R, Hoog A, Farnebo LO, Auer G, Larsson C & Backdahl M 1996 Genetic aberrations in adrenocortical tumors detected using comparative genomic hybridization correlate with tumor size and malignancy. *Cancer Research* 56 4219–4223.

Kjellman M, Roshani L, Teh BT, Kallioniemi OP, Hoog A, Gray S, Farnebo LO, Holst M, Backdahl M & Larsson C 1999 Genotyping of adrenocortical tumors: very frequent deletions of the MEN1 locus in 11q13 and of a 1-centimorgan region in 2p16. *Journal of Clinical Endocrinology and Metabolism* 84 730–735.

Kolomecki K, Stepken H & Narebski JM 2000 Vascular endothelial growth factor and basic fibroblast growth factor evaluation in blood serum of patients with hormonally active and inactive adrenal gland tumours. *Cytobios* 101 55–64.

Kolomecki K, Stepken H, Bartos M & Kuzdak K 2001 Usefulness of VEGF, MMP-2, MMP-3 and TIMP-2 serum level evaluation in patients with adrenal tumours. *Endocrine Regulations* 35 9–16.

Lam WW, Hatada I, Ohishi S, Mukai T, Joyce JA, Cole TR, Donnai D, Reik W, Schofield PN & Maher ER 1999 Analysis of germline CDKN1C (p57kip2) mutations in familial and sporadic Beckwith–Wiedemann syndrome (BWS) provides a novel genotype-phenotype correlation. *Journal of Medical Genetics* 36 518–523.

Latronico AC, Pinto EM, Domenice S, Fragoso MC, Martin RM, Zerbini MC, Lucon AM & Mendonca BB 2001 An inherited mutation outside the highly conserved DNA-binding domain of the p53 tumor suppressor protein in children and adults with sporadic adrenocortical tumors. *Journal of Clinical Endocrinology and Metabolism* 86 4970–4973.

Lebrouilleux S, Dromain C, Bonnialaud G, Auperin A, Caillou B, Lumbroso J, Sigal R, Baudin E & Schlumberger M 2006 Diagnostic and prognostic value of 18-fluorodeoxyglucose positron emission tomography in adrenocortical carcinoma: a prospective comparison with computed tomography. *Journal of Clinical Endocrinology and Metabolism* 91 920–925.

Lee MH, Reynisdottir I & Massague J 1995 Cloning of p57kip2, a cyclin-dependent kinase inhibitor with unique domain structure and tissue distribution. *Genes and Development* 9 639–649.

Libé R & Bertherat J 2005 Molecular genetics of adrenocortical tumours, from familial to sporadic diseases. *European Journal of Endocrinology* 153 477–487.

Lin SR, Lee YJ & Tsai JH 1994 Mutations of the p53 gene in human functional adrenal neoplasms. *Journal of Clinical Endocrinology and Metabolism* 78 483–491.

Lin SR, Tsai JH, Yang YC & Lee SC 1998 Mutations of K-ras oncogene in human adrenal tumours in Taiwan. *Breast Journal of Cancer* 77 1060–1065.

Lindholm J, Juul S, Jorgensen JO, Astrup J, Bjerre P, Feldt-Rasmussen U, Hagen C, Jorgensen J, Kosteljanetz M, Kristensen L et al. 2001 Incidence and late prognosis of cushing’s syndrome: a population-based study. *Journal of Clinical Endocrinology and Metabolism* 86 117–123.

Liu J, Kahri AI, Heikkila P & Voutilainen R 1997 Ribonucleic acid expression of the clustered imprinted genes, p57kip2, insulin-like growth factor II, and H19, in adrenal tumors and cultured adrenal cells. *Journal of Clinical Endocrinology and Metabolism* 82 1766–1771.

Logie A, Boulle N, Gaston V, Perin L, Boudou P, Le Bouc Y & Gicquel C 1999 Autocrine role of IGF-II in proliferation of human adrenocortical carcinoma NCI H295R cell line. *Journal of Molecular Endocrinology* 23 23–32.

Luton JP, Cerdas S, Billaud L, Thomas G, Guilhaume B, Bertagna X, Laudat MH, Louvel A, Chapuis Y, Blondeau P et al. 1990 Clinical features of adrenocortical carcinoma, prognostic factors, and the effect of mitotane therapy. *New England Journal of Medicine* 322 1195–1201.

Luton JP, Martinez M, Coste J & Bertherat J 2000 Outcome in patients with adrenal incidentaloma selected for surgery: an analysis of 88 cases investigated in a single clinical center. *European Journal of Endocrinology* 143 111–117.

Mac Farlane DA 1958 Cancer of the adrenal cortex: the natural history, prognosis and treatment in the study of 58 cases. *Annals of the Royal College of Surgery* 109 613–618.

Matsuoka S, Edwards MC, Bai C, Parker S, Zhang P, Baldini A, Harper JW & Elledge SJ 1995 P57kip2, a structurally distinct member of the p21CIP1 Cdk inhibitor family, is a candidate tumor suppressor gene. *Genes and Development* 9 650–662.

Maurea S, Klein M, Mainolfi C, Zivielo M & Salvatore M 2001 The diagnostic role of radionuclide imaging in evaluation of patients with nonhypersecreting adrenal masses. *Journal of Nuclear Medicine* 42 884–892.

Mesiano S, Mellon SH, Gospodarowicz D, Di Blasio AM & Jaffe RB 1991 Basic fibroblast growth factor expression is regulated by corticotropin in the human fetal adrenal: a model for adrenal growth regulation. *PNAS* 88 5428–5432.

Mesiano S, Mellon SH & Jaffe RB 1993 Mitogenic action, regulation, and localization of insulin-like growth factors in the human fetal adrenal gland. *Journal of Clinical Endocrinology and Metabolism* 76 968–976.

Moul JW, Bischoff JT, Theune SM & Chang EH 1993 Absent ras gene mutations in human adrenal cortical neoplasms and pheochromocytomas. *Journal of Urology* 149 1389–1394.

Naylor EW & Gardner EJ 1981 Adrenal adenomas in a patient with Gardner’s syndrome. *Clinical Genetics* 20 67–73.
Schulte KM, Mengel M, Heinze M, Simon D, Scheuring S, Kohrer K & Roher HD 2000 Complete sequencing and messenger ribonucleic acid expression analysis of the MEN 1 gene in adrenal cancer. Journal of Clinical Endocrinology and Metabolism 85 441–448.

Shields JM, Pruitt K, McFall A, Shaub A & Der CJ 2000 Understanding Ras: ‘it ain’t over til it’s over’. Trends in Cell Biology 10 147–154.

Sidhu S, Marsh DJ, Theodosopoulos G, Philips J, Bambach CP, Campbell P, Magarey CJ, Russell CF, Schulte KM, Roher HD et al. 2002 Comparative genomic hybridization analysis of adrenocortical tumors. Journal of Clinical Endocrinology and Metabolism 87 3467–3474.

van Slooten H, Moolenaar AJ, van Seters AP & Smekend 1984 The treatment of adrenocortical carcinoma with o.p.-DDD: prognostic implications of serum level monitoring. European Journal of Cancer and Clinical Oncology 20 47–53.

Soreide JA, Brabrand K & Thoresen SO 1992 Adrenal cortical carcinoma in Norway, 1970–1984. World Journal of Surgery 16 663–667 (discussion 668).

Stojadinovic A, Ghossein RA, Hoos A, Nissan A, Marshall D, Dudas M, Cordon-Cardo C, Jaques DP & Brennan MF 2002 Adrenocortical carcinoma: clinical, morphologic, and molecular characterization. Journal of Clinical Oncology 20 941–950.

Sullivan M, Boland GW, Hahn PF, Lee MJ & Mueller PR 2000 PCR-SSCP analysis of human adrenocortical adenomas: absence of K-ras gene mutations. Experimental and Clinical Endocrinology and Diabetes 108 513–514.

Ogawa O, Eccles MR, Szeto J, McNoe LA, Yun K, Maw MA, Smith PJ & Reeve AE 1993 Relaxation of insulin-like growth factor II gene imprinting implicated in Wilms’ tumour. Nature 362 749–751.

Ocker M, Sachse R, Eichfeld U, Schmidt F, Fuhrer D, Paschke R & Koch CA 2004 Does tumor heterogeneity limit the use of the Weiss criteria in the evaluation of adrenocortical tumors? Journal of Endocrinological Investigation 27 565–569.

Pena CS, Boland GW, Hahn PF, Lee MJ & Mueller PR 2000 Characterization of indeterminate (lipid-poor) adrenal masses: use of washout characteristics at contrast-enhanced CT. Radiology 217 798–802.

Pohlk C, Tannapfe A, Eichfeld U, Schmidt F, Fuhrer D, Paschke R & Koch CA 2004 Does tumor heterogeneity limit the use of the Weiss criteria in the evaluation of adrenocortical tumors? Journal of Endocrinological Investigation 27 565–569.

Rainier S, Johnson LA, Dobry CJ, Ping AJ, Grundy PE & Feinberg AP 1993 Relaxation of imprinted genes in human cancer. Nature 362 747–749.

Reincke M, Karl M, Travis WH, Mastorakos G, Alloio B, Linehan HM & Chrousos GP 1994 P53 mutations in human adrenocortical neoplasms: immunohistochemical and molecular studies. Journal of Clinical Endocrinology and Metabolism 78 790–794.

Reincke M, Wachenfeld C, Mora P, Thumser A, Jaurusch-Hancke C, Abdelhamid S, Chrousos GP & Alloio B 1996 P53 mutations in adrenocortical tumors: caucasian patients do not show the exon 4 ‘hot spot’ found in taiwan. Journal of Clinical Endocrinology and Metabolism 81 3636–3638.

Reincke M, Mora P, Beuschlein F, Arlt W, Chrousos GP & Alloio B 1997 Deletion of the adrenocorticotropin receptor gene in human adrenocortical tumors: implications for tumorigenesis. Journal of Clinical Endocrinology and Metabolism 82 3054–3058.

Reincke M, Beuschlein F, Menig G, Hofmockel G, Arlt W, Lehmann R, Karl M & Alloio B 1998 Localization and expression of adrenocorticotropic hormone receptor mRNA in normal and neoplastic human adrenal cortex. Journal of Endocrinology 156 415–423.

Ribeiro RC, Sandrini F, Figueiredo B, Zambetti GP, Michalkiewicz E, Lafferty AR, DeLacerda L, Rabin M, Cadwell C, Sampaio G et al. 2001 An inherited p53 mutation that contributes in a tissue-specific manner to pediatric adrenal cortical carcinoma. PNAS 98 9330–9335.

Schteingart DE, Doherty GM, Gauger PG, Giordano TJ, Hammer GD, Korobkin M & Worden FP 2005 Management of patients with adrenal cancer: recommendations of an international consensus conference. Endocrine-Related Cancer 12 667–680.

Thakker RV 1998 Multiple endocrine neoplasia syndromes of the twentieth century. Journal of Clinical Endocrinology and Metabolism 83 2617–2620.

Tissier F, Louvel A, Grabar S, Hagnere AM, Berthet J, Vacher-Lavenu MC, Dousset B, Chapuis Y, Bertagna X & Gicquel C 2004 Cyclin E correlates with malignancy of adrenocortical tumours: Preliminary results in 13 consecutive patients European Journal of Endocrinology 150 789–792.

Terzolo M, Bocuzzi A, Bovo S, Cappia S, De Giuli P, Ali A, Paccotti P, Porpiglia F, Fontana D & Angeli A 2001 Immunohistochemical assessment of Ki-67 in the differential diagnosis of adrenocortical tumors. Urology 57 176–182.

Turner HE, Harris AL, Melmed S & Wass JA 2003 Angiogenesis in endocrine tumors. Endocrine Reviews 24 600–632.
Varley JM, McGown G, Thorncroft M, James LA, Margison GP, Forster G, Evans DG, Harris M, Kelsey AM & Birch JM 1999 Are there low-penetrance TP53 alleles? Evidence from childhood adrenocortical tumors. *American Journal of Human Genetics* **65** 995–1006.

Venkatesh S, Hickey RC, Sellin RV, Fernandez JF & Samaan NA 1989 Adrenal cortical carcinoma. *Cancer* **64** 765–769.

Veugelers M, Wilkes D, Burton K, McDermott DA, Song Y, Goldstein MM, La Perle K, Vaughan CJ, O’Hagan A, Bennett KR et al. 2004 Comparative PRKAR1A genotype-phenotype analyses in humans with Carney complex and PRKAR1a haploinsufficient mice. *PNAS* **101** 14222–14227.

Wagner J, Portwine C, Rabin K, Leclerc JM, Narod SA & Malkin D 1994 High frequency of germline p53 mutations in childhood adrenocortical cancer. *Journal of the National Cancer Institute* **86** 1707–1710.

Wajchenberg BL, Albergaria Pereira MA, Medonca BB, Latronico AC, Campos Carneiro P, Alves VA, Zerbini MC, Liberman B, Carlos Gomes G & Kirschner MA 2000 Adrenocortical carcinoma: clinical and laboratory observations. *Cancer* **88** 711–736.

Wales MM, Biel MA, el Deiry W, Nelkin BD, Issa JP, Cavenee WK, Kuerbitz SJ & Baylin SB 1995 P53 activates expression of HIC-1, a new candidate tumour suppressor gene on 17p13.3. *Nature Medicine* **1** 570–577.

Weber MM, Auernhammer CJ, Kiess W & Engelhardt D 1997a Insulin-like growth factor receptors in normal and tumorous adult human adrenocortical glands. *European Journal of Endocrinology* **136** 296–303.

Weber MM, Michl P, Auernhammer CJ & Engelhardt D 1997b Interleukin-3 and interleukin-6 stimulate cortisol secretion from adult human adrenocortical cells. *Endocrinology* **138** 2207–2210.

Weiss LM 1984 Comparative histologic study of 43 metastasizing and nonmetastasizing adrenocortical tumors. *American Journal of Surgical Pathology* **8** 163–169.

Wiedmann HR 1983 Tumours and hemihypertrophy associated with Wiedemann-Beckwiyh syndrome. *European Journal of Pediatrics* **141** 129.

Wood BJ, Abraham J, Hvizda JL, Alexander HR & Fojo T 2003 Radiofrequency ablation of adrenal tumors and adrenocortical carcinoma metastases. *Cancer* **97** 554–560.

Wooten MD & King DK 1993 Adrenal cortical carcinoma. Epidemiology and treatment with mitotane and a review of the literature. *Cancer* **72** 3145–3155.

Yano T, Linehan M, Anglard P, Lerman MI, Daniel LN, Stein CA, Robertson CN, LaRocca R & Zbar B 1989 Genetic changes in human adrenocortical carcinomas. *Journal of the National Cancer Institute* **81** 518–523.

Zhao J, Speel EJ, Mulella-Feurer S, Rutimann K, Saremslani P, Roth J, Heitz PU & Komminoth P 1999 Analysis of genomic alterations in sporadic adrenocortical lesions. Gain of chromosome 17 is an early event in adrenocortical tumorigenesis. *American Journal of Pathology* **155** 1039–1045.