Rev Esp Endocrinol Pediatr 2024; Volumen 15. Número 1
10.3266/RevEspEndocrinolPediatr.pre2024.Feb.865

Adrenal hyperfunction in childhood

Hiperfunción suprarrenal durante la infancia

Constantine A. Stratakis

Hormones Editor-in-Chief. Hormones. Athens. Greece
Director. Human Genetics & Precision Medicine. IMBB. FORTH. Heraklion. Greece
Director. Medical Genetics. H. Dunant Hospital. Athens. Greece
Senior Associate. NIH Clinical Center. NICHD. NIH. Bethesda. MD. USA

Abstract

Advances in the past two decades have elucidated the genetic alterations leading to the development of adrenocortical tumors and/or hyperplasias, mostly appearing in children and adolescents. These molecular changes were initially discovered by studying rare familial tumor syndromes such as McCune-Albright Syndrome, Carney complex, Li-Fraumeni syndrome, and Beckwith-Wiedemann syndrome, and the identification of alterations in genes and molecular pathways subsequently leading to the discovery of aberrations in these or related genes and pathways in sporadic tumors. Genetic alterations in *GNAS*, *PRKAR1A*, *PRKACA*, *PRKACB*, *PDE11A*, and *PDE8B* leading to aberrant cyclic adenosine monophosphate-protein kinase A signaling were identified as playing a major role in the development of benign cortisol-producing adrenocortical tumors and/or hyperplasias, whereas genetic defects in *KCNJ5*, *ATP1A1*, *ATP2B3*, *CACNA1D*, *CACNA1H* and *CLCN2* were implicated in the development of benign aldosterone-producing tumors and/or hyperplasias through modification of intracellular calcium signaling. Germline *ARMC5* defects were found to cause the development of primary bilateral macronodular adrenal hyperplasia with cortisol and/or aldosterone oversecretion. Adrenocortical carcinoma was linked primarily to aberrant Wnt-β-catenin signaling, p53 signaling, and/or IGF2 overexpression, with frequent genetic alterations in TP53, ZNRF3, CTNNB1, and 11p15.

Correspondencia:
Constantine A. Stratakis, MD, D(med)Sci, PhD
Nikolaou Plastira 100. Vassilika Vouton. GR 700 13 Heraklion Crete. Greece
castratakis@verizon.net

Key words: adrenocortical tumors, genetics, Cushing syndrome, children.

Resumen

Los avances de las dos últimas décadas han esclarecido las alteraciones genéticas que provocan el desarrollo de tumores y/o hiperplasias corticosuprarrenales, que surgen, sobre todo, en niños y adolescentes. Estos cambios moleculares se descubrieron inicialmente estudiando síndromes tumorales familiares poco frecuentes, como el síndrome de McCune-Albright, el complejo de Carney, el síndrome de Li-Fraumeni y el síndrome de Beckwith-Wiedemann. Posteriormente, la identificación de alteraciones en genes y vías moleculares llevó al descubrimiento de anomalías en estos genes y vías o en genes relacionados en tumores esporádicos. Se identificaron alteraciones genéticas en *GNAS*, *PRKAR1A*, *PRKACA*, *PRKACB*, *PDE11A* y *PDE8B* que provocan una señalización anómala de la monofosfato de adenosina-proteína cinasa A cíclica y que desempeñan un papel importante en el desarrollo de tumores o hiperplasias corticosuprarrenales benignos productores de cortisol, mientras que los defectos genéticos en *KCNJ5*, *ATP1A1*, *ATP2B3*, *CACNA1D*, *CACNA1H* y *CLCN2* estaban relacionados con el desarrollo de tumores o hiperplasias benignos productores de aldosterona a través de la modificación de la señalización del calcio intracelular. Se descubrió que los defectos de la estirpe germinal *ARMC5* causan el desarrollo de hiperplasia suprarrenal macronodular bilateral primaria con hipersecreción de cortisol o aldosterona. El carcinoma corticosuprarrenal se relacionó principalmente con la señalización anómala de Wnt-β-catenina, la señalización de p53 o la sobreexpresión de IGF2, con frecuentes alteraciones genéticas en TP53, ZNRF3, CTNNB1 y 11p15.

Palabras clave: genética, niños, síndrome de Cushing, tumores corticosuprarrenales.

Introduction

Benign adrenocortical tumors (ACT) are a heterogeneous group of lesions of the adrenal cortex. Somatic and germline mutations in key molecular pathways including cyclic AMP (cAMP) and *Wnt*-signaling pathways have been shown to play pivotal roles in the development of ACT. Even in cases where there are no known mutations[1], the cAMP pathway in particular appears to be involved. The discovery of mutations in *GNAS*, which encodes the alpha subunit (Gsα) of the stimulatory guanine nucleotide-binding protein, first reported in benign cortisol-producing adenomas (CPA) in patients with McCune-Albright syndrome (MAS), paved the way for identifying the cAMP-signaling pathway as the most important one in the pathogenesis of benign cortisol-producing ACTs. This was rapidly followed by the discovery of mutations in the regulatory subunit type 1-α (RIα) of protein kinase A (PKA, *PRKAR1A* gene) and phosphodiesterase-11A and -8B (the *PDE11A* and *PDE8B* gene respectively) in Carney complex (CNC) and isolated adrenal hyperplasia, and the recently identified germline mutations in the tumor suppressor gene *ARMC5* (*armadillo repeat containing 5*) and somatic mutations in *KCNJ5*, which have been implicated in the majority of primary bilateral macronodular adrenocortical hyperplasia (PBMAH) [2,3] and aldosterone producing adenomas (APA) respectively. Other syndromes with increased predisposition to benign ACT include Carney triad (CT), Carney-Stratakis syndrome (CSS), familial adenomatous polyposis (FAP), and hereditary leiomyomatosis and renal cancer syndrome (HLRCS).

Classification of Benign Adrenocortical Tumors (ACT)

The first comprehensive classification of ACT was proposed in 2007[3]. Broadly, ACT can be divided into adrenocortical adenomas (ACA), adrenocortical hyperplasia, and adrenocortical cancer (ACC)[3]. These lesions can be unilateral or bilateral. ACA are classified on the basis of their radiographic and biochemical characteristics as being either functional or nonfunctional and benign or malignant. In a postmortem series, ACA were present in 5% of cases, while adrenocortical hyperplasia was present in 36%[4]. Conversely, PBMAH is estimated to affect 10% and 15% of Cushing syndrome (CS) patients in young adulthood and childhood respectively[3], with figures likely to be higher in subclinical CS. Approximately 75-90% of ACT leading to CS are due to a unilateral and benign CPA, with the remainder of the majority are primary pigmented nodular adrenocortical disease (PPNAD), isolated massive adrenocortical disease (iMAD), and PBMAH[5].

PBMAH was first described in 1964[6], and was previously called massive macronodular adrenocortical disease (MMAD), bilateral macronodular adrenal hyperplasia (BMAH), or ACTH-independent macronodular adrenocortical hyperplasia (AIMAH). The term PBMAH was proposed given the recent discovery of a local intra-adrenal secretion of adrenocorticotropic hormone (ACTH) with an autocrine/paracrine effect on cortisol secretion through aberrant G-protein-coupled receptors (GPCR)[7, 8]. Asynchronous involvement of only one adrenal gland in PBMAH is rare, but could pose a diagnostic challenge for clinicians. Secondary bilateral adrenocortical hyperplasia or adenomatous formation due to excess ACTH stimulation of the adrenal glands from either ectopic ACTH secretion or Cushing disease (dysregulated cortisol production from a pituitary ACTH-secreting tumor), which may become autonomously functioning, should be differentiated from primary ACT due to genetic causes as the workup and management differs.

Cortisol-producing bilateral adrenocortical hyperplasias (BAH) are divided into micronodular (<1 cm in diameter) or macrocronodular (>1cm in diameter)[3]. An additional feature is pigmentation (i.e.: mainly lipofuscin) within the lesion or the surrounding adrenal cortex, which characterizes a particular type of BAH called PPNAD. A careful histologic examination of the adrenal tissue by an experienced pathologist is therefore a critical step in subtyping the various types of BAH.

The micronodular subtypes are usually diagnosed in children and young adults, and are either pigmented (c-PPNAD, familial as seen in CNC, or isolated, i-PPNAD) or not pigmented (iMAD)[3]. The macronodular subtypes, which are usually diagnosed in adults over the age of 50, may be sporadic or familial. Syndromic forms are seen with mutations in *ARMC5, APC, MEN1, FH* and the CT, CSS and HLRCS. Other subtypes of macronodular PBMAH include primary bimorphic adrenocortical disease (PBAD), as seen in MAS, and lesions with GPCR that produce excess cortisol only in response to certain endogenous factors (e.g. gastrointestinal inhibitory polypeptide, GIP), as seen with food-dependent Cushing syndrome (FDCS).

Molecular Pathways in Benign Adrenocortical Tumors

Two major molecular pathways in adrenocortical development have been implicated in the formation

of ACT, and include the cAMP and *Wnt*-singling pathways. Briefly, GPCR (e.g. melanocortin 2 receptor, MC2R) undergo conformational changes in response to a variety of extracellular stimuli, including catecholamines, ACTH, or neurotransmitters. In the case of glucocorticoids, ACTH activates MC2R, a seven-transmembrane receptor, which leads to activation of adenyl cyclase (AC) through a Gsα subunit (encoded by *GNAS*). This step exchanges GDP for GTP, which in turn converts ATP into cAMP, activating protein kinase A (PKA). PKA is a holoenzyme that consists of a tetramer of two homo- or heterodimer regulatory subunits (R1α, R1β, R2α and R2β), and catalytic subunits (Cα, Cβ, Cγ and PRKX) that are encoded by several genes[9]. Their dissociation in the presence of cAMP enables phosphorylation of PKA targets, including gene expression to mediate cell growth, differentiation and hormone production (e.g.: cortisol). The major function of the regulatory subunits of PKA is to keep the catalytic subunits inactive in the absence of cAMP[9].

Alterations in any of these complex steps in the cAMP-dependent signaling pathway may predispose to the formation of ACT. The first alterations were reported in *GNAS* as seen in patients with MAS, followed by CNC through the inactivating mutations in *PRKAR1A* of PKA. This leads to constitutive activation of the pathway by increasing the availability of the PKA catalytic subunits. However, PKA signaling may either inhibit or stimulate cell proliferation depending on its specific role in the cell cycle[10,11]. The variability in PKA's cell growth control may partially explain why some biochemically active ACT are small and difficult to detect clinically.

The *Wnt*-signaling pathway consists of two major pathways; one is β-catenin-dependent and one is β-catenin-independent. The *Wnt*/β-catenin signaling pathway consists of binding of the ligand to a series of Frizzled family receptors, such as LRP6, which activates phosphoproteins that inhibit the phosphorylation of β-catenin. The nuclear accumulation of β-catenin leads to the transcription of important genes such as *WISP2*, *CTNNB1*, and *GSK3B*, as seen in PBMAH and PPNAD[12]. Both signaling pathways share the downstream activation of certain oncogenic signals, but differ substantially in their effects on others depending on the adrenocortical lesion[13]. This differential effect may partially explain why some somatic activating or inactivating mutations in the same signaling pathway lead to different types of ACT.

Carney Complex (CNC)

Carney complex (CNC) is a hereditary multiple neoplasia syndrome with an autosomal dominant (AD) inheritance due to alterations in *PRKAR1A* (17q22-24, CNC1 locus), an apparent tumor suppressor gene which encodes for the R1α subunit of PKA[14]. CNC is less commonly due to alterations of a yet-unidentified gene on chromosome 2p16 (CNC2) or *PRKACB* amplification. Over one hundred germline inactivating mutations in R1α of PKA spread along the whole coding sequence, with the majority leading to a premature stop codon by nonsense or frame shift, have been described in approximately 80% of CNC patients[15,16]. The overall penetrance of CNC among *PRKAR1A* mutations' carriers is over 95% by the age of 50.

The clinical manifestations of CNC are broad. The main manifestation is CS from PPNAD in approximately 60% of patients. Other tumors include cardiac myxomas, pigmented skin lesions (lentiginosis and blue nevi), somatotroph-pituitary adenomas, benign large cell calcifying Sertoli cell tumor (LCCSCT) of the testis, benign thyroid nodules, differentiated thyroid cancer, and melanocytic schwannomas. In-frame deletion of exon 3 and the c.708 +1G>T mutation appears to confer a more severe CNC phenotype, while the splice variant c.709(-7-2)del6 and the initiation-alternating substitution c.1A>G/p.M1Vp lead to an incomplete penetrance of CNC[17]. The hotspot c.491-492delTG mutation is more closely associated with lentigines, cardiac myxoma, and thyroid tumors than all other *PRKAR1A* mutations. Expressed RIα mutant proteins usually present with a more severe and aggressive CNC-phenotype. Conversely, CNC2 occurs later in life with a lower frequency of myxomas, schwannomas, thyroid tumors and LCCSCT. The clinical diagnosis of CNC is established if two or more of the aforementioned major manifestations are present. However, if a patient presents with a family history of CNC and one or more of these manifestations, genetic testing will help establish the diagnosis.

Some patients may present with mild disease with i-PPNAD, with or without accompanied lentiginosis. This "subtype" of CNC is usually diagnosed before 8 years of age and may be due to pathogenic mutations in *PRKAR1A*, particularly c.709 (-7-2) del6 or c.1A>G/p.M1V, in approximately 50% of cases. Mutations in *PDE11A,* or *PDE8B*, have also been described and are detailed later. The diagnosis of i-PPNAD should be considered after a thorough exclusion of CNC, with close surveillance of the other possible manifestations of CNC as they may evolve with time.

Conclusions

The identification of several genetic alterations leading to the formation of benign ACT has paved

our understanding of adrenocortical development and disease. Altered genes in the cAMP and *Wnt*-signaling pathways have uncovered new molecular pathways, which have been implicated in the proliferation of adrenocortical cells, with potential therapeutic implications using novel therapies. However, large-scale clinical and molecular studies are underway and needed to further expand our understanding of the genotype-phenotype-biochemical correlations and genetic counseling for affected individuals and their families.

References

1. Bimpaki EI, Nesterova M, Stratakis CA. Abnormalities of cAMP signaling are present in adrenocortical lesions associated with ACTH-independent Cushing syndrome despite the absence of mutations in known genes. Eur J Endocrinol. 2009;161(1):153-61.

2. Horvath A, Stratakis CA. Unraveling the molecular basis of micronodular adrenal hyperplasia. Curr Opin Endocrinol Diabetes Obes. 2008;15(3):227-33.

3. Stratakis CA, Boikos SA. Genetics of adrenal tumors associated with Cushing's syndrome: a new classification for bilateral adrenocortical hyperplasias. Nat Clin Pract Endocrinol Metab. 2007;3(11):748-57.

4. Saeger W, Reinhard K, Reinhard C. Hyperplastic and tumorous lesions of the adrenals in an unselected autopsy series. Endocr Pathol. 1998;9(3):235-9.

5. Lodish M, Stratakis CA. A genetic and molecular update on adrenocortical causes of Cushing syndrome. Nat Rev Endocrinol. 2016;12(5):255-62.

6. Kirschner MA, Powell RD, Jr., Lipsett MB. Cushing's syndrome: nodular cortical hyperplasia of adrenal glands with clinical and pathological features suggesting adrenocortical tumor. J Clin Endocrinol Metab. 1964;24:947-55.

7. Louiset E, Duparc C, Young J, Renouf S, Tetsi Nomigni M, Boutelet I, et al. Intraadrenal corticotropin in bilateral macronodular adrenal hyperplasia. N Engl J Med. 2013;369(22):2115-25.

8. Lefebvre H, Duparc C, Chartrel N, Jegou S, Pellerin A, Laquerriere A, et al. Intraadrenal adrenocorticotropin production in a case of bilateral macronodular adrenal hyperplasia causing Cushing's syndrome. J Clin Endocrinol Metab. 2003;88(7):3035-42.

9. Taylor SS, Ilouz R, Zhang P, Kornev AP. Assembly of allosteric macromolecular switches: lessons from PKA. Nat Rev Mol Cell Biol. 2012;13(10):646-58.

10. Robinson-White A, Meoli E, Stergiopoulos S, Horvath A, Boikos S, Bossis I, et al. PRKAR1A Mutations and protein kinase A interactions with other signaling pathways in the adrenal cortex. J Clin Endocrinol Metab. 2006;91(6):2380-8.

11. Lania AG, Mantovani G, Ferrero S, Pellegrini C, Bondioni S, Peverelli E, et al. Proliferation of transformed somatotroph cells related to low or absent expression of protein kinase a regulatory subunit 1A protein. Cancer Res. 2004;64(24):9193-8.

12. Bourdeau I, Antonini SR, Lacroix A, Kirschner LS, Matyakhina L, Lorang D, et al. Gene array analysis of macronodular adrenal hyperplasia confirms clinical heterogeneity and identifies several candidate genes as molecular mediators. Oncogene. 2004;23(8):1575-85.

13. Almeida MQ, Azevedo MF, Xekouki P, Bimpaki EI, Horvath A, Collins MT, et al. Activation of cyclic AMP signaling leads to different pathway alterations in lesions of the adrenal cortex caused by germline PRKAR1A defects versus those due to somatic GNAS mutations. J Clin Endocrinol Metab. 2012;97(4):E687-93.

14. Stratakis CA, Kirschner LS, Carney JA. Clinical and molecular features of the Carney complex: diagnostic criteria and recommendations for patient evaluation. J Clin Endocrinol Metab. 2001;86(9):4041-6.

15. Kirschner LS, Carney JA, Pack SD, Taymans SE, Giatzakis C, Cho YS, et al. Mutations of the gene encoding the protein kinase A type I-alpha regulatory subunit in patients with the Carney complex. Nat Genet. 2000;26(1):89-92.

16. Horvath A, Bertherat J, Groussin L, Guillaud-Bataille M, Tsang K, Cazabat L, et al. Mutations and polymorphisms in the gene encoding regulatory subunit type 1-alpha of protein kinase A (PRKAR1A): an update. Hum Mutat. 2010;31(4):369-79.

17. Groussin L, Horvath A, Jullian E, Boikos S, Rene-Corail F, Lefebvre H, et al. A PRKAR1A mutation associated with primary pigmented nodular adrenocortical disease in 12 kindreds. J Clin Endocrinol Metab. 2006;91(5):1943-9.