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 \textit{TP53} and Beckwith–Wiedemann syndrome, which is caused by dysregulation of the imprinted \textit{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 \textit{menin} gene. Cushing’s syndrome due to primary pigmented nodular adrenocortical disease (PPNAD) has been observed in Carney complex patients presenting inactivating germline \textit{PRKAR1A} mutations. Interestingly, allelic losses at 17p13 and 11p15 have been demonstrated in sporadic adrenocortical cancer and somatic \textit{PRKAR1A} mutations have been found in secreting adrenocortical adenomas. More rarely, mutations in \textit{Gsp} protein (\textit{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...
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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 clonal populations of cells and that nodular hyperplasia 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 kindred there is no report of ACC (31). Germline mutations in TP53 have been reported in Li–Fraumeni syndrome patients (31). 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 kindred there is no report of ACC (31). Germline mutations in TP53 have been reported in Li–Fraumeni syndrome patients (31).

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. 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).

Menin 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 (lentigiosis), 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...
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 mutation of PRKAR1A have been also demonstrated in sporadic secreting AA, with clinical, hormonal and pathological characteristics similar to those of PPNAD (52).

LOH at 17q22-24 has been also observed in sporadic AA and seems to be restricted to the PRKAR1A locus, suggesting the possible involvement of this tumour-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 ACT growth.

**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 AA 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 lost in ACC (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 tumorigenesis 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 sub-populations 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, ACTHR). 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 tumourgenesis. 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 cAMP-response-element-binding protein (CREB) expression (94–96).

**Figure 2** cAMP pathway alterations in various causes of Cushing’s syndrome. The figure shows alterations of the cAMP pathway from the extracellular (1 and 2) to the intracellular and nuclear levels (2–4), in various forms of Cushing’s syndrome associated with adrenal hyperplasia, adrenal tumours, macro- or micronodular adrenocortical disease (AIMAH and PPNAD). It should be noted that 2–4 are associated with benign tumours. (1) Eutopic (Cushing’s disease) or ectopic ACTH secretion. (2) Activating mutation of the ACTH-R and abnormal expression of membrane receptors GIP-R, β-adrenergic receptor (β) and luteinizing hormone (LH) receptor. (3) Activating mutation of the α subunit of the Gs protein, termed oncogene gsp in McCune–Albright syndrome (MAS). (4) Inactivating germline mutation of the PRKAR1A gene in Carney complex (CNC). AC, adenylyl cyclase; AMPc, cyclic AMP; CREB, cAMP-response-element-binding protein.
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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