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Genetics of Cushing's Syndrome

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Cushing's disease · Cushing's syndrome · Multiple endocrine neoplasia type 1 · Carney complex · Familial isolated pituitary adenomas · Aryl hydrocarbon receptor-interacting protein

Abstract
Cushing's syndrome (CS) is characterized by pathologically elevated free glucocorticoid levels. Endogenous hypercortisolism is usually due to ACTH-secreting pituitary corticotropic adenomas and less often due to ectopic ACTH-secreting neuroendocrine neoplasms or ACTH-independent adrenal cortisol hypersecretion. CS is a serious chronic disease leading to a several-fold increase in cardiovascular morbidity and mortality. Multiple genetic alterations have been described in the setting of sporadic corticotropinoma formation. Changes in the expression profiles have been demonstrated in growth factors and their receptors, cell-cycle regulators and in various genes related to hormonal gene transcription, synthesis and secretion. Sporadic adrenal adenomas and carcinomas may demonstrate dysfunction in genes such as *TP53* among others. Cushing's disease can be an inherited condition also. Multiple endocrine neoplasia type 1 (MEN1) and familial isolated pituitary adenomas (FIPA) together account for 5% of pituitary adenomas. Cushing's disease occurs infrequently in an inherited setting in both of these conditions. To date only 2 cases of Cushing's disease have been described in association with mutations in *AIP*. One case of Cushing's disease has been reported as part of MEN4, a rare MEN1-like syndrome due to mutation in the *CDKN1B* gene. Carney complex (CNC) due to *PRKAR1A* mutations in most cases is associated with CS, mainly as a cause of bilateral adrenal hyperplasia. The cAMP signaling pathway is affected in this setting. In recent times the involvement of genes such as *PDE11A, PDE8B* and others have expanded the spectrum of the genetic pathophysiology of CS.

Introduction

Cushing's syndrome (CS) has been described for about a century and represents a classical syndrome in endocrinology. Recent years have been marked by progress in elucidating the etiology and the pathogenesis of the disease. In this short review, we summarize some of the molecular genetic information of most relevance to the causation of CS.

Cushing's Disease

An ACTH-secreting pituitary adenoma (corticotropinoma) is the commonest cause of CS, accounting for approximately 75–80% of cases. A number of studies have examined the genetic alterations underlying sporadic corticotropinoma formation. Several changes in the pituitary-specific signaling pathways have been found. For instance, Morris et al. [1] found loss of expression of the

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ACTH-receptor gene in corticotropic adenomas in patients with Cushing's disease. However, mutations in the ACTH receptor have not been found in this setting [1]. Similarly, while the glucocorticoid receptor (GR) and the CRH receptor have increased expression in corticotropic adenomas, they are not characteristically associated with mutations in Cushing's disease [2, 3]. While the vasopressin receptor subtype 3 (V3R) is expressed in all corticotropic adenomas, no mutation of the V3R gene has been demonstrated to date [4]. It was found that the function of 11 β-hydroxysteroid dehydrogenase (11β-HSD), an enzyme that could have a significant pre-receptor role in regulating the corticosteroid hormone action, is altered in corticotropic adenomas. However, the role of these functional derangements remains unclear [5].

The hallmark of ACTH-secreting pituitary adenomas is their resistance to glucocorticoids (GC) and their unresponsiveness to normal GC negative feedback. Molecular analysis of the normal mechanism of GC feedback led to identification of two essential proteins for pro-opiomelanocortin repression, the expression of which are often dysregulated in corticotropic adenomas thus providing a potential molecular explanation for GC resistance [6]. These two proteins, Brg1 (the ATPase subunit of the Swi/Snf complex) and histone deacetylase 2 (HDAC2), are involved in chromatin remodeling and may also participate in the tumorigenic process, as Brg1 is a tumor suppressor [7]. In some adenomas Brg1 is expressed but delocalized to the cytoplasm of the tumor cells. These tumors retained responsiveness to high-dose GC.

Most often Cushing's disease occurs as a sporadic, isolated condition. Rarely, it can appear as a component of genetic syndromes such as multiple endocrine neoplasia type 1 (MEN1), Carney complex (CNC) and in the setting of familial isolated pituitary adenomas (FIPA). MEN1 is a familial disorder with autosomal-dominant transmission, due to an inactivating mutation in the *MEN1* gene located in chromosome 11q13 [8]. The prevalence of pituitary disease among MEN1 patients is about 40% [9]. Pituitary adenomas are mainly prolactin-secreting, of which 85% are macroadenomas. Corticotropinomas in MEN1 are rare (6/136 in the series of Verges et al. [9]). No significant difference was found in their prevalence comparing a cohort of patients with sporadic pituitary adenomas [9]. Regarding extrapituitary CS, genetic abnormalities in the region of the *MEN1* gene are common, with loss of heterozygosity (LOH) at 11q13 being observed in more than 90% of adrenocortical carcinomas and 20% of adrenocortical adenomas.

Recently, it was shown that patients with a MEN1-like phenotype but negative for a *MEN1* mutation could harbor mutations in the *cyclin-dependent kinase inhibitor 1B* gene (*CDKN1B*, expressing p27kip1) [10]. *CDKN1B* is located in chromosome 12. Patients with these mutations have a pluritumoral syndrome, termed multiple endocrine neoplasia type 4 (MEN4), however, the condition is extremely rare. Cushing's disease may be part of this syndrome (described in only 1 patient with MEN4) [11].

FIPA is a clinical entity first described in the late 1990s in which two or more cases of pituitary adenomas occur in related members of a kindred in the absence of MEN1 or CNC. Mutation of the *aryl hydrocarbon receptor-interacting protein* (*AIP*) gene located on chromosome 11q13–32 has been described in 15% of these patients. CD is rare in FIPA families. In a study comprising 138 patients (within 64 families), eight patients were affected by CD in five FIPA families (in two families, there was homogeneous expression of Cushing's disease in affected members) [12]. Patients with Cushing's disease within FIPA were negative for *AIP* mutations, but sporadic cases of Cushing's disease have been reported in association with germline *AIP* mutations, one of them a child [13].

Extrapituitary Disease

Two other inherited diseases, McCune-Albright syndrome (MAS) and CNC are associated with CS, mainly bilateral adrenal hyperplasia. The c-AMP signaling pathway is affected in both conditions. CNC is a multiple neoplasia syndrome with an autosomal dominant transmission. Inactivating germline mutations in the gene coding for *PRKAR1A* – one of the regulatory subunits of PKA – have been found [14]. The gene coding for PRKAR1A is located on chromosome 17q22–23. All described mutations (small or large deletions, base substitutions, etc.) usually lead to a premature stop codon [15]. It has been noted that mutation can also result in an expression of abnormal protein, thus demonstrating that alteration of PRKAR1A function (not merely its absence) could augment PKA activity leading to tumorigenesis in tissues affected by CNC [16]. More than 60% of CNC patients harbor pathogenic *PRKAR1A* mutations. Patients with sporadic primary pigmented nodular adrenocortical disease (PPNAD) may also harbor this mutation [17]. Other loci located on chromosome 2 (2p16, 2q31.2 regions) have been implicated in the tumorigenesis of CNC. Inactivating mutations in the *phosphodiesterase 11A (PDE11A)* gene, located on chromosome 2q31.2, has been described

Table 1. Genetic abnormalities in pituitary adenomas

Gene	Molecular defect	Pituitary tumor
RB1	Promoter methylation, loss of 13q14, decreased Rb expression	Various pituitary adenomas, invasive growth
Cyclin D1 (CCND1)	Allelic imbalance, overexpression	Somatotropinomas, non-secreting adenomas, invasive growth
Cyclin D3 (CCND3)	Overexpression	Various pitutiatary adenomas
Cyclin E (CCNE)	Overexpression	Corticotropinomas
Cyclin A (CCNA1)	Overexpression	Various pituitary adenomas
p15Ink4b (CDKN2B)	Promoter methylation, underexpression	Various pituitary adenomas
P16Ink4a (CDKN2A)	Promoter methylation, underexpression	Various pituitary adenomas
P18Ink4c (CDKN2c)	Underexpression	Various pituitary adenomas
P21cip1a (CDKN1a)	Underexpression Overexpression	Non-functioning adenomas Various pituitary adenomas
PTTG	Overexpression	Various pituitary adenomas, invasive growth
ZAC1	Promoter methylation, underexpression	Non-functioning adenomas
GADD-45	Promoter methylation, underexpression	Various pituitary adenomas
Pdt-FGFR4	Alternative transcription initiation, overexpression	Various pituitary adenomas, invasive growth
FGFR2	Promoter methylation, underexpression	Various pituitary adenomas
MAGE-A3	Hypomethylation, underexpression	Various pituitary adenomas
BMP-4	Underexpression	Prolactinoma
EGFR	Overexpression	Corticotropinomas
PKC	Overexpression, point mutations	Invasive growth
PIK3CA	Somatic mutations, gene amplification	Invasive growth
RAS	Point mutations, gene amplification	Invasive growth
WIF-1	Promoter methylation, underexpression	Various pituitary adenomas
MEG3a	Promoter methylation, underexpression	Non-functioning adenomas
PTAG	Promoter methylation, underexpression	Various pituitary adenomas
FR	Overexpression Underexpression	Non-functioning adenomas Prolactinomas, somatotropinomas
CMPtk	Overexpression Underexpression	Corticotropinomas Prolactinomas
ODC	Overexpression Underexpression	Somatotropinomas Corticotropinomas
LAPTM4B	Overexpression	Non-functioning adenomas, corticotropinomas
BAG1	Overexpression	Various pituitary adenomas
GNAS	Inactivation mutations, mother imprinting	40% of somatotropinomas, MAS
MEN1	Inactivating mutations, underexpression	All pituitary adenoma types
P27kip1 (CDKN1B)	Germline nonsense mutation Underexpression	MEN1-like syndrome Sporadic adenomas
PRKAR1A	Germline mutations	Somatolactotrope hyperplasia and adenomas in CNC
AIP	Germline mutations and LOH	15% of FIPA and rare in sporadic adenomas, Cushing disease very infrequent; aggressive behavior

in patients with PPNAD negative for *PRKAR1A* mutations [14, 18]. These patients present with micronodular adrenocortical disease with limited or absent pigment, mostly occurring as an isolated or sporadic disease in early childhood. Inactivating mutations in *PDE11A* lead to a disruption of the ability to hydrolyze cAMP and cGMP. Some of the mutated sequences are frequent in the general population, thus revealing the *PDE11A* gene mutation as possible predisposing factor to a number of tumors. A mutation in the gene coding for *PDE8B* (locus 5q13) has been described resulting in disrupted ability for cAMP hydrolysis.

ACTH-independent macronodular adrenal hyperplasia (AIMAH) is responsible for less than 1% of endogenous CS [19]. Most often it appears as a sporadic disease. There are several reports of familial forms of AIMAH with autosomal dominant transmission. Substantial progress has been made in elucidating the mechanism of cortisol production in AIMAH, previously described as 'autonomous'. It has been found that hypercortisolism can be the result of ectopic expression of aberrant receptors, such as glucose-dependent insulinotropic peptide (GIP) receptor, β-adrenergic receptors, vasopressin (V2–V3) receptor, serotonin (5-HT7), angiotensin-II receptor (AT1R) and glucagon receptors. An increased expression or altered activity of eutopic receptors such as gonadotropin (LH/hCGR), serotonin (5-HT4) and probably leptin receptors has also been associated with AIMAH [19]. The presence of aberrant receptors in AIMAH can be explained by a germline mutation (in the familial forms) or by a somatic mutation at an early stage of embryogenesis that involves both adrenal cortices [19]. The protein kinase A (PKA) signaling pathway was shown to be altered in AIMAH. Somatic losses of the 17q22–24 region, alterations of the PKA subunit expression, and activity changes have been demonstrated [20]. A variation in the gene encoding phosphodiesterase 11A4 implicated in the regulation of cyclic nucleotide levels has been described [21].

Adrenocortical adenomas (ADA) and carcinomas (ACC), the most frequent cause of adrenal CS, may appear isolated or in a familial setting (Li-Fraumeni syndrome, Beckwith-Wiedemann syndrome, familial adenomatous polyposis coli and MEN1). ACC are of monoclonal origin, whereas ADA might be monoclonal as well as polyclonal [22]. Chromosomal alterations are observed in 28% of benign tumors and up to 62% of ACC. Changes in expression of the *insulin-like growth factor II* gene *(IGF-II)*, located at 11p15, may play a role in adrenocortical tumorigenesis [23]. In sporadic ACC, IGF-II is overexpressed in approximately 90% of the cases. Genetic *(CDKN1C)* or epigenetic (KCNQ10T, H19) changes in the imprinted region of 11p15 have been shown to cause Beckwith-Wiedemann syndrome [24]. Uniparental disomy is more frequent in ACC than in adrenal adenomas, correlates with Weiss score and is associated with high risk of tumor recurrence [25]. It has been proposed as a strong biological marker for predicting ACC malignancy after surgery [25]. The tumor suppressor gene *TP53*, located in chromosome 17p13, plays an important role in cell proliferation. A somatic mutation of *TP53* has been demonstrated in 25–35% of adults with sporadic ACC [26]. In children with apparently sporadic ACC this mutation is more frequent – 50–80%. A specific germline mutation of the *TP53* gene (R337) has been demonstrated in Brazilian pediatric patients [27]. In 70% of the families with Li-Fraumeni syndrome, germline mutation in *TP53* is found. Allelic loss (LOH) at the *TP53* locus (17p13) is observed in 85% of ACC and in less than 30% of benign adenomas thus revealing that 17p13 could be used as a molecular marker of malignancy in adrenocortical tumors [23].

The Wnt/β-catenin signaling pathway is another important factor in adrenal cortex functioning. Germline mutation of the *APC* (adenomatous polyposis coli) gene leads to activation of the Wnt/β-catenin pathway and thus to the development of familial adenomatous polyposis coli. These patients can harbor adrenal cortical tumors. *β-catenin* somatic mutations that alter the glycogen synthase kinase 3-β (GSK3-β) phospohorylation site may be found in 25–30% of ACC patients [28]. These mutations are also seen in adrenocortical adenomas and PPNAD.

Conclusion

Significant progress is being made in understanding the molecular genetic mechanisms of tumor pathogenesis CS, especially in the field of adrenal CS (table 1). Nevertheless, in both pituitary and extrapituitary disease, a wealth of opportunities remain to delineate and characterize novel genetic and epigenetic mechanisms of tumorigenesis and growth in the settings of sporadic and familial CS.

Disclosure Statement

The authors declare no conflicts of interest.

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