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Review article

Consensus statement by the French Society of Endocrinology (SFE) and French Society of Pediatric Endocrinology & Diabetology (SFEDP) for the diagnosis of Cushing's syndrome: Genetics of Cushing's syndrome

Laetitia Martinerie[a], Jérôme Bouligand[b], Marie-Odile North[c], Jérôme Bertherat[d], Guillaume Assié[d], Stéphanie Espiard[e,*]

[a] *Department of Pediatric Endocrinology, CHU Robert-Debré, AP–HP, Paris, France*
[b] *Faculté de médecine Paris-Saclay, Inserm Unit UMRS1185 Endocrine Physiology and Physiopathology, Paris, France*
[c] *Department of Genetics and Molecular Biology, hôpital Cochin, AP–HP, University of Paris, Paris, France*
[d] *Endocrinology Department, centre de référence maladies rares de la surrénale (CRMRS), hôpital Cochin, AP–HP, University of Paris, Paris, France*
[e] *Service d'endocrinologie, diabétologie, métabolisme et nutrition, CHU de Lille, 59000 Lille, France*

INFO ARTICLE

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ABSTRACT

Cushing's syndrome is due to overproduction of cortisol, leading to abnormal and prolonged exposure to cortisol. The most common etiology is Cushing disease, while adrenal causes are rarer. Knowledge of the genetics of Cushing's syndrome, and particularly the adrenal causes, has improved considerably over the last 10 years, thanks in particular to technical advances in high-throughput sequencing. The present study, by a group of experts from the French Society of Endocrinology and the French Society of Pediatric Endocrinology and Diabetology, reviewed the literature on germline genetic alterations leading to a predisposition to develop Cushing's syndrome. The review led to a consensus statement on genetic screening for Cushing disease and adrenal Cushing's syndrome.

1. Introduction

Knowledge of the genetics of Cushing's syndrome (CS) has improved considerably over the last 10 years, particularly with technical advances in high-throughput sequencing. This knowledge concerns both germline genetic alterations (present in leukocyte DNA) and somatic alterations (present only in tumor DNA).

The aim of the present study was to carry out a review of the literature in order to draw up recommendations for indications and application of genetic screening for Cushing's syndrome. The review focused on germline alterations leading to a genetic predisposition to develop the diseases and on associated familial cases. Somatic genetic alterations are not discussed in these recommendations. The level of evidence for each recommendation was classified as: ++++, high; +++, moderate; ++, low; and +, very low. These recommendations were recently published alongside other recommendations concerning the diagnosis of Cushing's syndrome [1].

It is important to point out that genetic testing has not previously been included in recommendations for the diagnosis of Cushing's syndrome; the most recent international consensus dates back to 2008. Technical advances and the recent discovery of the many genes involved have changed the approach to Cushing's syndrome. Gene mutation testing is now part of both individual diagnosis and screening for familial cases.

2. Cushing's syndrome of adrenal origin

The germline genetic alterations described in patients with Cushing's syndrome of adrenal origin are summarized in Table 1.

The majority of germline genetic alterations are mutations in tumor-suppressor genes. A suppressor gene is inactivated according to Knudson's theory. At the germline level, there is an initial loss-of-function mutation in a first allele, constituting a predisposition. At leukocyte level, the affected individual presents the gene mutation in the heterozygous state. At somatic level (i.e., in the tumor), the second allele is inactivated by a second event, which may be a point mutation but is more often a gene deletion, resulting in what is classically known as "loss of heterozygosity".

* Corresponding author: Service d'endocrinologie, métabolisme, hôpital C.-Huriez, Lille University Hospital, rue Polonovski, 59037 Lille cedex, France.
Adresse e-mail : stephanie.espiard@live.fr (S. Espiard).

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Table 1
Genes and genetic syndromes (constitutional genetics/germline events) associated with adrenal Cushing's syndrome.

Gene	Genetic syndrome	Phenotype	Frequency of adrenal damage in case of mutation	Genetics	References
PRKAR1A	Isolated PPNAD Carney complex	Isolated PPNAD (~12%) PPNAD associated with: Myxoma of the heart, skin or breast Lentigines Pituitary adenoma or hyperplasia (GH ± PRL) Other tumors	26–60%	Private mutations distributed throughout the gene 3 hotspots (c.709(-7-2)del6; c.491-492delTG; c82C > T) Large deletions reported	[2–7]
ARMC5	–	PMAH Meningioma (several cases reported)	ND, penetrance not complete in the families described	Private mutations distributed throughout the gene	[8–14]
KDM1A	–	Food-dependent PMAH Multiple myeloma, monoclonal gammopathy of undetermined significance	ND, penetrance not complete in the families described	Mutations distributed throughout the gene. No hotspots reported	[15,16]
MEN1	NEM1	Cortisol-producing adenoma PMAH Adrenocortical carcinoma Pituitary adenoma Primary hyperparathyroidism Pancreatic neuroendocrine tumor	0.6–8.7%	Private mutations distributed throughout the gene or large deletions	[17–23]
Fumarate hydratase	HLRCC	PMAH, unilateral adrenal adenoma Cutaneous or uterine leiomyoma, papillary clear-cell renal cell carcinoma	0.8%	Private mutations distributed throughout the gene	[24–27]
APC	Familial polyposis coli	PMAH, adenoma Adrenocortical carcinoma Colonic polyps	Clinical cases	Gene-wide mutations	[24,28–31]
PRKACA	–	PPNAD PMAH Macroglossia	Clinical cases (adult and pediatric)	Gene amplification	[32,33]
GNAS	McCune Albright syndrome	Isolated neonatal PMAH (1 year) Adrenal adenoma Peripheral precocious puberty (ovarian cyst) Café-au-lait spots Fibrous bone dysplasia Phosphate diabetes Somatotropic adenoma and/or prolactinoma Multinodular goiter, hyperthyroidism	Estimated at 5% of patients for neonatal PMAH (clinical cases or small series)	Somatic mutations Hotspots (p.R201H and p.C174Y)	[24,34–37]
Chromosome 11p15.5 (*IGF2, H9, CDKI*)	Beckwith Widemann syndrome	Adrenocortical cancer PMAH Hemi-hypertrophy, organomegaly, neonatal hypoglycemia, umbilical hernia/omphalocele, macroglossia Wilms' tumor, neuroblastoma, hepatoblastoma	Clinical cases Young children only, < 5 years	Parental imprint anomaly	[38,39]
TP53	Li Fraumeni	Adrenocortical carcinoma Predisposition to numerous cancers: osteosarcoma, soft-tissue sarcoma, breast cancer, brain tumor, leukemia	Mutation found in 70% of childhood adrenocortical carcinomas	Germline mutation Hotspot p.R337H in Brazil	[38,40]

PMAH: bilateral macronodular adrenal hyperplasia; HLRCC: Hereditary leiomyomatosis and renal cell carcinoma; ND: not determined; NEM1: multiple endocrine neoplasia type 1; PPNAD: Primary Pigmented Nodular Adrenal Hyperplasia.

Two main pathways are altered in adrenal tumorigenesis: the β-catenin pathway and the cAMP/PKA pathway. Activation of the β-catenin pathway is mainly involved in adrenal tumor, particularly non-functional adenoma associated with moderate autonomous cortisol secretion [41], and in adrenocortical carcinoma. Its role as a driver of adrenal tumorigenesis has been demonstrated in vitro and in vivo [42]. The cAMP/PKA pathway is essential for maintenance of the adrenal cortex and synthesis and secretion of glucocorticoids. Numerous alterations in this pathway have been described in cortisol-secreting adrenal tumors [43].

- primary macronodular adrenal hyperplasia (PMAH) is often diagnosed in the fifth or sixth decade after several years' progression. Onset of Cushing's syndrome and tumor growth are progressive [45];
- primary pigmented nodular adrenal dysplasia (PPNAD) can develop as early as the first 2–3 years of life, but for the majority of patients, it develops in adulthood; with a peak in the 2nd and 3rd decades [46].

2.1. Bilateral adrenal hyperplasia

Bilateral cortisol-secreting adrenal hyperplasia is a rare disease. There are two main forms [44]:

The bilateral and multifocal nature of the adrenal affection and reported familial forms clearly suggest a germline predisposition to these pathologies. Currently, a genetic origin has been identified in around 25% of cases of PMAH and just over 70% of cases of PPNAD.

R1. We recommend screening for a genetic cause of adrenal Cushing's syndrome in all children and adults presenting bilateral micro- or macronodular adrenal hyperplasia. (++)

2.1.1. Micronodular adrenal hyperplasia and dysplasia

In most cases, PPNAD is part of the Carney complex (CNC). It may be isolated in 12% of cases [47]. CNC is a multiple endocrine and non-endocrine neoplasia. PPNAD is observed in 26–60% of CNC cases [2,3,46]. Other endocrine tumors observed comprise pituitary adenoma, mostly somatotropic, calcified testicular Sertoli cell tumor, thyroid nodules or well-differentiated vesicular thyroid cancer. The main non-endocrine manifestations are cardiac myxoma, skin lesions (lentiginosis, myxoma, blue nevi), breast tumor (myxoma or adenofibroma), and schwannoma. More rarely, other tumors are observed, such as osteochondromyxoma or pancreatic tumor [3,48].

2.1.1.1. PRKAR1A. In around 70% of cases, Carney complex is linked to mutations in the *PRKAR1A* gene encoding the R1α regulatory subunit of protein kinase A (*PRKAR1A*) located at locus 17p22-24 [49,50]. Transmission is autosomal dominant. Another locus on chromosome 2p16 was associated with CNC on genetic linkage studies [51,52]; however, to date, no candidate gene has been found at this locus.

Thirty-seven percent of sporadic CNC cases are mutated for *PRKAR1A*. Twenty percent of these mutations occur *de novo* [4]. A *PRK1R1A* mutation is observed in around 80% of CNC families [2]. The overall penetrance of manifestations is around 95% at 50 years. Only two mutations (c.709(-7-2)del6 and c.1A>G) have incomplete penetrance [4,53].

Mutations in the *PRKAR1A* gene are distributed across all 10 coding exons and adjacent intronic sequences, at the mRNA splice site. They are mainly point mutations and deletions of a few base pairs [2,4]. Large deletions of the gene have more rarely been reported [5]. Most mutations are private, found only in one family. Three mutations, c.82C>T (exon 2), c.491_492delTG (exon 5) and c.709(-7-2)del6 (intron 7), are nevertheless found in several families and can be considered as hotspots [2].

Genotype-phenotype correlations have been established [2]. In particular, exon mutations are more often associated with acromegaly, cardiac myxoma, lentigines and schwannoma, while intronic splice site mutations have a less severe phenotype. The c.709(-7-2)del6 hotspot mutation and the c.1A>G mutation are more often associated with isolated PPNAD.

2.1.1.2. Phosphodiesterase. In addition to *PRKAR1A* mutations, SNP microarray analysis has led to suspicion of a role for the *PDE11A* gene, located at the 2q31-2q35 locus, in onset of PPNAD [54]. Phosphodiesterases (PDEs) hydrolyze cAMP, reducing its intracellular level and limiting PKA activation. Rare variants in the general population can reduce PDE11A enzymatic activity, conferring a predisposition to micronodular adrenal hyperplasia [55]. Finally, mutations in the *PDE8B* gene are an exceptional cause of PPNAD [56,57].

2.1.2. Macronodular adrenal hyperplasia

Activation of the PKA pathway by aberrant expression of G-protein-coupled receptors was one of the first mechanisms described in PMAH [58]. To date, no germline abnormalities in these receptors have been reported. Molecular explanations have only been provided for food-dependent PMAH in association with aberrant expression of the Gastrointestinal Peptide (GIP) receptor [15,16]. Interestingly, transcriptome analysis of PMAH shows three clusters: the first includes PMAH mutated for the *ARMC5* gene, the most frequent genetic cause currently identified, the second includes food-dependent PMAH linked to mutations in the *KDM1A* gene; in the third cluster, genetic alterations have not yet been characterized [16].

While PMAH is most often isolated, it has also been reported in certain syndromic diseases. Unlike PPNAD, the etiologies of PMAH are different in adults and children.

2.1.2.1. Apparently isolated macronodular adrenal hyperplasia.

2.1.2.1.1. ARMC5. PMAH is linked to a mutation in the *ARMC5* gene in around 25% of sporadic cases and nearly 80% of familial forms. In 2013, this gene was discovered to be responsible for PMAH, thanks to an approach combining analysis of chromosomal abnormalities by an SNP chip and whole genome sequencing [59].

This first study, together with the National Institute of Health's PMAH cohort study [60] suggested that *ARMC5* mutations were associated with more severe forms of the disease. The study of a large French and German cohort confirmed that *ARMC5* mutations were associated with greater biological hypercortisolism, higher prevalence of hypertension, and greater adrenal hyperplasia with a higher number of nodules [61]. *ARMC5*-mutated patients are therefore more likely to undergo surgery than non-mutated patients. The presence of an abnormal response to vasopressin or orthostasis was reported in *ARMC5*-mutated PMAH, while no food-dependent Cushing's disease has been reported. The association of *ARMC5* mutations with more severe cases was confirmed on a European scale in a cohort of 352 patients. In this cohort, patients mutated for *ARMC5* had at least bilateral adrenal lesions and pathological dexamethasone suppression test (cortisol after test > 50 nmol/L) [62].

Data in the literature suggest that *ARMC5* is responsible for nearly 80% of familial forms [8,63,64]. Familial studies show that the penetrance of the disease is fairly high, but that the phenotype is variable, ranging from an isolated adrenal CT abnormality to subclinical hypercortisolism [8]. The association of PMAH with meningioma was reported in patients mutated for *ARMC5* [8,64,65] and proved by observation of loss of heterozygosity or mutation of the other allele in the brain tumor [64,65].

Mutations in the *ARMC5* gene are distributed along the gene. There are no real hotspots, although some mutations have been found in several unrelated patients. Deletions of the gene have been more rarely reported [61,66]. The function of *ARMC5* is not well understood. Recent studies showed that *ARMC5* is a tumor-suppressor gene involved in apoptosis and the cell cycle [59,61,67].

2.1.2.1.2. KDM1A. Aberrant cortisol response to food, the first described in the literature [68,69], is in fact relatively rare in PMAH. It was the least frequent response in a series of patients screened for a large panel of aberrant responses [70]. In two sporadic cases of food-dependent Cushing's syndrome, there was somatic duplication/rearrangement of the locus of the *GIPR* gene, encoding the GIP receptor. The alterations heightened the response of the glucocorticoid response elements located in the promotor of the genes driving its overexpression [71].

In 2021, *KDM1A* was identified as the causative gene for these food-dependent PMAHs by two French teams using an exome approach [15,16]. Inactivation of this gene follows the pattern of a tumor-suppressor gene such as *ARMC5*. There is a heterozygous mutation at leukocyte and adrenal level, and loss of heterozygosity of the gene locus (1p) is observed. *KDM1A* acts by demethylating histones at chromatin level, thus acting as a transcriptional repressor. Inactivation results in altered expression of several G-protein-coupled receptors, *GIPR* being the gene the expression of which is the most altered, with drastically elevated expression compared to unmutated PMAH [15,16].

The clinical presentation is linked to the food-dependent nature of these PMAHs: 8 a.m. cortisol is low in the majority of cases, in line with the fasting state, while midnight and 24-hour urinary free

cortisol are high. Adrenal size does not differ from other forms of PMAH. Interestingly, this gene was previously identified in families with several cases of multiple myeloma [72]. Cases of myeloma or monoclonal gammopathy of undetermined significance were also found in patients with PMAH or their relatives [15,16].

2.1.2.1.3. PRKACA. Three cases of duplications of the region of chromosome 19 comprising the gene encoding the alpha subunit of protein kinase A (*PRKACA*) have been reported in patients with PMAH [73,74]. These involved two adults, a mother and her son [73] and a 2-year-old child with neonatal hypoglycemia and macroglossia [74].

2.1.2.1.4. MC2R. Mutations in the ACTH receptor (MC2R) were reported in two isolated cases by the same team [75,76]. However, no other studies identified mutations in this gene, making it difficult to conclude that there is a causal link between these mutations and disease onset.

2.1.2.1.5. Phosphodiesterases. As with PPNAD, the presence of *PDE11A* variants seems to confer a genetic predisposition for PMAH. In a series of 46 patients, the frequency of *PDE11A* variants was higher than in the control population [77]. This observation was subsequently confirmed in an independent cohort [78]. In vitro analysis of the two most frequent variants showed an increase in cAMP levels and an increase in PKA [78].

2.1.2.2. Macronodular adrenal hyperplasia in a syndromic context.

2.1.2.2.1. MEN1. Multiple endocrine neoplasia type 1 (*MEN1*) is an autosomal dominant disease linked to mutations in the *MEN1* gene (11q13). It includes primary hyperparathyroidism (95%), pancreatic neuroendocrine tumor (50%), pituitary adenoma (40%) and thymic carcinoid tumor [79]. Adrenal lesions (hyperplasia or nodules) have been reported in up to 50% of patients with *MEN1* [17,18,80,81]. Cushing's syndrome of adrenal origin is fairly rare, reported in only 0.6% of patients in the cohort of the French Endocrine Tumor Research Group [81]. PMAH was reported in only two patients with *MEN1* [19,82]. The causal link between *MEN1* and adrenal tumor is corroborated by the development of adrenal tumor or hyperplasia in mice carrying deletions of certain exons of the *MEN1* gene [83].

2.1.2.2.2. Fumarate hydratase. Autosomal dominant mutations in the *fumarate hydratase* (*FH*) gene (1q43) are responsible for hereditary leiomyomatosis and renal cell carcinoma (HLRCC). FH is a Krebs cycle enzyme that converts fumarate to malate. The frequency of adrenal lesions in HLRCC in a large series of HLRCC patients was 7.8%, with multifocal nodules in 20% of cases [84]. Approximately 10 cases of PMAH have been reported and histologically proven [84,85]. The observation of loss of heterozygosity in the gene locus in the tumor tissue of an operated patient provided a strong argument for a causal link between FH mutations and onset of PMAH [85]. Interestingly, an FH mutation was also reported in a sporadic case of PMAH [24].

2.1.2.2.3. Familial polyposis coli. Familial polyposis coli or Gardner's syndrome is characterized by multiple colon polyps and colon cancers at an early age. Patients may also present pigmented retinal lesions, desmoid tumor, osteoma, thyroid nodules or cribriform thyroid cancer and other malignancies [86]. Cases of PMAH were also reported [24,28,87]. Here again, the observation of second events at tumor level provides strong arguments for a causal link between *APC* gene mutations and PMAH [28,87].

2.1.2.3. Macronodular adrenal hyperplasia in children. In children, the most frequent cause of Cushing's syndrome of adrenal origin is McCune Albright syndrome (MAS), linked to post-zygotic mutations of the *GNAS* gene, encoding the Gαs subunit of heterotrimeric G protein [88,89]. The disease is characterized by fibrous bone dysplasia, café-au-lait spots and endocrine activation, usually leading to early onset of puberty. Neonatal Cushing's syndrome can be observed in newborns with a particular form of nodular adrenal hyperplasia characterized by areas of nodular hyperplasia and cortical atrophy, and by persistence of fetal cells [34]. In these situations, Cushing's syndrome is often the first manifestation of the syndrome. It typically occurs during the first year of life, and may resolve spontaneously. It should be noted that somatic *GNAS* mutations can also be observed in adult PMAH nodules [90,91].

Finally, pediatric clinical cases of Cushing's syndrome related to micro- or macronodular adrenal hyperplasia have been reported in patients with Beckwith-Wiedemann syndrome [92] or mutations in the *PRKACA* [74] or *PDE8B* gene [57].

2.2. Unilateral adrenal lesions

Unilateral adrenal cortisol-producing tumor (cortisol-producing adenoma and adrenocortical carcinoma) is often sporadic and mainly linked to somatic alterations. To date, a genetic predisposition has been reported only in *MEN1* and familial polyposis coli. In children, these adrenal tumors are particularly rare, and onset of cortisol-producing adenoma may reveal *MEN1*, while onset of adrenocortical carcinoma may reveal Li Fraumeni syndrome.

> R2. We recommend systematic screening for a genetic cause in case of unilateral adrenocortical tumor only in children. (+)

2.2.1. Cortisol-producing adenoma

In more than 40% of cases, cortisol-producing adenomas are linked to activating somatic mutations of the alpha subunit of protein kinase A (*PRKACA*). *PRKACA* mutation was first characterized in 2014 by Beuschlein et al. [73], shortly followed by 3 other teams [93–95]. It has been shown that adenomas linked to *PRKACA* mutations are smaller, with a higher level of cortisol secretion, and occur at a younger age than those without mutations [73,94,95]. As the treatment of choice is adrenalectomy, which achieves cure, screening for *PRKACA* mutations would not affect management. In rare cases, cortisol-producing adenomas are part of a genetic predisposition syndrome.

2.2.1.1. NEM1A. Cortisol-producing adenoma is more frequent than PMAH in *NEM1*, but nevertheless rare, reported for example in only 3 of the 715 patients in the cohort of the French Endocrine Tumor Research Group. Adrenal lesions often develop years after the first manifestation of the disease [18]. In a single case, a cortisol-producing adenoma led to diagnosis of the disease in a 16-year-old girl whose work-up revealed hyperparathyroidism and a prolactinoma [20].

2.2.2. Familial polyposis coli

Cortisol-producing adenoma has been reported during the course of familial polyposis coli or leading to its diagnosis [87,96].

2.3. Adrenocortical carcinoma

Adrenocortical carcinoma (ACC) is usually sporadic. Numerous chromosomal, genetic and epigenetic alterations have been described in ACC [97]. Their characterization can help establish prognosis. In addition, genetic abnormalities may be characterized in therapeutic trials for targeted therapies [98]. ACC is found in certain genetic predisposition syndromes.

2.3.1. Li Fraumini syndrome

This is an autosomal dominant disease characterized by predisposition to and early onset of cancer: sarcoma, mammary carcinoma, brain tumor, leukemia and ACC [99,100]. The *TP53* gene encodes the p53 protein, known as the "guardian of the genome". This protein plays a fundamental role in the cell response to stress or DNA damage, by regulating the cell cycle and apoptosis. In children, ACC was linked to germline mutations in *TP53* in over 70% of cases in Europe and North America [101]. There is a region in southern Brazil where the prevalence of the *TP53 p*.R337H mutation, and thus the incidence of ACC in children, is particularly high [102]. In adults, germline mutations of *TP53* are present in 3.9–5.8% of cases of sporadic ACC. In some cases, the patient does not meet the diagnostic criteria for Li Fraumeni syndrome [103,104]. Twenty-five percent of *TP53* mutations are *de novo* [105].

2.3.2. Carney complex

ACC was reported in two patients with CNC. However, the causal link between *PRKAR1A* mutations and tumor is unclear, and may be fortuitous [106–108]. Findings of somatic mutations in *PRKAR1A* in sporadic ACC are an argument in favor of the gene's involvement in ACC [109,110].

2.3.3. NEM1

ACC is the most common adrenal lesion in *NEM1* [18,21,80,81]. In the cohort of the French Endocrine Tumor Research Group cohort, 1.1% of patients presented ACC [81]. Inactivation of the second allele of the gene in ACC supports the role of menin mutations in the development of these tumors [111].

2.3.4. Familial polyposis coli

ACC was reported in patients with familial polyposis coli, and a second mutation in the adrenal tumor supports an association between this disease and ACC [87].

2.4. Sequencing strategy

Next-generation sequencing (NGS) is currently preferred by the majority of laboratories. Point mutations and large rearrangements (reported for *ARMC5*, *PRKACA*, *PRKAR1A*, *MEN1* and *APC*) can be identified by NGS. It is important to ensure that the NGS analysis enables detection of copy number anomalies. If this is not the case, an additional MLPA analysis should be performed in case of negativity.

R3.1 We recommend a gene panel including the most frequently implicated genes: (+)

- *ARMC5* in adults with macronodular adrenal hyperplasia.
- *GNAS* in children with neonatal Cushing's syndrome.
- *PRKAR1A* in children or adults with micronodular adrenal hyperplasia.

Choice of genes can also be guided by the syndromic context.

At the time of the submission of these guidelines, the *KDM1A* gene had not yet been identified. Screening for mutations in this gene in patients with food-dependent PMAH or with personal or family history of multiple myeloma is justified by recent data in the literature.

If the candidate gene approach fails to identify a genetic abnormality, it is worth considering a broader genomic approach of the exome type, after tumor board discussion. This approach could identify new causes of adrenal Cushing's syndrome.

R3.2 In second line, genome-wide study may be discussed (e.g., *France Médecine Génomique* 2025). (+).

2.5. Family screening

When a *PRKAR1A* gene mutation is found, family screening is usually suggested as early as 3 years of age, given the early onset of cardiac myxoma [6].

When an *ARMC5*, *KDM1A* or *PRKACA* duplication mutation is found, the benefit of genetic screening for management of relatives has not been studied. However, given the possibility of subclinical Cushing's syndrome, several teams suggest genetic screening from the age of 18.

For other genetic alterations responsible for syndromic disease, family genetic screening must be carried out in a way that is adapted to each pathology, in accordance with any recommendations specifically proposed for each.

3. Cushing's disease

3.1. Genetic etiologies

Cushing's disease seems to be essentially linked to somatic abnormalities: primarily mutations in the *USP8* gene, implicated in around 20–30% of childhood and 35–60% of adult Cushing's disease cases [112–115]. These mutations are predominantly found in women and are associated with a smaller tumor size. They can predict response to somatostatin analogue therapy [116].

Cushing's disease can, however, be caused by germline genetic alterations in the context of multiple endocrine neoplasia or, more rarely, in isolation. The germline alterations summarized in Table 2 mainly concern pediatric populations.

R4. We recommend screening for a genetic cause of Cushing's syndrome in case of: (+)

- associated clinical manifestations suggestive of a particular genetic syndrome.
- family history of pituitary adenoma.
- corticotroph macro-adenoma in subjects < 30 years of age at diagnosis (TENGEN recommendation).
- corticotroph micro-adenoma in children (TENGEN recommendation)

3.1.1. Cushing's disease in multiple neoplasia

3.1.1.1. Carney complex. In CNC, the somatotropic compartment is mainly abnormal, with hyperplasia more often than adenoma. Hyperprolactinemia is sometimes observed [142]. Corticotroph adenomas have been reported, but are a rare manifestation of CNC [3,131].

3.1.1.2. NEM1. Cushing's disease is one of the pituitary manifestations of *NEM1*. While no Cushing's disease was observed in an initial Australian series of adult patients with NEM1 [121], the same group recently showed, in their pediatric series, that 2 of the 13 adenomas were corticotropic [122]. In the Dutch series [123] and the Franco-Belgian series of the Endocrine Tumor Research Group [124] respectively, corticotroph adenomas accounted for only 3.3

Table 2
Genes and genetic syndromes (constitutional genetics/germline events) associated with Cushing's disease.

Gene	Genetic syndrome	Phenotype	Mutation frequency in Cushing's disease	Genetics	References
AIP	FIPA	Corticotroph micro- or macro-adenoma (2.9–16% of FIPAs, diagnosed between 6 and 50 years of age) Usually somatotropic adenoma ++	0–6.8%	Point mutations with several hotspots Large deletions reported	[117–120]
NEM1	NEM1	Corticotroph micro- ++ or macro-adenoma Cf. Table 1	2.5% (pediatric cohort) (1–2% of NME1)	See Table 1	[21,117,121–127]
CDKN1B	NEM4	Corticotroph micro-adenoma Isolated Or NEM1-like phenotype	Clinical cases 2.6% of a pediatric cohort	Point mutations	[128–130]
PRKAR1A	Carney complex	Corticotroph micro-adenoma Cf. Table 1	Clinical cases	See Table 1	[3,131]
CABLES 1	-	Corticotroph macro-adenoma	4 cases reported (i.e., 1.4% of a pediatric cohort and 5.7% of an adult cohort)	Point mutations	[132]
DICER1	DICER1 syndrome	Corticotroph pituitary blastoma Cystic tumor of the kidney, Thyroid cancer, Sertoli/Leydig cell ovarian tumor, cervical sarcoma, pineoblastoma, medulloepithelioma, pituitary adenoma	1% Children < 2 years	Point mutations	[133–136]
USP8	USP8 germline syndrome	Corticotroph micro-adenoma Growth retardation, dysmorphia, ichthyosiform hyperkeratosis, chronic lung disease, renal failure, dilated cardiomyopathy, hyperglycemia, partial GH deficiency	1 case of germline mutation	Mutation p.S719P	[137]
Tuberous sclerosis	Tuberous sclerosis of Bourneville	Corticotroph micro-adenoma Multiple hamartoma Hyperparathyroidism Insulinoma Other pituitary adenomas	1 adult and 1 pediatric case reported	Point mutations	[138,139]
RET	NEM2	Corticotroph micro-adenoma Medullary thyroid cancer Pheochromocytoma Hyperparathyroidism	1 pediatric case 1 adult case	Point mutations Hotspot, the most common p.C634R	[140,141]

FIPA: Familial Isolated Pituitary Adenoma; NEM 1: multiple endocrine neoplasia type 1; NEM2: multiple endocrine neoplasia type 2; NEM4: multiple endocrine neoplasia type 4.

and 4.4% of all adenomas diagnosed in *NEM1* patients. The majority of adenomas are micro-adenomas, but cases of corticotroph macro-adenoma have also been observed [21,124]. In more than three-quarters of cases of Cushing's syndrome in patients with *NEM1*, the etiology is Cushing's disease [21]. In children and young adults, Cushing's disease may be the mode of revelation of NEM1 [125–127].

3.1.1.3. NEM4. NEM4 is a much rarer form of multiple endocrine neoplasia linked to *CKN1B* mutations, leading to a phenotype similar to *NEM1*. Corticotroph adenoma was also reported in adult patients with other manifestations of the disease [128,129]. Isolated Cushing's disease is possible in children. In a large, mainly pediatric cohort tested by complete exome sequencing, a *CDKN1B* mutation was found in 2.6% of cases [130]. These 5 patients had developed Cushing's disease around the age of 10 years.

3.1.1.4. DICER1. *DICER1* syndrome is a rare form of multiple endocrine neoplasia linked to mutations in the *DICER1* gene encoding the Dicer protein, and responsible for various tumors of the kidney, thyroid, gonads, pleuropulmonary blastoma, pineoblastoma, medulloepithelioma or, more rarely, pituitary blastoma. A *DICER1* mutation is observed in less than 1% of cases of Cushing's diseases (fewer than 15 cases reported in the literature) in the pathognomonic form of a pituitary blastoma [133] and only in children under 2 years of age [133,134].

3.1.1.5. Tuberous sclerosis of Bourneville. Cushing's disease (microadenoma) was reported in an adult [138] and in a child [139] with tuberous sclerosis of Bourneville, a disease linked to mutations in the *TCS1* and *TCS2* genes. However, no causal link has been established.

3.1.1.6. Others. In NEM2 linked to *RET* oncogene mutations Cushing's disease was reported in multiple endocrine neoplasia type 2 (1 pediatric case and 1 adult case) [140,141], and in a patient with a germline mutation of USP8 [137].

3.1.2. Isolated Cushing's disease

3.1.2.1. CABLES1. Mutations in the *CABLES1* gene, a regulator of corticotropic cell proliferation [143], were described in 2 of the 146 patients in the National Institute of Heath's pediatric Cushing's disease cohort [132]. In this study, a further 35 adult patients were tested, with the identification of two additional variants in 2 adults. All patients were carriers of a macro-adenoma. No other studies have yet confirmed the prevalence of *CABLES1* mutations in corticotroph adenoma in children or adults.

3.1.2.2. AIP. Germline mutations of the *AIP* gene have been described in isolated forms of pediatric and adult Cushing's disease (before the age of 50), but much more rarely than in GH or prolactin adenoma [117–120]. In an initial pediatric cohort [117] then another pediatric and adult cohort [118] respectively, 1.4 and 6.8% of corticotroph adenomas were mutated for *AIP*. It should be noted, however, that in these cohorts, germline mutations for *AIP* in corticotroph adenoma were identified in sporadic cases. In the absence of familial forms, variable penetrance and/or expressivity can be assumed, giving these rare germline mutations a low level of predisposition.

3.2. Sequencing strategy

The strategy for genetic investigation is the same as for Cushing's syndrome of adrenal origin, with initial NGS to explore the most frequent genetic causes, followed by discussion of a genome-wide exome approach.

R5. We recommend a gene panel including at least the *MEN1*and *AIP* genes. Choice can also be guided by the syndromic context. (+)

R7. In second line, genome-wide study may be discussed (e.g., *France Médecine Génomique* 2025). (+)

3.3. Family screening

Given the rarity of genetic forms of Cushing's disease, there are currently insufficient data to recommend family screening for mutations in genes responsible for isolated Cushing's disease, particularly in case of *AIP* mutations identified mainly in a sporadic context. In other genetic alterations responsible for syndromic disease, family genetic screening should be carried out according to the specific recommendations for each pathology.

4. Conclusion

Thanks to recent technological advances, more and more genes are being identified as associated with Cushing's syndrome. Germline mutation is observed in around 50% of Cushing's syndromes of adrenal origin, depending on etiology, although this still concerns fewer than 5% of cases of Cushing's diseases. In conclusion, genetic testing is currently recommended in all cases of bilateral adrenal damage, as well as in all familial and/or pediatric forms of Cushing's syndrome of adrenal or pituitary origin. For a number of these genes responsible for Cushing's syndrome, however, it is unclear how far taking these genetic alterations into account could modify the management of patients and their families.

Disclosure of interest

The authors declare that they have no competing interest.

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