Genetics of primary bilateral macronodular adrenal hyperplasia: a model for early diagnosis of Cushing's syndrome?

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Abstract

Long-term consequences of cortisol excess are frequent despite appropriate treatment after cure of Cushing's syndrome. This might be due to diagnostic delay, often difficult to reduce in rare diseases. The identification of a genetic predisposing factor might help to improve early diagnosis by familial screening. Primary bilateral macronodular adrenal hyperplasia (PBMAH) is a rare cause of Cushing's syndrome. Hypercortisolism in PBMAH is most often diagnosed between the fifth and sixth decades of life. The bilateral nature of the adrenocortical tumors and the occurrence of rare clear familial forms suggest a genetic origin. Indeed, a limited subset of PBMAH can be observed as part of multiple tumors syndromes due to alterations of the *APC*, *Menin* or *Fumarate Hydratase* genes. Rare variants of the phosphodiesterases *PDE11A* have been associated with PBMAH. The recent identification of *ARMC5* germline alterations in 25–50% of PBMAH patients without obvious familial history or associated tumors opens new perspectives. *ARMC5* alterations follow the model of a tumor suppressor gene: a first germline inactivating mutation of this 16p located gene is followed by a somatic secondary hit on the other allele (inactivating mutation or allelic loss). Functional studies demonstrate that *ARMC5* controls apoptosis and steroid synthesis. The phenotype of index cases patients with the mutation seems more severe than the one of WT index cases. However, phenotype variability within a family is often observed. This review summarizes the genetics of PBMAH, focusing on *ARMC5*, which offer new perspectives for early diagnosis of Cushing's syndrome.

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Introduction

Primary bilateral macronodular adrenal hyperplasia (PBMAH) is an adrenal cause of Cushing's syndrome (CS) due to the bilateral development of adrenocortical nodules (1). Previously called massive macronodular

adrenocortical disease, primary macronodular adrenal hyperplasia or bilateral macronodular hyperplasia, bilateral form are more likely genetic. CS due to primary adrenal hypersecretion is classified as ACTH-independent

Invited Author's profile

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CS, and PBMAH was also named ACTH-independent macronodular adrenal hyperplasia (AIMAH). Louiset *et al.* (2013) reported that the cortisol secretion in PBMAH is not truly ACTH-independent (2), since the adrenocortical tumor cells express ACTH, and this locally produced ACTH stimulates in a paracrine and autocrine fashion the cortisol secretion (Fig. 1). Considering that ACTH could play a role in this disease, it was then suggested to name it PBMAH, to avoid the confusing reference to 'ACTH-independence' (3). The concept of illegitimate receptor expression was mostly demonstrated after the investigation of PBMAH patients, and this phenomenon is observed in the majority of PBMAH patients (1, 4, 5, 6).

PBMAH can be diagnosed in patients with clinical signs of cortisol excess, usually in the fifth or sixth decade of life

(1). PBMAH is also nowadays often diagnosed after the investigation of an adrenal incidentaloma. Adrenal incidentalomas can be observed in 0.4–5% of the general population, and are bilateral in 10–15% of the cases, corresponding mostly to PBMAH (7). CS can be diagnosed in about 35% of these bilateral incidentalomas (7, 8). CS in PBMAH is often mild and insidious, even if serious forms can be encountered. PBMAH seems to be predominant in females as observed with other causes of CS (9, 10, 11). Finally, the exact prevalence of PBMAH in the general population is unknown and dependent of the diagnosis criteria.

CS is responsible for many manifestations, including cardiovascular disease, neuropsychiatric disorders, osteoporosis, glucose intolerance or diabetes. The many consequences of CS lead to significant impairment of quality of life. Several studies suggest that despite the cure

Figure 1

Pathophysiology of Primary Bilateral Macronodular Adrenal Hyperplasia (PBMAH). Mutations of proteins are represented by stars. Corticotropin adrenal cells: a cluster of gonadal-like adrenal cells secreting local ACTH responsible for an autocrine or paracrine stimulation of the PKA pathway. Aberrant receptors: the abnormal expression of ectopic or eutopic receptors leading to an activation of the PKA pathway in response to the corresponding stimuli. PKA pathway: other mechanisms leading to an activation of the PKA signaling pathway have been described. Wnt pathway: the Wnt/ β -catenin signaling pathway is involved in adrenocortical tumors. Mutations of APC in PBMAH lead to dissociation of the

β -catenin from the destruction complex. The β -catenin protein accumulates in the cytoplasm and in the nucleus where it stimulates target gene expression. Krebs cycle: mutations of fumarate hydratase (FH) suggest the involvement of the Krebs cycle in PBMAH. Steroidogenesis: steroidogenesis is paradoxically less efficient in PBMAH cells. Steroidogenic enzymes expression is decreased after ARMC5 inactivation. Apoptosis: The mutations of ARMC5 lead to a decreased apoptosis of the adrenocortical cells and probably to their accumulation, explaining the increase of cortisol secretion despite a reduction of cortisol production at the cell level.

of cortisol excess, a significant morbidity persists and that even in cured patients life expectancy is reduced (12, 13, 14). Duration of cortisol excess, especially before CS diagnosis, is probably an important factor to reduce as far as possible for improvement of the recovery after cure of cortisol excess (15). Considering the rarity of CS and the difficulties for its screening and diagnosis by non-specialized physicians, this is challenging. Endocrine tumors of genetic origin offer a good opportunity for early diagnosis by familial screening.

Several observations argue for a genetic origin of the PBMAH: the report of rare familial forms and the bilateral and multifocal characteristics of the adrenal nodules. Candidate gene approaches showed that actors of the cAMP/protein kinase A (PKA) signaling pathway or genes causing an hereditary familial tumor syndrome including adenomatous polyposis coli gene (*APC*), *menin* (*MEN1*) and fumarate hydratase (*FH*) can favor or be responsible for the development of PBMAH (Fig. 1 and Table 1). Recently, the use of combined pan-genomic approaches led to the identification of a new gene, *ARMC5*, as a frequent cause of sporadic or familial PBMAH (16). This demonstrates that PBMAH is often genetically determined and brought some new perspectives for early diagnosis of the disease.

Alterations in the cAMP/PKA signaling pathway in PBMAH

Stimulation of the PKA pathway by adrenocorticotropin (ACTH) is essential for the adrenal cortex maintenance

and the synthesis and secretion of glucocorticoids and adrenal androgens (Fig. 2A). Various molecular and cellular alterations of the cAMP pathway have been described in adrenal tumors, including PBMAH (17). Activation of the PKA pathway by aberrant expression of G-protein-coupled receptors (GPCR) was one of the first mechanisms described in PBMAH (18) (Fig. 1). An abnormal expression of ectopic receptors (i.e., receptors that are not present on the surface of a normal adrenocortical cells) or eutopic receptors (i.e., normally present in an adrenocortical cells) leads to the activation of the PKA pathway in response to their corresponding stimulus (Fig. 2B) (18). However, to date, no genetics mechanisms enable to explain these abnormal expressions. The observation that some molecular defects as *ARMC5* mutations (see below) seems to be associated with particular profile of GPCR expression suggests that it could be indirectly the consequence of primary genetic alterations not present on the respective gene of these receptors nor a component of its signaling pathway (16). Association of rare variants of the *PDE11A* and encoding the isoform 11A of the phosphodiesterase enzyme with PBMAH have been observed (19, 20). *PDE11A* probably plays a role in the development of the disease by altering cAMP degradation. However, it is not *per se* a causal gene. Finally, only rare mutations of the melanocortin receptors type 2 gene (*MC2R*) and of the guanine nucleotide-binding protein, alpha-stimulating activity polypeptide gene (*GNAS*), as well as the duplication of the catalytic subunit alpha of the PKA (*PRKACA*) have been described in PBMAH. They are all predicted to over-activate the PKA

Table 1 Genes identified in PBMAH.

Gene	Locus	Function of the WT protein	Associated manifestations
<i>ARMC5</i>	16p11	No known function, potential role in regulation of apoptosis and steroidogenesis	Meningioma?
<i>Menin</i>	11q13	Regulator of gene transcription, cell proliferation, apoptosis, and genome stability	Multiple endocrine neoplasia type 1 (MEN1): hyperparathyroidism, pituitary adenomas, pancreatic neuroendocrine tumors
<i>FH</i>	1q42	Krebs cycle, amino acid metabolism	Hereditary leiomyomatosis and renal cell carcinoma (HLRCC)
<i>PDE11A</i>	2q31-35	Hydrolysis of cAMP and cGMP	Isolated
<i>GNAS1</i>	20q13	Stimulation of adenyl cyclase, activation of the cAMP/PKA pathway	McCune Albright syndrome: fibrous bone dysplasia, café-au-lait spots, precocious puberty, acromegaly, toxic multinodular goiter
<i>APC</i>	5q12-22	Prevent β -catenin accumulation, inhibition of the Wnt/ β -catenin pathway	Familial adenomatous polyposis: colon adenomas and carcinomas, pigmented retinal lesions, desmoids tumors, other malignant tumors as adrenocortical carcinomas
<i>MC2R</i>	18p11	ACTH receptor, activation of the cAMP/PKA pathway	Isolated
<i>PRKACA</i>	19p13.1	Catalytic subunit of PKA, activation of the cAMP/PKA pathway

Figure 2

Activation of the PKA pathway in PBMAH. (A) Physiology of the cAMP/PKA signaling pathway from the cell surface to the nucleus in adrenocortical cells: in normal adrenocortical cells, the ACTH binds to MC2R resulting in the activation of the Gs protein. Then the activated subunit alpha stimulates adenylyl cyclase (AC), which produces cAMP. Four cAMP molecules bind to the dimer of regulatory subunits (R) that set free catalytic subunits (C). Free catalytic subunits phosphorylate the transcription factor CREB which then activate the transcription of cAMP-regulated (CRE) genes. The phosphodiesterases (PDE) regulate the pathway by degrading the cAMP. (B) Dysregulation

of the cAMP/PKA signaling pathway in Cushing's syndrome: several molecular alterations of components of the cAMP/PKA signaling pathway have been described in PBMAH: i) aberrant expression of GPCR leading to a specific activation of the PKA pathway depending on the receptor and its ligand; ii) activating mutation of MC2R leading to higher activation of the pathway when stimulated by ACTH, iii) activating mutations of GNAS; iv) inactivating mutations of PDE11A leading to an accumulation of cAMP, v) duplication of the PRKACA gene leading to an accumulation of the PKA catalytic subunits alpha.

pathway, but they have been observed in a limited number of patients.

ACTH binds its receptor, the G-protein-coupled melanocortin 2 receptor (MC2R), and activates the PKA pathway (Fig. 2A). Mutation in MC2R is a rare event (21), described in two reports only (22, 23). In the first one, a patient presenting with episodic cortisol excess and PBMAH harbored the germline mutation F278C leading *in vitro* to an increase of the accumulation of cAMP and the activation of the PKA after stimulation by ACTH (22) (Fig. 2B). The second one concerns a patient carrying two pathogenic variants in the same allele but without PBMAH (23).

The guanine nucleotide-binding protein, alpha-stimulating activity polypeptide (GNAS) gene encodes for the subunit alpha of the G protein. G protein couples hormone receptors to adenylyl cyclase and leads, via the stimulation of this enzyme, to the production of cAMP

(Fig. 2A). Post-zygotic mutations of GNAS have been reported in a patient with McCune Albright syndrome (24). This disease is characterized by a congenital polyostotic fibrous bone dysplasia, the presence of *café-au-lait* skin spot and precocious puberty. Neonatal CS by nodular adrenal hyperplasia could also occur (24). Beside early post-zygotic events, somatic mutations of GNAS in adrenal nodules of patients with PBMAH have been described in few patients (10, 25, 26). Two mutants were described, R201H (10, 25, 26) and R201S (25), and their deleterious effect were proved in GH-producing pituitary tumors. Substitutions occurring in the arginine 201 led to an activation of cAMP formation, by inhibition of the intrinsic GTPase activity of the G-protein alpha subunit. GNAS defects in adult PBMAH patients are also a rare event (27).

In 2014, somatic activating mutation of PRKACA, coding for the catalytic subunit alpha of PKA, has been

discovered by whole exome sequencing (WES) as a frequent cause of overt-cortisol producing adenomas, observed in 40% of such unilateral tumors (17, 28). To date, no germline mutation of *PRKACA* has been described (17, 29). However, germline duplications on a genomic region on chromosome 19 including *PRKACA* were found in PBMAH patients (28, 30) (Fig. 2B). Three cases have been reported so far: a mother and her son who presented mild insidious CS due to PBMAH diagnosed at the third and fourth decade respectively (28); the third one was a 2-year-old boy operated for PBMAH. In this last case, the PBMAH was associated with neonatal hypoglycemia and macroglossia (30). Further studies are needed to determine the frequency of *PRKACA* duplication in large cohorts of PBMAH patients.

Multiple tumors syndromes associated with PBMAH

Familial adenomatous polyposis coli and mutations of APC ▶ Activation of the Wnt/ β -catenin signaling pathway is involved in adrenal tumors, especially non-cortisol-producing adenomas and adrenocortical carcinomas (31). Patients with familial adenomatous polyposis (FAP) (OMIM #175100) present multiple colonic polyps and an increased risk of early colon carcinomas and various adrenocortical tumors, including non-functional cortisol producing adenoma, adrenocortical cancer (31) and bilateral macronodular adrenal hyperplasia (10, 32). FAP is caused by germline inactivating mutation of *APC* (5q22), a tumor suppressor gene that inhibits Wnt/ β -catenin signaling. In the tumor tissue, the second allele is inactivated by a second event, a mutation (32) or a loss of heterozygosity (LOH) of the *APC* locus (33) (Fig. 3A). Interestingly, the second event differs in function of the nodules in the patient with PBMAH (32). The role of the activation of β -catenin as a driver of adrenocortical tumorigenesis has been clearly demonstrated *in vitro* and *in vivo* (31).

Multiple endocrine neoplasia type 1 ▶ Germline inactivating alterations of the *MEN1* gene (11q13) cause multiple endocrine neoplasia type 1, characterized by development of endocrine or non-endocrine tumors (OMIM #131100). The most frequent features are primary hyperparathyroidism, pancreatic endocrine tumors and pituitary adenomas. Adrenal lesions have been described and vary: either bilateral or unilateral, including adrenal enlargement, adenomas, macronodular adrenal hyperplasia and adrenal cancer. The frequency of adrenal lesions range between 9 and 73% depending on the screening methods used (34).

Bilateral lesions represent almost half of the patients with adrenal abnormalities. Recently, a retrospective analysis of the French and Belgium multicenter database (Groupe d'étude des Tumeurs Endocrines) including 715 MEN1 patients reported adrenal enlargement in 20.4% of the patients (34). Patients with bilateral tumors represented only 1.3% of the total cohort. Interestingly, the frequency

Figure 3

Model of *bi-allelic* inactivation of a tumor suppressor gene in Primary Bilateral Macronodular Adrenal Hyperplasia (PBMAH) development. (A) For a complete loss-of-function, the inactivation of a tumor suppressor gene has to inactivate both alleles. According to the Knudson model it is occurring by a two steps mechanism. First, a germline event inactivates the first allele. Latter a somatic inactivation of the second allele by either a LOH or a second mutation (represented by stars) leads to the development of the nodules. (B) In a patient with a germline *ARMC5* mutation a secondary somatic alterations (16p LOH without copy number change or mutation) occur latter in various adrenocortical cells on both side. This leads to the slow development of adrenal macronodules. Most often the diagnosis is made between the age of 50 and 60 years, typically after investigation of an incidentaloma or clinical signs of Cushing's syndrome progressing for several years.

of CS was higher than observed for adrenal incidentalomas. In particular, one of the patients with CS had bilateral tumors compatible with a PBMAH. *MEN1* is a tumor suppressor gene involved in cellular functions as cell cycle and proliferation (35). Knockout mice with deletion of certain exons developed adrenal tumors or hyperplasia that could be bilateral or associated with corticosterone secretion (36). Finally, PBMAH could be considered as one of the manifestations of *MEN1*. Clinical and biological signs of CS must be attentively seek in *MEN1* patients with bilateral adrenal lesions.

Fumarate hydratase ► Autosomal dominant inactivating mutation of the fumarate hydratase (FH) gene (1q43) causes hereditary leiomyomatosis and renal cell carcinoma (HLRCC) (OMIM # 150800). FH is an enzyme that converts fumarate to malate during the Krebs cycle. The frequency of adrenal lesions in HLRCC has been estimated to 7.8% in a series of 255 patients. The nodules were multifocal and bilateral in 20% (four patients) and 15% (three patients) respectively (37). Only one of the three patients with bilateral lesions presented a clinical CS (37, 38). In this patient, a LOH occurred in the adrenal lesions leading to a *bi-allelic* inactivation of the gene (Fig. 3A) (38). Thus, if the frequency of PBMAH among patients with HLRCC is low, it could be considered as a cause of PBMAH.

Armadillo repeat containing 5, the main genetic cause of PBMAH

Identification of *ARMC5* as a new tumor suppressor gene in PBMAH ► Before 2013, all genetic defects described in PBMAH and resumed above explained only a few cases. However, the bilateral character and the multifocal nodules of the adrenal disease, as well as the report of few cases of familial PBMAH, suggests a major role of genetic factors in PBMAH development. In keeping with this hypothesis, we reported in 2013 the identification of a new gene responsible for PBMAH using an integrated genomics approach (16). The analysis of a series of adrenal tumor tissues from 33 PBMAH patients by single-nucleotide polymorphism (SNP) array identified the copy-neutral LOH at chromosome 16p as the most frequent chromosomal alteration. This 16p LOH was found in the tumor tissue from a quarter of the patients. Then, using whole genome sequencing of paired germline-somatic DNA, germline and somatic inactivating mutations in the armadillo repeat containing 5 (*ARMC5*) gene were discovered. The *ARMC5* gene is located at chromosome 16p11.2. For the analysis of tumor and

leucocyte DNA it appear that the germline *ARMC5* alteration is detected in all the adrenal nodules in an *ARMC5* mutated patient, whereas the somatic alteration differ for each adrenal nodule (16). The latter is a somatic mutation in 68% of the nodules or a LOH at 16p in 32% (39). These characteristics are those of a tumor suppressor gene (Fig. 3A). In this first series of operated PBMAH patients, *ARMC5* germline mutations or deletions were observed in 55% of the cases. This clearly established that PBMAH is frequently of genetic origin in adults, even in the absence of a multiple tumor syndrome. Subsequently, two studies have confirmed that *ARMC5* mutations are frequent in PBMAH. *ARMC5* mutations were reported in almost 25% of PBMAH index cases in these series, which include operated as well as non-operated patients (9, 40).

***ARMC5* mutations and phenotype in PBMAH** ► The first studies by Assié *et al.* (16) and Faucez *et al.* (9) suggested that *ARMC5* defects are associated with a more severe disease. This was confirmed by a recent European study on 98 index cases PBMAH patients with different severities of Cushing's syndrome (39). Twenty-five percent of these index case patients presented with an *ARMC5* defect, and mutated patients had higher cortisol levels, larger adrenal glands and higher numbers of nodules on computed tomography (CT)-scan. They were also more often operated than non-mutated patients. Interestingly, mutated patients did not present a cortisol response to food ingestion, suggesting the association of *ARMC5* mutation with specific patterns of illegitimate receptors. Another interesting observation is that *ARMC5*-mutated patients have hypertension more often than WT patients. A more severe hypercortisolism is probably one of the major explanations, but it has been recently suggested that *ARMC5* mutation could favor hypertension with low renin hyperaldosteronism in the African-American population (41). The investigation of a cohort of 56 patients with primary hyperaldosteronism showed 10.7% of *ARMC5* variants predicted pathogenic by *in silico* analysis in African-American patients. Two of these patients had bilateral adrenal hyperplasia and co-secretion of aldosterone and cortisol (41). Whether this applies to other populations remains to be demonstrated.

Three recent studies demonstrate that *ARMC5* mutation is a very frequent cause of clear familial forms of PBMAH. These studies included PBMAH patients with relatives clearly diagnosed with the disease and presenting frequently a Cushing's syndrome on the basis of investigations done before any genetic screen. In eight out of the ten large families reported so far, an *ARMC5* germline

mutation could be found (40, 42, 43). Alencar *et al.* (40) investigated 47 patients from a large Brazilian PBMAH family on up to three generations and found a germline mutation in all 16 affected members. The sporadic cases are more frequently reported than familial cases but the familial nature of the disease is probably underestimated because of the phenotypic variability. Subclinical forms of Cushing's syndrome are underdiagnosed if a systematic familial screen is not performed. This is supported by familial studies demonstrating that in some of the *ARMC5* adults mutation carriers identified by familial screening the adrenal alterations are subtle (40). With the data already published, it seems that the penetrance of the disease is very high in adults who are mutation carriers. Indeed, almost all of them present adrenal imaging and/or hormonal alterations (15, 39). However, the severity of the disease might vary and some relatives of the index cases can present with only CT-scan alterations of sub-clinical Cushing's.

Combining all the *ARMC5* studies reported to date, a total of 29 germline and 32 somatic pathogenic mutations have been identified (9, 16, 39, 40, 41, 42, 43) (Fig. 4). Two additional mutations were identified in tumor DNA but the germline or somatic origin was not determinate (39). Among these mutations, a few of them can be found in non-related index cases, as the germline mutations p.I58Nfs44* (9, 40), p.R267X (9, 16, 39), p.R593W (9, 42), p.R898W (9, 16, 39) and the p.A106Rfs31* (39, 43). The *ARMC5* mutations are spread equally along the sequence. This multitude of described mutations suggests that most of the pathogenic mutations are private and that there is no clear hot spot for *ARMC5* mutations.

It remains to be established whether *ARMC5* can be responsible for a broader tumor predisposition syndrome as observed in MEN1, FAP and McCune–Albright syndrome. Indeed, two studies described occurrence of meningiomas in several *ARMC5*-mutated patients (40, 43). In one of these study, Elbelt *et al.* brought strong evidence for the involvement of *ARMC5* in meningioma development by the description of a somatic *ARMC5* mutation in the DNA from the meningioma tumor. For this 69-year-old woman, several different mutations were found in her adrenal nodules (43). In the second study, Alencar *et al.* reported the co-occurrence of meningioma in three out of seven members (43%) of the same family with macronodular adrenal hyperplasia due to *ARMC5* mutation. The authors suggest that the *ARMC5* gene will be a causal gene in both PBMAH and meningioma.

Function of *ARMC5* and its implication in PBMAH development ► The initiation and development

mechanisms of PBMAH are not explained, but the discovery of the involvement of *ARMC5* alterations opens new research perspectives. The understanding of the role of *ARMC5* is essential for a better characterization of the molecular mechanisms involved in the disease. However, to date, very little is known about *ARMC5* structure or function. It is very difficult to predict the biological role of the *ARMC5* protein from its peptidic sequence. It has been termed after its sequence homology with other members of the ARMC family of proteins. All the members of this family contain two domains involved in proteins-proteins interaction: the armadillo repeat domains (ARM repeat) and the BTB/POZ (broad complex Tramtrack bric-a-brac/Pox virus and zinc finger) (Fig. 4A). These two domains are highly conserved during evolution from drosophila to human. Despite the presence of these domains, it is not possible to predict the partners of the *ARMC5* protein because the recognition mechanism of these domains with substrate and/or target proteins is not fully characterized. However, the existence of these domains suggests that *ARMC5* can interact with various proteins, and by doing so be involved in several biological processes.

Inactivation of *ARMC5* in PBMAH follows the 'two-hit' model of a tumor suppressor gene responsible for a hereditary neoplasia syndrome. Tumor suppressor genes are known to control cell proliferation in order to limit clonal cell expansion. Inactivating mutations lead to either the expression of an inactive mutant protein or the absence of the protein. In the case of *ARMC5*, the model suggests that the loss of cell control in the adrenal cortex secondary to *ARMC5* mutation leads to nodular hyperplasia development. Initial studies showed two potential roles of *ARMC5* that could be important for PBMAH development. On one hand, the transient overexpression of the normal *ARMC5* protein induces early cell death in human adrenocortical cells, while *ARMC5* missense mutants lose the ability to induce apoptosis. On the other hand, in adrenocortical cells the expression of various major actors of steroidogenesis is altered following the *in vitro* inactivation of *ARMC5* expression by siRNA (Fig. 1). Indeed, the levels of the mRNA of the MC2R receptor, the transcription factor SF1 and the steroidogenic enzymes CYP17 and CYP21 are lower in *ARMC5*-deficient cells. This is observed both in basal condition and after stimulation of the cAMP production by forskolin. The decreased expression of several actors of the steroidogenic cascade induces a decrease of cortisol production (16). In view of the *ARMC5* protein sequence, it could be suggested that *ARMC5* possibly interacts with proteins regulating the

Figure 4

Locations and frequency of *ARMC5* mutations identified in patients with Primary Bilateral Macronodular Adrenal Hyperplasia (PBMAH). (A) In the upper part of the *ARMC5* sequence are shown germline mutations (written in red) and in the lower part of the *ARMC5* sequence somatic mutations (written in purple) and non-determinate (written in black italic, ND) mutations. *indicates mutations identified in meningioma,

indicates mutations identified in patients with primary hyperaldosteronism. (B) The diagrams show the frequency of each type (frameshift, deletion, missense, and nonsense) of mutations identified on germline DNA (left), or tumor DNA (somatic) (center) or the cumulative description of all the identified mutations (right).

apoptosis and steroidogenic processes. Dissemination of mutations along the gene in PBMAH patients suggests that most of the mutations could lead to a conformational change of the ARMC5 protein and so disrupt interaction with different partners and function. Further studies will be important to understand the exact role of this protein in these two cellular processes, in order to know how ARMC5 is involved in the initiation and/or development of PBMAH. The understanding of the ARMC5 protein interaction network will provide a genome-scale resource for elucidating normal functions of ARMC5, other pathogenic mechanisms of ARMC5 mutant forms, as well as deeper mechanistic insights into known signaling pathways altered in PBMAH (for instance, the cAMP pathway).

In PBMAH patients with ARMC5 mutation, CT scans show multiple macronodules that are more numerous than in WT patients (39). Similarly, the adrenal weight from operated patients is higher in ARMC5-mutated patients. Considering the *in vitro* data obtained on ARMC5 function, this would suggest that adrenal hyperplasia and nodules appear progressively due to decreased apoptosis following ARMC5 inactivation. At the same time, tumor cell differentiation is responsible for a lower but pituitary-autonomous cortisol synthesis and secretion capacity by each adrenal cell. When the adrenal mass is large enough, the balance between steroidogenesis capacity and cortisol autonomy results in cortisol excess (Fig. 3B). This process takes years to develop since PBMAH is a benign tumor probably with a very slow growth rate. This would explain why Cushing's syndrome in PBMAH patients is most often diagnosed late in adult life.

Perspectives in the genetic of PBMAH

Despite ARMC5 being the first gene discovered to be frequently involved in PBMAH, three-quarters of all PBMAH adult patients non-selected on the basis of the severity of Cushing's syndrome nor familial history are apparently not explained. If PBMAH in these patients is also of genetic origin, the causal gene(s) remains to be identified. Pan-genomic studies, especially WES, are powerful tools to advance in the understanding of the genetics of PBMAH and identify new candidate genes. Considering our first whole genome sequencing study (16) and studies reported by others using WES (43, 44, 45), it is likely that either each of the other genes will be implicated in a small subset of patients or that a large portion of remaining patients do not have a single monogenic disorder. Recently, another WES study from China in

patients with various types of adrenocortical tumors, including PBMAH, has suggested new candidate genes. Somatic mutations of DOT1-like histone H3K79 methyltransferase gene (*DOT1L*) were reported in two out of seven PBMAH. *DOT1L* encodes for a histone H3 lysine methyltransferase with important cellular functions, as regulation of gene transcription and cellular proliferation. In this cohort, a PBMAH presented with a mutation in the histone deacetylase 9 gene (*HDAC9*) (44). A variant (S420T) in the endothelin receptor type A gene (*EDNRA*) was identified by WES and discussed as a potential cause in two patients from the same family and one sporadic case (45). *EDNRA* encodes for the GPCR endothelin receptor type A, involved in cardiovascular or polycystic kidney disease. Sequencing of large cohorts of patients and functional studies are needed to confirm the involvement of this gene in PBMAH (45). It is also likely that future pan-genomic studies will suggest new PBMAH candidate genes. In the likely hypothesis that PBMAH could be a heterogeneous disease, integrated genomic characterization of PBMAH as performed recently in adrenocortical carcinomas could lead to a better molecular classification of PBMAH (16) that might help in the identification of new causative gene(s).

Conclusion

Major advances in the genetics of PBMAH have been possible the past 3 years with the use of pan-genomic approaches. These advances open new perspectives for understanding the pathophysiology of the disease. The knowledge of ARMC5 functions could help understand PBMAH development.

Already, genetic screening for ARMC5 germline mutation could help better diagnose and classify patients with PBMAH. Familial screening would lead to the identification of the relatives of an index case with ARMC5 mutation at risk of Cushing's syndrome development. Prospective follow-up will allow a better analysis of the development of PBMAH in such families. How this would translate into early cures or even prevention of Cushing's syndrome, and how this would improve the long-term manifestations of cortisol excess, is an exciting question that can now be addressed.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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