Molecular genetics of adrenocortical tumours, from familial to sporadic diseases

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Abstract

Adrenal masses can be detected in up to 4% of the population, and are mostly of adrenocortical origin. Adrenocortical tumours (ACTs) may be responsible for excess steroid production and, in the case of adrenocortical cancers, for morbidity or mortality due to tumour growth. Our understanding of the pathogenesis of ACTs is more limited than that for other tumours. However, studies of the genetics of ACTs have led to major advances in this field in the last decade. The identification of germline molecular defects in the hereditary syndrome responsible for ACTs has facilitated progress. Indeed, similar molecular defects have since been identified as somatic alterations in sporadic tumours. The familial diseases concerned are Li–Fraumeni syndrome, which may be due to germline mutation of the tumour-suppressor gene TP53 and Beckwith–Wiedemann syndrome, which is caused by dysregulation of the imprinted IGF-II locus at 11p15. ACTs also occur in type 1 multiple endocrine neoplasia (MEN 1), which is characterized by a germline mutation of the menin gene. Cushing’s syndrome due to primary pigmented nodular adrenocortical disease (PPNAD) has been observed in Carney complex patients presenting inactivating germline PRKAR1A mutations. Interestingly, allelic losses at 17p13 and 11p15 have been demonstrated in sporadic adrenocortical cancer and somatic PRKAR1A mutations have been found in secreting adrenocortical adenomas. More rarely, mutations in Gs protein (gsp) and the gene for ACTH receptor have been observed in ACTs. The genetics of another group of adrenal diseases that can lead to adrenal nodular hyperplasia – congenital adrenal hyperplasia (CAH) and glucocorticoid-remediable aldosteronism (GRA) – have also been studied extensively. This review summarizes recent advances in the genetics of ACTs, highlighting both improvements in our understanding of the pathophysiology and the diagnosis of these tumours.

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Introduction

Adrenal masses affect 1–4% of the population (1). Most turn out to be benign adrenocortical adenomas (AAs), that can cause hypersecretion (hypercortisolism in Cushing’s syndrome, mineralocorticoid excess in Conn’s adenoma) or be non-functional. Adrenocortical cancers (ACCs) can also cause morbidity and mortality secondary to tumour growth and metastasis.

Progress in this field has been slower than that for most other cancers, mainly because of the limited number of tumours treated surgically. However, considerable advances toward understanding the molecular mechanisms of adrenocortical tumour (ACT) development have recently been made. The study of rare genetic syndromes associated with ACT has greatly facilitated progress and has increased our understanding of sporadic adrenal tumours (Table 1). Furthermore, several observations have demonstrated that genetic alterations are frequent in both benign and malignant ACT. We present here evidence for the importance of genetic alterations in the pathophysiology of ACT and review the most important genetic defects identified to date.

The clonal origin of ACTs

The study of tumour clonality is an important prerequisite for establishing the cellular origins of neoplasms and identifying the mechanisms underlying tumour progression. Polyclonality suggests that tumour cells are affected by local or systemic stimuli, whereas monoclonality indicates that tumour progression is the end result of an intrinsic genetic mutation. In two different studies, analysis of the
Table 1 Summary of the genetics of ACTs. This table describes genetic diseases responsible for ACTs and other tumoural and non-tumoural manifestations. The molecular alterations observed in sporadic (mostly at the somatic level) are listed in the ‘Sporadic tumours’ column. PPNAD, primary pigmented nodular adrenocortical disease; ACC, adrenocortical cancer; AA, adrenocortical benign adenoma; GH, growth hormone; PRL, prolactin; IGF, insulin-like growth factor; AIMAH, ACTH-independent macronodular adrenal hyperplasia; LCCSCT, large cell calcifying Sertoli cell tumour; LOH, allelic loss. The most important references for each mutation in sporadic tumours are listed in the last column.

| Genetic disease                                | Gene and chromosomal localization | Tumours and associated manifestations                                                                 | Sporadic tumours                                                                                   |
|------------------------------------------------|-----------------------------------|---------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------|
| Li–Fraumeni syndrome (LFS)                     | TP53 (17q13) (hCHK2)              | Soft tissue sarcoma, breast cancers, brain tumours, leukaemia, ACC                                      | TP53 germline mutation in paediatric ACC (32, 33)                                                 |
|                                                |                                   |                                                                                                         | TP53 somatic mutations in sporadic ACC (37, 38)                                                    |
|                                                |                                   |                                                                                                         | 17p13 LOH in sporadic ACC (26, 28)                                                                |
| Multiple endocrine neoplasia type 1 (MEN 1)   | Menin (11q13)                     | Parathyroid, pituitary, pancreas tumours; adrenal cortex; adenoma, hyperplasia, rare carcinoma         | Very rare menin gene mutations in sporadic ACTs (24, 25)                                           |
|                                                |                                   |                                                                                                         | Frequent 11q13 LOH in ACC (23–25)                                                                 |
| Carney complex (CNC)                           | PRKARIA (17q22-24) Other loci? (2p16) | PPNAD, GH- and PRL-secreting tumours, thyroid tumours, testicular tumours (LCCSCT), ovarian cysts, lentiginosis, cardiac myxomas | Sporadic PPNAD, germline de novo PRKARIA mutation (51)                                              |
|                                                |                                   |                                                                                                         | Secreting AA, somatic PRKARIA mutation (52)                                                        |
|                                                |                                   |                                                                                                         | Sporadic AA and ACC, 17q22–24 LOH (52)                                                            |
|                                                | p57kip2 (CDKN1C; genetic defect)  | Omphalocele, macroglossia, macrosomia, hemihypertrophy, Wilms’ tumour, ACC                            | 11p15 LOH (28)                                                                                    |
| Beckwith–Wiedemann syndrome (BWS)              | KCNQ10T (epigenetic defect) H19 (epigenetic defect) 11p15 locus alterations IGF-II overexpression         |                                                                                                         | IGF-II overexpression (61–63)                                                                     |
|                                                |                                   |                                                                                                         |                                                                                                   |
| McCune–Albright syndrome (MAS)                 | GNAS 1 (20q13)                    | Polysyndactyly, fibrous dysplasia, café-au-lait spots, precocious puberty, hyperfunction of endocrine glands (thyroid, adrenal glands, pituitary gland) | A few GNAS1 mutations in sporadic adrenocortical tumours (75, 76)                                   |
|                                                |                                   |                                                                                                         | GNAS1 mutation in AIMAH without MAS features (77)                                                  |
| Congenital adrenal hyperplasia (CAH)           | CYP21 (6p)                        | Adrenal hyperplasia; Classical form; early onset (virilization of externa genitalia in females, hypocortisolism, precocious puberty in both sexes); Non classical form, late onset | CYP21 gene mutation in AA (82)                                                                    |
| Glucocorticoid-remediable aldosteronism (GRA)  | CYP11B1 (8q21)                    | AA, micronodular, homogeneous hyperplasia                                                          |                                                                                                   |
| Hereditary isolated glucocorticoid deficiency syndrome | MC2-R (18p11) | Inactivating mutations, glucocorticoid deficiency                                                   | Activating mutation of MC2-R in AIMAH (91)                                                          |
|                                                |                                   |                                                                                                         | 18p11 LOH in ACC (93)                                                                             |
pattern of X-chromosome inactivation in heterozygous female tissue has shown that ACCs consist of monoclonal populations of cells and that nodular hyperplastic adrenal tissue consists of a polyclonal population of cells. According to one of these studies (2), AAs may be monoclonal (43%) or polyclonal (28.5%), with various intermediate forms (28.5%). But in the other study almost all AAs were monoclonal (3). The genetic heterogeneity observed in AA may be explained in two different ways: (1) different types of tumour may have fundamentally different pathogenic mechanisms or (2) different tumours may correspond to different stages of a common multistep pathway. The unusual case of bilateral macronodular hyperplasia may provide a natural illustration of the second model, with heterogeneous clonal patterns expressed simultaneously at different locations. The initial event in the multistep process is probably initiation of the growth of a polyclonal tumour, with the maintenance of a normal pattern of steroid secretion. This event may be local, reflecting the action of extrinsic factors such as mitogens or growth factors (e.g. epidermal growth factor, basic fibroblast growth factor and insulin-like growth factors (IGFs)), which may increase cell proliferation, increasing the susceptibility of the cell to oncogene or tumour-suppressor gene mutations (4–6). Once a subclone acquires a genetic advantage over competing subclones, selective proliferation may occur, with the advantaged clone replacing other cells in the tumour.

Pituitary tumours were initially reported to have a monoclonal origin (7, 8), but Farrell & Clayton (9) suggested a different clonal composition for these endocrine tumours. Indeed, although pituitary tumours are generally benign adenomas, they can recur and grow after initial surgery. This led Farrell & Clayton (9) to suggest that invasiveness and biological aggressiveness may result from the accumulation of losses of tumour-suppressor gene functions. An initial stimulus leading to the hyperplasia of specific cell subtypes in the pituitary gland gives rise to a number of different clones, each with variable potential to develop into a discrete tumour, depending on rates of cell division and/or apoptosis (10). It is unclear whether these observations on endocrine tumours of the pituitary gland can also be applied to ACTs.

A potential example of this dynamic process involving local/systemic stimuli, the accumulation of successive genetic alterations and clonality in adrenal tumourigenesis is the ectopic or abnormal expression of hormone receptors observed in adrenocorticotrophic (ACTH)-independent macronodular adrenal hyperplasia (AIMAH) and in unilateral ACTs (11). This process has been studied more extensively in food-dependent adrenal Cushing’s syndrome (FD-ACS), due to the ectopic expression of gastric inhibitory polypeptide receptor (GIP-R): to date, a few cases of AA and slightly more cases of AIMAH (11–14) with FD-ACS have been reported. A cortisol response to GIP has been observed in vivo and in vitro in FD-ACS. GIP is as potent as ACTH in stimulating cortisol secretion from fragments of AA or AIMAH in vitro (15, 16). Binding and reverse transcriptase PCR studies have shown that the GIP-R is expressed in adrenal tumours from patients with FD-ACS, whereas little or no expression of this receptor is observed in adjacent non-tumoural or normal adrenal tissue (17, 18). Groussin et al. (14) demonstrated that GIP-R expression is frequent in AIMAH and may not necessarily be associated with low fasting plasma cortisol levels. This suggests that the maintenance of hypercortisolism in cases of GIP-R-expressing AIMAH is not solely dependent on GIP-R and that other membrane receptors may also be expressed abnormally in this condition (16).

The importance of genetic alterations in sporadic ACTs

Monoclonal tumours result from genetic alterations conferring a growth advantage on 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 screening for such chromosomal alterations. These approaches have identified alterations affecting various chromosomes and loci (19–23). Kjellman et al. (19) demonstrated by CGH that chromosomal alterations are observed in 28% of AAs. Most of the changes observed concern losses on chromosomes 2, 11q and 17p and gains on chromosomes 4 and 5. In more recent studies, CGH identified changes in 61% of AAs and the most common gains observed were on chromosomes 5, 12, 19 and 4. Losses were observed at 1p, 17p, 22p, 22q, 2q and 11q in up to 62% of cases of ACC (22). Studies using microsatellite markers have demonstrated high percentages of loss of heterozygosity (LOH)/allelic imbalance at 11q13 (100%) (23–25) and 2p16 (92%) (23) in carcinomas. Moreover, LOH of the 17p13 locus (26, 27) has been reported to be highly specific to malignant tumours and to be of prognostic value for the recurrence of localized tumours (28).

Genetic alterations, from familial syndromes to sporadic diseases

TP53 and locus 17p13

TP53 is a tumour-suppressor gene, located at 17p13, and involved in the control of cell proliferation. Acquired mutations in TP53 are common tumour-specific genetic alterations in humans, and have been identified in most of the major types of cancer (29).
Germline mutations in TP53 are identified in 70% of families with Li–Fraumeni syndrome. This syndrome displays dominant inheritance and confers susceptibility to breast carcinoma, soft tissue sarcoma, brain tumours, osteosarcoma, leukaemia and ACC (30). Other possible component tumours include melanoma, gonadal germ cells tumours and carcinoma of the lung, pancreas and prostate. These tumours 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 Li–Fraumeni syndrome patients (31). However, in these few kindreds there is no report of ACC (31). Germline mutations in TP53 have been observed in 50–80% of children with apparently sporadic ACC in North America and Europe (32, 33). The incidence of paediatric ACC is about 10 times higher in southern Brazil than in the rest of the world, and a specific diatric ACC is about 10 times higher in southern America and Europe (32, 33). 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 (34, 35). Molecular studies about this mutation have shown that the tissue-specific effects of this mutation may be due to a pH-dependent effect caused by the replacement of an arginine by a histidine in the tetramerization domain of TP53 (36).

In sporadic ACC in adults, somatic mutations of TP53 are found in only 25% of ACC cases and are located in four ‘hot-spot regions’ within exons 5 and 8, as first demonstrated by Ohgaki et al. (37) and Reincke et al. (38) in a small series. An Italian group recently reported a TP53 mutation rate of 70% in 10 ACCs (39). Lin et al. (40) reported TP53 mutations in the AA of 73% of Taiwanese patients studied, with 82% of these mutations located in exon 4. Reincke et al. (41) sequenced exon 4 in 19 AAs 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 AA (26, 28). LOH at 17p13 was recently reported to occur in 85% of malignant tumours and only in 30% of benign adenomas. LOH at 17p13 is correlated with Weiss score, an index of cytopathological alterations used to determine the malignancy of ACT. It has therefore been suggested that 17p13 LOH could be used as a molecular marker of malignancy in ACT: in a large prospective study of ACT patients, 17p13 LOH was demonstrated to be an independent variable predictive of recurrence after complete surgical removal of localized ACT (28).

The discrepancy between the frequencies of TP53 mutation and 17p13 LOH may be accounted for by the existence of another tumour-suppressor gene in this region. The HIC-1 (hypermethylated in cancer) gene is such a candidate. It encodes a transcription factor triggered by TP53 and inactivated by hypermethylation or allelic losses in various cancers (42).

Menin gene and locus 11q13
The menin gene, located at the 11q13 locus, is thought to be a tumour-suppressor gene. A heterozygous inactivating germline mutation of menin is found in about 90% of families affected by multiple endocrine neoplasia type 1 (MEN 1). This is an autosomal dominant syndrome with high penetrance and an equal sex distribution. The principal clinical features include parathyroid (95%), endocrine pancreas (45%) and pituitary (45%) tumours, thymic carcinoids and thyroid adenomas (43). ACTs and/or hyperplasia are observed in 25–40% of MEN 1 patients (23, 25). In most cases, they are non-functional AAs that can be managed conservatively with radiological/hormonal follow-up. 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 menin gene is very rare: one mutation was identified in a series of 41 AAs in one study (24) and one mutation in a series of ACCs was found in another (25). By contrast, LOH at 11q13 was identified in more than 90% of informative ACC in three different series whereas it has been reported in fewer than 20% of informative adenomas (23–25). However, LOH in ACC involves almost all of the 11q domain, suggesting that an as-yet-unidentified tumour-suppressor gene located on the long arm of the chromosome is involved in ACC formation.

PRKAR1A gene and locus 17q22-24
The regulatory R1A subunit of protein kinase A (PRKAR1A) is a key component of the cAMP signalling pathway that has been implicated in endocrine tumourigenesis (44, 45). This gene maps to 17q22-24, a locus that has been implicated, by linkage analysis, in a dominantly multiple neoplasia inherited syndrome with many clinical and pathological manifestations, the Carney complex (CNC) (46, 47). Heterozygous inactivating germline mutations of PRKAR1A have been demonstrated in about 45–65% of CNC families (47, 48). LOH at 17q22-24 is observed in tumours from CNC patients, suggesting that PRKAR1A is a tumour-suppressor gene. The main features of CNC include spotty skin pigmentation (lentigines, endocrine overactivity and cardiac myxomas (49). The tumours observed in CNC patients include growth hormone (GH)-secreting pituitary adenoma, thyroid adenomas or carcinomas, testicular tumours (large-cell calcifying Sertoli cell tumours), ovarian cysts, melanocytic schwannomas, breast ductal adenomas and adrenocortical lesions. ACTH-independent Cushing’s syndrome caused by primary pigmented nodular adrenocortical disease (PPNAD) is observed in 25–30% of patients with CNC. PPNAD is caused by a primary bilateral adrenal defect and may occur in patients with no other CNC features and no family history of
CNC. ACTH-independent Cushing’s syndrome is often atypical in PPNAD: it may be cyclic, associated with a paradoxical increase in cortisol concentration after dexamethasone administration and may be found in patients with normal computed tomography scans. The frequency of PRKAR1A mutations is about 80% in CNC patients with Cushing’s syndrome, suggesting that 17q22-24 defects are more likely to be found in families with PPNAD (50). Moreover, patients with isolated PPNAD and no family history of CNC may present de novo germline mutation of PRKAR1A (51). Somatic PRKAR1A mutations have been also demonstrated in sporadic secreting AA, with clinical, hormonal and pathological characteristics similar to those of PPNAD (52).

**IGF-II and 11p15 alterations**

The 11p15 region is organized into two different clusters: a telomeric domain including the IGF-II gene (53), H19 (54) and a centromeric domain including CDKN1C (p57kip2) (55, 56). The IGF-II gene encodes an important foetal growth factor, is maternally imprinted and is therefore expressed only from the paternal allele (53). 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 parentally imprinted and are therefore expressed from the maternal allele only (fig. 1). 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 (BWS) (57). This overgrowth disorder is characterized by macrosomia, macroglossia, organomegaly and developmental abnormalities (in particular abdominal-wall defects with exomphalos). It predisposes patients to the development of embryonal tumours – such as Wilms’ tumour – ACC (58–60), neuroblastoma and hepatoblastoma.

IGF-II mRNA is efficiently translated and malignant tumours contain large amounts of IGF-II protein, some of which is in the prohormone form. The IGF system is involved in the development and maintenance of differentiated adrenocortical functions and its role has been largely documented in ACTs (6, 27, 28). Many studies have demonstrated that IGF-II is often strongly overexpressed in malignant ACTs, with such overexpression observed in approximately 90% of ACCs (61–63). Transcriptome analysis of ACT has demonstrated that IGF-II is the gene most overexpressed in ACC by comparison with AAs or normal adrenal glands (64–66). The mechanism underlying IGF-II overexpression is paternal isodisomy (loss of the maternal allele and duplication of the paternal allele) or, less frequently, loss of imprinting (67, 68) (with maintenance of both parental alleles but a paternal-like IGF-II gene expression pattern; fig. 1) (62).

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 (69). The mitogenic effect of IGF-II is dependent on the IGF-I receptor, as reported by Logié et al. (70), who demonstrated that IGF-II is involved in NCI H295R cell line proliferation and acts via the IGF-I receptor. IGF-II effects are restricted to tumours 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 ACTs with IGF-II overproduction have been shown to contain large amounts of IGFBP-2 (61), suggesting that IGFBP-2 may regulate IGF-II effects in ACC. Furthermore, IGFBP-2 levels have been shown to correlate with tumour stage in ACC (71). In ACC, only the maternal H19 allele is expressed, so expression of this gene is abolished in most ACCs displaying paternal isodisomy (62). Methylation of the H19 promoter has been shown to be involved in the abnormal expression of both H19 and IGF-II in human ACC (72). Expression of p57kip2 is also abolished in ACC (73), but the precise role of the product encoded by this gene in the cell-cycle machinery and tumourigenesis requires confirmation. Like 17p13 LOH, 11p15 LOH is associated with a higher risk of tumour recurrence, is more frequent in ACC than in AA (78.5 versus 9.5%) and correlates with Weiss score (28). These genetic abnormalities generate a mosaic-like pattern in some tumours, suggesting that the tumour is made up of different subpopulations of cells. Thus, 11p15 alterations could be used as a biological marker for predicting ACC malignancy after surgical removal of the tumour (28). However, 11p15 LOH seems to have a lower predictive value than 17p13 LOH.

**GNAS1 gene**

The trimeric G-protein (α, β and γ subunits) is responsible for transmembrane signal transduction following ligand activation of a G-protein-coupled seven-transmembrane domain receptor (ACTH receptor, ACTH-R). Somatic activating mutations of the GNAS1 gene (mutant Gs protein, termed gsp) responsible for excess activity of the cAMP signalling pathway have been reported in McCune–Albright syndrome (MAS) (74). This disease is characterized by polyostotic fibrous dysplasia, café-au-lait spots, precocious puberty and hyperfunction of multiple endocrine glands (thyroid, adrenal glands, pituitary gland). Hypercortisolism occurs in 5% of patients and is due to AIMAH (74). In MAS, the gsp mutation occurs during embryonic development, as demonstrated by its mosaic pattern of distribution in various tissues. Few somatic GNAS mutations have been found in ACTs; only one mutation in one sporadic aldosterone-secreting tumour and in one cortisol-secreting tumour have been reported (75, 76).

Two different gsp mutations have been reported in three patients with Cushing’s syndrome due to AIMAH without MAS features (77). The authors speculated as to whether these patients presented a disease in the spectrum of MAS, with a late somatic mutation leading to a single defect, or whether they were the first reported cases of isolated AIMAH with gsp mutations involved in molecular pathogenesis (77).

**Congenital adrenal hyperplasia (CAH)**

CAH is one of the most frequent genetic endocrine diseases, inherited as an autosomal recessive trait. It is caused by the loss or severe decrease in activity of one of the steroidogenic enzymes involved in cortisol biosynthesis (mostly 21-hydroxylase (21-OH), 11β-hydroxylase (11β-OH) and 3β-hydroxysteroid dehydrogenase). Deficiencies in 21-OH (CYP21) are the most common causes of CAH, accounting for 90–95% of cases. All the known biochemical defects impair cortisol secretion, resulting in the stimulation of pituitary corticotrophs, leading to compensatory hypersecretion of ACTH resulting in hyperplasia of the adrenal cortex. This effect is ACTH-dependent, whereas extrinsic factors, such as growth factors or mitogens, may be involved in adrenal hyperplasia. These two different mechanisms may account for the heterogeneous clonal pattern of CAH.

In the past, both homozygous and heterozygous patients with CAH have been reported to have substantially enlarged adrenal glands and a prevalence of adrenal incidentalomas (78, 79). Beuschlein et al. (80) investigated the mutational spectrum and mRNA levels for the CYP21 gene in six aldosterone-producing adenomas, four adrenal carcinomas and two adrenocortical incidentalomas. They found that neither of the two adrenocortical incidentalomas had homozygous or heterozygous CYP21 mutations, although the mRNA contents of the two tumours were markedly lower than those of aldosterone-producing adenomas. No mutation in CYP21 was detected in a recent study of leukocyte DNA from a series of 27 patients, whereas two heterozygous CYP21 mutations were found in adrenal tumour DNA (81). By contrast, in another series, a higher frequency of classic CAH carriers (16%) and of manifest CAH (2%) was reported among patients with AAs than in the general population (82). ACC with 21-OH deficiency is a possible, albeit rare event, as suggested by Merke et al. (83) in a patient carrying 21-OH deficiency and adrenal lymphocytic infiltration with histological features of adrenal carcinoma. Few data concerning CYP11B gene mutations in ACTs have been reported: 11β-OH deficiency may be involved in adrenal tumourigenesis, but no CYP11B gene mutation has been observed (84).
Glucocorticoid-remediable aldosteronism (GRA)

GRA was the first described familial form of hyperaldosteronism. This disorder is characterized by the chronic regulation of aldosterone secretory function by ACTH. Aldosterone hypersecretion can therefore be blocked chronically by exogenous glucocorticoids, such as dexamethasone. This autosomal dominant disorder has been shown to be caused by a hybrid gene formed by crossover between the ACTH-responsive regulatory portion of the 11β-OH (CYP11B1) gene and the coding region of the aldosterone synthase gene (CYP11B2). Adrenal tumours, together with micronodular and homogeneous hyperplasia of the adrenal cortex, have been observed in the familial cases (85, 86).

ACTH-R gene

ACTH-R belongs to a subgroup of five receptors of the G-protein-coupled receptor superfamily. This subgroup consists of ACTH-R (or melanocortin 2 receptor (MCR-2)), melanocyte stimulating hormone receptor (MSH-R) (or MCR-1) and three other receptors (MCR-3–5). It is encoded by an intron-less gene on chromosome 18p11.2. Inactivating mutations in ACTH-R have been identified in several families with hereditary isolated glucocorticoid deficiency (87). Screening for ACTH-R mutations in a variety of adrenal tumours has identified no somatic activating mutations to date (88, 89). One potential activating germline mutation of ACTH-R has been reported in an abstract by Aloi et al. (90), in a patient with bilateral adrenal hyperplasia and Cushing’s syndrome. Swords et al. (91) reported the functional characterization of this mutant receptor and demonstrated that it displays high levels of basal activity due to a defect in receptor desensitization. Hiroi et al. (92) described the case of a woman with two germline mutations in ACTH-R, leading to a previously undescribed syndrome, ‘ACTH hypersensitivity syndrome’, although no functional studies were reported to confirm this. ACTH-R LOH has also been investigated in AAs and ACTs: it was observed in two of four informative cancers, but not in 15 hyperfunctioning adenomas, suggesting a role for ACTH-R in cellular differentiation (93).

Conclusion

Studies of clonality show clearly that genetic alterations play a major role in adrenal cortex tumourigenesis. Studies of hereditary neoplasia syndromes have led to the identification of various loci or chromosomal regions and genes responsible for ACT development. The same molecular defects are observed in the germ-line DNA in cases of hereditary disease and as somatic defects in tumour DNA in cases of sporadic ACT. For a given genetic defect, the tumour phenotype observed in sporadic tumours displays some similarities to the tumour phenotype observed in familial diseases. This may have important clinical implications as the molecular study of tumour DNA could provide important information for diagnostic and/or prognostic purposes. Interestingly, in sporadic tumours, there is almost no overlap between the genetic alterations observed in cancers and those found in AAs. For instance, LOH at 17p13 or 11q15, and p53 mutations are observed in cancers but not in the rare benign adenomas with mutations in PRKAR1A or GNAS. The same applies to certain cellular defects, such as loss of cAMPresponse-element-binding protein (CREB) expression (94–96).
or ectopic expression of GIP-R, the first of which is observed in cancer and not in secreting adenomas and the second of which has been identified to date only in secreting AAs and AIMAH. The lack of a known molecular defect identified consistently in both benign and malignant tumours raises questions about the development of benign and malignant ACTs. The development of tumours in other tissues, such as the digestive tract, is thought to be based on the accumulation of numerous molecular defects, resulting in progression from benign polyp to colon cancer. Some rare tumours in which a malignant and a benign zone are associated within the same adrenal gland are consistent with this model (97). However, from what we have learned so far from the genetics of ACT, it would seem premature to suggest that such a model could be applied to the adrenal cortex. However, it is tempting to speculate that genetic defects might stimulate the growth of some benign cortisol-secreting tumours with such a level of cellular differentiation that progression towards a malignant dedifferentiated tumour would be prevented. This is illustrated by the various cellular and molecular defects activating the cAMP signalling pathway that have been observed in benign hyperplasia or tumours causing Cushing’s syndrome (fig. 2). Nevertheless, this hypothesis is consistent with an apparently benign adenoma with a lower level of differentiation, not responsible for overt cortisol secretion, being able to progress toward a malignant tumour. However, the high frequency of such adenomas, which are usually discovered by chance, contrasts with the rarity of adrenal cancer, suggesting that this multistep progression from benign to malignant tumours might be very rare (fig. 3). Clearly, despite progress in studies of the genetics of ACT, much remains to be done if we are to identify the many molecular alterations involved.

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References

1 Bertherat J, Mosnier-Pudar H & Bertagna X. Adrenal incidentalomas. Current Opinion in Oncology 2002 14 58–63.
2 Gicquel C, Leblond-Francillard M, Bertagna X, Louvel A, Chapuis Y, Luton JP, Girard F & Le Bouc Y. Clonal analysis of human adrenocortical carcinomas and secreting-adenomas. Clinical Endocrinology 1994 40 465–477.
3 Beuschlein F, Reinicke M, Karl M, Travis WD, Jaursch-Hancke C, Abdelhamid S, Chrousos GP & Allopolo B. Clonal composition of human adrenocortical neoplasms. Cancer Research 1994 54 4927–4932.
4 Feige JJ, Cochet C, Savona C, Shi DL, Keramidas M, Defaye G & Chambaz EM. Transforming growth factor beta 1: an autocrine regulator of adrenocortical steroidogenesis. Endocrine Research 1991 17 267–279.
5 Mesiano S, Mellon SH, Gospodarowicz D, Di Blasio AM & Jaffe RB. Basic fibroblast growth factor expression is regulated by corticotropin in the human fetal adrenal: a model for adrenal growth regulation. PNAS 1991 88 5428–5432.
6 Mesiano S, Mellon SH & Jaffe RB. Mitogenic action, regulation, and localization of insulin-like growth factors in the human...
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Delbridge L & Robinson BG. Comparative genomic hybridization analysis of adrenocortical tumors. *Journal of Clinical Endocrinology and Metabolism* 2002 87 3467–3474.

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

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

Schulte KM, Mengel M, Heinze M, Simon D, Scheuring S, Kohrer K & Rohrer HD. Complete sequencing and messenger ribonucleic acid expression analysis of the MEN 1 gene in adrenal cancer. *Journal of Clinical Endocrinology and Metabolism* 2000 85 441–448.

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

Gicquel C, Bertagna X & Le Bouc Y. Recent advances in the pathogenesis of adrenocortical tumours. *European Journal of Endocrinology* 1995 133 133–143.

Gicquel C, Bertagna X, Gaston V, Coste J, Louvel A, Baudin E, Berthet J, Chapuis Y, Duclos JM, Schlumberger M, Plouin PF, Luton JP & Le Bouc Y. Molecular markers and long-term recurrences in a large cohort of patients with sporadic adrenocortical tumours. *Cancer Research* 2001 61 6762–6767.

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

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

Auer G, Larsson C & Backdahl M. Genetic aberrations in adrenocortical tumors: very frequent deletions of the MEN1 locus in 11q13 and of a 1-centimorgan region in 2p16. *European Journal of Endocrinology* 1999 141 483–489.

Varley JM, McGown G, Thorncroft M, James LA, Margison GP, Forster G, Evans DG, Harris M, Kelsey AM & Birch JM. p53 mutations in childhood adrenocortical cancer. *Journal of Pathology* 1995 170 7–12.

Varley JM, McGown G, Thorncroft M, James LA, Margison GP, Forster G, Evans DG, Harris M, Kelsey AM & Birch JM. Are there low-penetrance TP53 alleles? Evidence from childhood adrenocortical tumours. *American Journal of Human Genetics* 1999 65 995–1006.

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

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

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

DiCamilmarino EL, Lee AS, Cadwell C, Zhang W, Rothner B, Ribeiro RC, Zambetti G & Krtiwacki RW. A novel mechanism of tumorigenesis involving ph-dependent destabilization of a mutant p53 tetramer. *Nature Structural Biology* 2002 9 1–12.

Ohgaki H, Kleihues P & Heitz PU. p53 mutations in sporadic adrenocortical tumors. *International Journal of Cancer* 1993 54 408–410.

Reincke M, Karl M, Travis WH, Mastorakos G, Allolio B, Linehan HM & Chrousos GP. p53 mutations in human adrenocortical neoplasms: immunohistochemical and molecular studies.
Journal of Clinical Endocrinology and Metabolism 1994 78 790–794.

39 Barzon L, Chiossi M, Fallo F, Martinogoni G, Montagna L, Pulu G & Boscaro M. Molecular analysis of CDKN1C and TP53 in sporadic adrenal neoplasms. European Journal of Endocrinology 2001 145 207–212.

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

41 Reincke M, Wachenfeld C, Mora P, Thumser A, Jaursch-Reincke M, el Deiry W, Nelkin BD, Issa JP, Cavenee WK, Thakker RV. Multiple endocrine neoplasia syndromes of the twentieth century. Journal of Clinical Endocrinology and Metabolism 1998 83 2617–2620.

42 Bertherat J. Protein kinase A in Carney complex: a new example of cAMP pathway alteration in endocrine tumors. European Journal of Endocrinology 2001 144 209–213.

43 Bossi I & Stratakis CA. Minireview: Phakelias 1a: normal and abnormal functions. Endocrinology 2004 145 5452–5458.

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

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

46 Veugelers M, Wilkes D, Burton K, McDermott DA, Song Y, Goldstein MM, La Perle K, Vaughan CJ, O’Hagan A, Bennett KR, Meyer BJ, Legius E, Kartunen M, Norel R, Kaariainen H, Lavyne M, Neau JP, Richter G, Kirali K, Farnsworth A, Stapleton K, Morelli P, Takanashi Y, Barnforth JS, Etelberger F, Noszian I, Manfroi W, Powers J, Imai T, Ko GT, D’Souza CA, Goldmuntz E, Edelberg JM, Collins A, Eccles D, Irving AD, McKnight GS & Basson CT. Comparative PRKAR1A genotype-phenotype analyses in humans with Carney complex and PRKAR1A haploinsufficient mice. PNAS 2004 101 14222–14227.

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

48 Groussin L, Kirschner LS, Vincent-Dejean C, Perlemoine K, Jullian E, Delemer B, Zachariaves S, Pignatelli D, Carney JA, Laton JP, Bertagna X, Stratakis CA & Bertherat J. 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 pathophysiologic: Augmented PKA signaling is associated with adrenal tumorigenesis in PPNAD. American Journal of Human Genetics 2002 71 1433–1442.

49 Groussin L, Jullian E, Perlemoine K, Louvel A, Leheup B, Laton JP, Bertagna X & Bertherat J. Mutations of the PRKAR1A gene in Cushing’s syndrome due to sporadic primary pigmented nodular adrenocortical disease. Journal of Clinical Endocrinology and Metabolism 2002 87 4324–4329.

50 Bertherat J, Groussin L, Sandrini F, Matyakhina L, Beri T, Stergiopoulos S, Papageorgiou T, Bourdeau I, Kirschner LS, Vincent-Dejean C, Perlemoine K, Gicquel C, Bertagna X & Stratakis CA. 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 2003 63 5308–5319.

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

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

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

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

55 Lam MM, Watada I, Oshishi S, Mukai T, Joyce JA, Cole TR, Donnai D, Reik W, Schofield PN & Maher ER. Analysis of germline CDKN1C (p57kip2) mutations in familial and sporadic Beckwith-Wiedemann syndrome (BWS) provides a novel genotype-phenotype correlation. Journal of Medical Genetics 1998 36 518–523.

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

57 Sbragia-Neto L, Melo-Filho AA, Guerra Junior G, Valente de Lemos Martini SH, Baptista MT, Sabino de Matos P, De Oliveira-Filho AG & Bittorf-Sella JM. Beckwith-Wiedemann syndrome and virilizing cortical adrenal tumor in a child. Journal of Pediatric Surgery 2004 39 1269–1271.

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

59 Boule N, Logie A, Gicquel C, Perin L & Le Bocu Y. 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 1998 83 1713–1720.

60 Gicquel C, Rafflin-Sansom ML, Gaston V, Bertagna X, Plouin PF, Schlumberger M, Louvel A, Laton JP & Le Bocu Y. 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 1997 82 2559–2565.

61 Ilvesmaki V, Kahri AI, Miettinen PJ & Voutilainen R. 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 1997 82 852–858.

62 Giordano TJ, Thomas DG, Kuick R, Lignyess M, Misiek DE, Smith AL, Sanders D, Aljundi RT, Gouger PG, Thompson NW, Taylor JM & Hanash SM. Distinct transcriptional profiles of adrenocortical tumors uncovered by DNA microarray analysis. American Journal of Pathology 2003 162 521–531.

63 Bourdeau I, Antonini SR, Lacroix A, Kirschner LS, Matyakhina L, Lorang D, Libutti SK & Stratakis CA. Gene array analysis of macro-nodular adrenal hyperplasia confirms clinical heterogeneity and identifies several candidate genes as molecular mediators. Oncology 2004 20 1575–1585.

64 de Frainport F, El Attifi M, Cherradi N, Le Moigne G, Defaye G, Houlgatte R, Bertherat J, Bertagna X, Plouin PF, Baudrin E, Berger F, Gicquel C, Chabre O & Fiege JJ. Gene expression profiling of human adrenocortical tumors using cDNA microarray identifies several candidate genes as markers of malignancy. Journal of Clinical Endocrinology and Metabolism 2005 90 1819–1829.

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

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

67 Weber MM, Auernhammer CJ, Kiess W & Engelhardt D. Insulin-like growth factor receptors in normal and tumorous adult
human adrenocortical glands. *European Journal of Endocrinology* 1997 136 296–303.
70 Logie A, Boulle N, Gaston V, Perin L, Boudou P, Le Bouc Y & Gicquel C. Autocrine role of IGF-II in proliferation of human adre- nocortical carcinoma NCI H295R cell line. *Journal of Molecular Endocrinology* 1999 23 23–32.
71 Boulle N, Gicquel C, Logie A, Christol R, Feige JJ & Le Bouc Y. Fibroblast growth factor-2 inhibits the maturation of pro-insul- lin-like growth factor-II (pro-IGF-II) and the expression of insu- lin-like growth factor binding protein-2 (IGFBP-2) in the human adrenocortical tumor cell line NCI-H295R. *Endocrinology* 2000 141 3127–3136.
72 Gao ZH, Suppola S, Liu J, Heikkila P, Janne J & Voutilainen R. Association of H19 promoter methylation with the expression of H19 and IGF-II genes in adrenocortical tumors. *Journal of Clinical Endocrinology and Metabolism* 2002 87 1170–1176.
73 Liu J, Kahri AI, Heikkila P & Voutilainen R. 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* 1997 82 1766–1771.
74 Weinstein LS, Shenker A, Gejman PV, Merino MJ, Friedman E & Spiegel AM. Activating mutations of the stimulatory G protein in the McCune-Albright syndrome. *New England Journal of Medi- cine* 1991 325 1688–1695.
75 Yoshimoto K, Iwahana H, Fukuda A, Sano T & Itakura M. Rare European Journal of Endocrinology 2000
76 Dall’Asta C, Ballare E, Mantovani G, Ambrosi B, Spada A, Jaresch S, Kornely E, Kley HK & Schlaghecke R. Adrenal inciden-
77 Fragoso MC, Domenice S, Latronico AC, Martin RM, Pereira MA, Baumgartner-Parzer SM, Pauschenwein S, Waldhausl W, Jaresch S, Kornely E, Kley HK & Schlaghecke R. Adrenal incidence-
78 Dall’Asta C, Ballare E, Mantovani G, Ambrosi B, Spada A, Jaresch S, Kornely E, Kley HK & Schlaghecke R. Adrenal incidence-
79 Jaresch S, Kornely E, Kley HK & Schlaghecke R. Adrenal incidence-
80 Beuschlein F, Schulze E, Mora P, Gensheimer HP, Maser-Gluth C, Allocco B & Reincke M. Steroid 21-hydroxylase mutations and 21-hydroxylase messenger ribonucleic acid expression in human adrenocortical tumors. *Journal of Clinical Endocrinology and Metabolism* 1998 83 2585–2588.
81 Kjeliman M, Holst M, Backdahl M, Larsson C, Farnebo LO & Wedell A. No overrepresentation of congenital adrenal hyperplasia in patients with adrenocortical tumors. *Clinical Endocrinology* 1999 50 343–346.
82 Baumgartner-Parzer SM, Pauschenwein S, Waldhausl W, Pöhlker K, Nowotny P & Vierhapper H. Increased prevalence of heterozygous 21-OH germline mutations in patients with adrenal incidentalomas. *Clinical Endocrinology* 2002 56 811–816.
83 Merke DP, Bornstein SR, Braddock D & Chrousos GP. Adrenal lymphocytic infiltration and adrenocortical tumors in a patient with 21-hydroxylase deficiency. *New England Journal of Medicine* 1999 340 1121–1122.
84 Maser-Gluth C, Reincke M, Allocco B & Schulze E. Metabolism of glucocorticoids and mineralocorticoids in patients with adrenocortical tumors. *European Journal of Clinical Investigation* 2000 30 (Suppl 3) 81–86.