*European Journal of Endocrinology*, 2023, **189**, 242–251
https://doi.org/10.1093/ejendo/lvad088

**Original Research**

# Prevalence and clinical features of armadillo repeat-containing 5 mutations carriers in a single center cohort of patients with bilateral adrenal incidentalomas

**Valentina Morelli,[1,†] Francesca Marta Elli,[2,†] Sofia Frigerio,[2,3] Walter Vena,[4] Serena Palmieri,[2,*] Camilla Lucca,[2] Maria Antonia Maffini,[2] Andrea Contarino,[2,3] Francesca Bagnaresi,[2] Giovanna Mantovani,[2,3] and Maura Arosio[2,3]**

[1]Unit for Bone Metabolism Diseases and Diabetes and Lab of Endocrine and Metabolic Research, IRCCS, Istituto Auxologico Italiano, 20149 Milan, Italy
[2]Endocrinology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, 20122 Milan, Italy
[3]Department of Clinical Sciences and Community Health, University of Milan, 20122 Milan, Italy
[4]Diabetes Center, Humanitas Gavazzeni Institute, 24125 Bergamo, Italy

***Corresponding author:** Endocrinology Unit, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Via Francesco Sforza 35, Milan 20122, Italy. Email: serena.palmieri@policlinico.mi.it

## Abstract

**Objective:** We aimed to evaluate the prevalence of armadillo repeat-containing 5 (*ARMC5*) genetic defects in our cohort of bilateral adrenal incidentaloma (BAI) patients and to evaluate the possible existence of genotype–phenotype correlations.

**Design:** Cross-sectional study.

**Setting:** Tertiary care center.

**Participants:** 72 BAI patients.

**Main Outcome Measure(s):** The following data have been collected: morning adrenocorticotropic hormone (ACTH) concentrations; cortisol levels after 1 mg overnight dexamethasone suppression test (F-1mgDST); urinary free cortisol (UFC) levels; diameter of the adrenal masses; and the association with overweight/obesity, arterial hypertension, diabetes mellitus, dyslipidemia, cardiovascular events, unrelated neoplasia, osteoporosis, thyroid nodular disease, and primary hyperparathyroidism. A search for *ARMC5* germline and somatic pathogenic variants was performed in all patients and in the adrenal tissue of patients operated on, respectively.

**Results:** The prevalence of germline *ARMC5* pathogenic variants among patients with mild autonomous cortisol secretion (MACS+, defined as F-1mgDST > 1.8 µg/dL) was 18.8%. No germline pathogenic variants were detected in patients without MACS. Moreover, somatic *ARMC5* pathogenic variants were also found in the adrenal tissue of six patients without germline *ARMC5* variants. The F-1mgDST levels >5 µg/dL predicted with a poor sensitivity but a 90.5% specificity in identifying the presence of *ARMC5* germline pathogenic variants. We did not find any clinical parameter predictive of the *ARMC5* mutation presence.

**Conclusions:** In MACS+ BAI patients, germline *ARMC5* gene pathogenic variants are frequent. Further studies are needed to elucidate the pathophysiological role of somatic *ARMC5* pathogenic variants on adrenal tumor development in otherwise wild-type (WT) patients.

**Keywords:** primary bilateral macronodular adrenal hyperplasia (PBMAH), armadillo repeat-containing 5 (*ARMC5*), bilateral adrenal incidentaloma, autonomous cortisol secretion, adrenal tumors

### Significance

Our work provides interesting information both for the finding of a high prevalence of germline armadillo repeat-containing 5 (*ARMC5*) pathogenic variants in patients with bilateral adrenal incidentalomas and mild autonomous cortisol secretion and, above all, for the novel detection of somatic mutations in the adrenal tissue of patients without germline *ARMC5* pathogenic variants. Although the sample size of our cohort is quite small, due to the monocentric nature of the study, a thorough genetic analysis was performed associated with an extensive hormonal, clinical, and comorbidity assessment. We think that these findings can stimulate in the future further studies to elucidate the pathophysiological role of somatic *ARMC5* variants in the adrenal tumor development in otherwise wild-type patients and the possible clinical implications.

† M.V. and E.F.M. contributed equally.

**Received:** February 23, 2023. **Revised:** May 18, 2023. **Editorial Decision:** June 27, 2023. **Accepted:** June 27, 2023

## Introduction

Primary bilateral macronodular adrenal hyperplasia (PBMAH) or, accordingly to the recent World Health Organization (WHO) classification,[1] bilateral macronodular adrenocortical disease was described for the first time in 1964 as a rare cause of Cushing's syndrome (CS), characterized by an increased cortisol release independent from the pituitary adrenocorticotropic hormone (ACTH).[2] Adrenocortical nodules found in PBMAH are typically larger than 10 mm in diameter and progress slowly.[1,2] Although PBMAH with CS is found mostly in fifth or sixth decade, milder forms of PBMAH can be diagnosed earlier, after an incidental finding of bilateral adrenal tumors (bilateral adrenal incidentalomas [BAI[).[3–5] Although about two-thirds of BAI is considered hormonally inactive, mild autonomous cortisol secretion (MACS) is frequently found, associated or not with moderate symptoms, thus making diagnosis challenging.[6,7] The most sensitive cutoff for morning serum cortisol after 1 mg overnight dexamethasone suppression test (F-1mgDST) for the identification of patients with MACS has been defined at 1.8 µg/dL.[7] It remains to be understood whether all BAI cases represent different presentations of PBMAH or whether the definition should be restricted to cases with multiple adrenal nodules and evidence of autonomous cortisol secretion.[8]

Although the pathogenic mechanism underlying PBMAH has not yet been fully understood, different genes have been associated with the PBMAH phenotype including phosphodiesterase 11A (*PDE11A*), phosphodiesterase 8B (*PDE8B*), melanocortin 2 receptor (*MC2R*), menin 1 (*MEN1*), protein kinase CAMP-activated catalytic subunit alpha (*PRKACA*), alpha subunit of the stimulatory guanyl-nucleotide binding protein (*GNAS*), polyposis adenomatous coli (*APC*), and fumarate hydratase (*FH*) and the most recent armadillo repeat-containing 5 (*ARMC5*) and lysine-specific demethylase 1 (*LSD1/KDM1A*).[4,5,9–15]

The *ARMC5* gene, which plays a role in the regulation of apoptosis and steroidogenesis, was found to be the most frequently mutated gene in PBMAH, with an observed frequency of pathogenic germline allelic variants in up to 65% of patients with overt CS and bilateral adrenal nodules.[15,16] In patients with BAI and MACS, a lower prevalence has been described (2.4%).[16] According to the two-hit model of oncosuppressor genes, the clinical phenotype derives from the presence of a second *ARMC5* somatic genetic alteration in the adrenal tissue.[17] Armadillo repeat-containing 5 pathogenic variants seem to be associated with more severe cortisol hypersecretion and adrenal hyperplasia, as confirmed by in vitro studies showing altered steroidogenesis and impaired cell survival.[18–21] Beyond PBMAH, biallelic inactivation of the *ARMC5* gene due to damaging germline and tumor-specific somatic pathogenic variants has been shown in meningioma, suggesting that *ARMC5*-associated oncogenesis may not be limited to the adrenal cortex.[22] Indeed, *ARMC5* is ubiquitously expressed in many tissues, such as the thyroid, the brain, and the lymphocyte,[23] and *ARMC5* variants have been found among sporadic neuroendocrine tumors or multiple endocrine neoplasia type 1 patients.[24] Armadillo repeat-containing 5 is part of a RING E3 ubiquitin ligase complex and serves as an adaptor to bind multiples ligands leading to their further degradation by the proteasome, like RPB1, SREBF, and NRF1.[25]

The present study aims to evaluate the prevalence of pathogenic *ARMC5* genetic defects and to characterize the genotype–phenotype correlation in our cohort of BAI. In particular, we focused on the coexistence of *ARMC5* pathogenic variants with other endocrine/metabolic diseases.

## Patients and methods

### Patients

We enrolled 72 patients (named P1, P2… P72) consecutively referred to the Endocrinology Unit of Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico of Milan between 2012 and 2022 with benign bilateral adrenal nodules with a diameter of at least 1 cm at computed tomography and/or nuclear magnetic resonance scan performed for unrelated diseases (BAI). No patient presented any clinical sign of CS. Patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency or active malignancy have been excluded (Figure 1). In patients with a cancer history, the secondary nature of the adrenal lesions was excluded by benign radiological features and dimensional stability over time.

The study (ID2137) was approved by the Ethical Committee of Milan Area B (resolution n.325_2021bis) and was carried out in accordance with the Declaration of Helsinki. All subjects involved in the study subscribed to the informed consent.

### Methods

For all patients, the following data were collected from ambulatory medical records: morning fasting blood ACTH and cortisol concentrations, cortisol levels after 1 mg overnight dexamethasone suppression test (F-1mgDST), urinary free cortisol (UFC) levels, the diameter of the adrenal masses at radiological imaging, body mass index (BMI), and arterial blood pressure. Moreover, we recorded the presence of arterial hypertension (AH), diabetes mellitus (DM), dyslipidemia (DL), osteoporosis (OP), thyroid nodular disease (TND), and primary hyperparathyroidism (PHPT). Cardiovascular events (CVEs) (myocardial infarction, stroke, transient ischemic attack, and pulmonary embolism) and unrelated neoplasia occurrence were evaluated during the 10 years before the BAI finding and during the follow-up period.

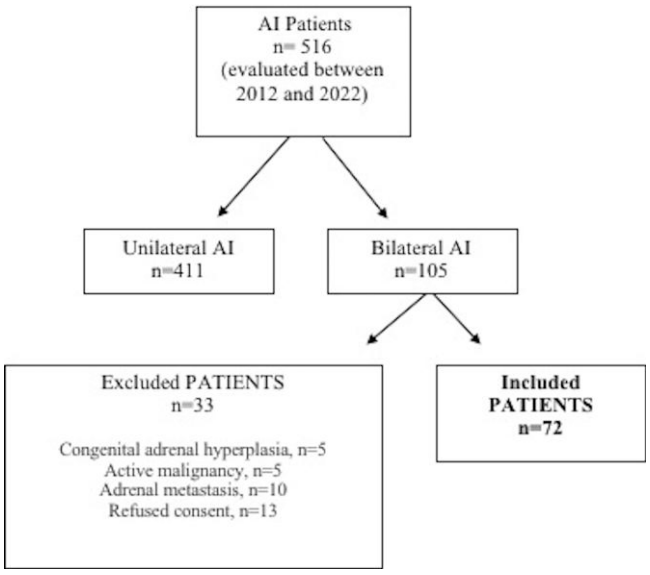


**Figure 1.** Study population. AI, adrenal incidentaloma.

Mild autonomous cortisol secretion was diagnosed in the presence of F-1mgDST > 1.8 μg/dL.[6] Arterial hypertension was defined as the detection of systolic and/or diastolic blood pressure (BP) ≥ 140 and 90 mmHg, respectively, and/or the need for any antihypertensive treatment.[26] Diabetes mellitus was diagnosed using the American Diabetes Association's current clinical practice recommendations.[27] Dyslipidemia was defined as stated in ATP-III.[28] Osteoporosis was defined as the presence of morphometric vertebral fractures or vertebral and/or lumbar T-score ≤ −2.5 standard deviation scores (SDS) at dual-energy X-ray absorptiometry.[29] The total diameter of the adrenal masses was defined as the sum of the diameters of the right and the left major nodule.[30] A thyroid ultrasound was performed to evaluate the presence of a TND in *ARMC5* mutation carriers. Thyroid nodules assessment of the risk of malignancy was conducted accordingly to the European Thyroid Association guidelines.[31] Primary hyperparathyroidism was diagnosed by the presence of hypercalcemia and elevated or inappropriately normal PTH levels, after the exclusion of familial hypocalciuric hypercalcemia.[32]

Plasma ACTH levels at 8:00 AM were measured by chemiluminescence (Immulite 2000, Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA, normal values 10-55 ng/L). Serum cortisol concentrations were measured by the second-generation monoclonal immunoassay Elecsys Cortisol II (Elecsys Cortisol Immunoassay, Roche Diagnostics, Mannheim, Germany, on CobaS E 602). The determination of UFC (reference range up to 43 μg/24 h) was performed by liquid chromatography–tandem mass spectrometry (LC-MS/MS). All tests were performed in the same laboratory.

All patients underwent genetic analysis on peripheral blood for research of *ARMC5* pathogenic variants. In addition, fresh-frozen or formalin-fixed paraffin-embedded (FFPE) adrenal tissues from 10 out of 16 patients that had been operated on were studied. All samples were analyzed to identify pathogenic single nucleotide variants (SNVs) of the *ARMC5* gene by Sanger sequencing and/or structural rearrangements (duplications/deletions, termed as copy number variants [CNVs]) involving part or the whole *ARMC5* gene by TaqMan CNV qPCR. The existence of transcript-specific genetic alterations has been determined not only in the commonly studied NM_001105247 but also in the extra-long NM_001288767 (mainly expressed in the liver, lung, tonsils, and ureter), the short NM_024742 (preferentially observed in lung, uterus, fat, liver, and lymph nodes), and the endocrine NM_001301820 (expressed specifically in uterus, pituitary, pancreas, adrenal, prostate, and lung).[23] In selected patients, Sanger sequencing of coding and splicing regions of the *MEN1* gene was performed considering the reference sequence NM_130799 as the wild-type (WT) reference. All the protocols used are summarized in the Supplementary Material section.

### Statistical analysis

Based on literature data regarding *ARMC5* pathogenic variants among PBMAH patients,[15] the sample size evaluated in the present study consented to have a power of 0.8 (type I error 0.05). Statistical analysis was performed by SPSS version 27.0 statistical package (IBM SPSS, Italy). The normality of data distribution was tested by Kolmogorov–Smirnov test. The results were expressed as median values with interquartile ranges in parenthesis or as absolute frequencies and percentages for categorical variables. The comparisons of continuous variables between mutated and WT patients were performed by using Mann–Whitney test or Kruskal–Wallis test followed by Dunn's post hoc test. The categorical variables were compared by using the $\chi^2$ test or Fisher exact test, as appropriate. The receiver operating characteristic (ROC) curve analysis was used to assess the cutoff of the F-1mgDST with the best sensibility (SN) and specificity (SP) for detecting the presence of *ARMC5* germline pathogenic variants. *P*-values < .05 were considered statistically significant.

## Results

### Clinical and biochemical characteristics of the whole cohort of patients

The study cohort was composed of 72 subjects, with a median age of 71.5 (64-77) years; 44.4% were males, and 55.6% were females. Median BMI was 27.1 (24.3-31) kg/m$^2$, and the total diameter of the adrenal lesions was 4.15 cm (3-5.3). Median F-1mgDST levels were 2.45 (1.5-3.5) μg/dL, and 48 patients (66.6%) were categorized as having mild cortisol hypersecretion (MACS+). Adrenocorticotropic hormone concentrations were 12.3 (7-18) ng/L, and high UFC levels were present in 13.9% of cases (all patients MACS+). The prevalence of the main cardiovascular and metabolic complications was the following: AH, 76.4%; DM, 31.9%; DL, 51.4%; and CVE, 25%. A history of unrelated cancer was reported in 43%, OP in 62.5%, TND in 76.4%, and PHPT in 13.9% of cases.

### Genetic analysis

In our cohort of BAI patients, we identified nine (12.5%) patients affected by a germline *ARMC5* variant (Table 1). Moreover, we identified at least one somatic *ARMC5*

**Table 1.** Summary of germline and somatic variants found in the studied cohort of BAI patients.

| pt ID | Germline | Somatic |
|---|---|---|
| 1 | WT | Exons 4-6 deletion |
| 6 | c.1123del, p.Met375Trpfs*86 | NA |
| 8 | 5′UTR deletion | NA |
| 11 | WT | Whole gene deletion |
| 16 | WT | Whole gene deletion |
| 17 | c.2436del, p.Cys813Valfs*104 | c.231_265del, p.Ala78Argfs*13 |
| 20 | 5′UTR del + Ex1-3 deletion | NA |
| 23 | WT | Whole gene deletion/c.682C > T, p.Gln228Ter |
| 29 | WT | Whole gene deletion/c.2486G > A, p.Gly829Asp |
| 31 | c.1586dup, p.Ser530Valfs*8 | NA |
| 34 | c.2635C > T, p.Arg879Trp | Whole gene deletion |
| 37 | WT | Whole gene deletion |
| 43 | c.2192C > G, p.Pro731Arg | NA |
| 61 | c.682C > T, p.Gln228Ter | NA |
| 71 | c.2192C > G, p.Pro731Arg | NA |

Abbreviations: pt ID, patient identification number; NA, not assessed; WT, wild type.

mutation in 8 out of 10 adrenal tissue analyzed (Table 1). The opportunity to test more slices of FFPE-embedded adrenal nodules from the same patient allowed to appreciate a certain degree of genetic mosaicism within the same tissue and that mutation acquisition was a multistep process. The analysis in parallel of four different slices of tissue from patient 23 found a heterozygous *ARMC5* deletion together with the co-existence of two different nondeleted alleles, one carrying a heterozygous SNP in the genomic position 31′460′057 and the other with the heterozygous truncating mutation p.Gln228Ter. The rare presence of two different nondeleted alleles, one heterozygous and the other homozygous for the SNP in position 31′460′057, was further confirmed in patient 34. The presence of mosaicism together with the low sensitivity of selected molecular biology techniques when testing low-quality, highly fragmented DNA from FFPE-extracted nucleic acids and the fact that we did not exclude germinal and/or acquired variants in the *ARMC5* regulatory regions did not allow to rule out whether the detection of a single somatic mutation was a technical limit rather than the occurrence of pure somatic alterations.[18] Moreover, the analysis of blood and tissue samples confirmed the presence in the cohort of patients affected by familial (one germinal and one somatic in patient 17) or by sporadic (two somatic defects in patient 23) BAI. In 2 out of 10 adrenal tissue samples obtained from operated patients (P51 and P72), no somatic pathogenic variants were found.

In particular, the search for point mutations in the coding region or intronic flanking sequences identified eight different SNVs in nine patients (Table 2 and Figure 2). Of these eight SNVs, four were previously described, that is, the c.682C > T p.Gln228Ter variant,[33] affecting the ARM3 domain in exon 3, the c.2192C > G p.Pro731Arg,[17] the c.2436del p.Cys813Valfs*104, and the c.231_265del, p.Ala78Argfs*13 variants[34] localized in exon 6, inside or few amino acids upstream of the CUL3-interacting BTB domain (aa 748-816). Three out four novel SNVs were identified only in lymphocyte-extracted DNA, while the fourth was identified only in the adrenal tissue sample. The four new SNVs were prioritized using pathogenicity scores of pre-classification sensitivity (pathogenic/likely pathogenic prediction) calculated using a subset of 18 out of the 28 American College of Medical Genetics and Genomics (ACMG)/Association for Molecular Pathology (AMP) criteria for variant pathogenicity assessment. Their frequency and allelic distribution were controlled from population databases (Genome Aggregation Database—gnomAD, ExAC, and 1000 Genomes Project—1000GP), and their pathogenic effect was predicted using different functional, conservational, and evolutionary scores predicting damaging variants (Table 2). As expected from the literature, all variants were mainly frameshift or nonsense, leading to a prematurely truncated protein and spread along the whole gene region (exons 1, 3, 4, and 6). Specifically, we found two frameshift protein-truncating variants, namely, the c.1123del, p.Met375Trpfs*86 deletion in exon 3, within the ARM6 domain (P6) and a single-base duplication c.1586dup, p.Ser530Valfs*8 in exon 4, within the protein common region (P31) and a missense variant, located in the low complexity region encoded by exon 6, the c.2635C > T, p.Arg879Trp transversion (P34). The novel somatic SNV c.2486G > A p.Gly829Asp was a missense transition located in the low complexity region encoded by exon 6 of the not deleted allele recovered from the tissue sample of P29. Missense defects might alter the protein activity according to the affected amino acid residue. This could modify protein–protein interactions, posttranslational protein modification and/or the three-dimensional protein structure. The deleterious effect of our variants could be explained by their location around the BTB domain and ARM domain, which could impair the function of adapter in RING E3 ubiquitin ligases complexes.

**Table 2.** Summary of single nucleotide variants found in the studied cohort of BAI patients.

| Nucleotide variation | Exonic location | Protein variation | Protein domain | Expression | pt ID | gnomAD allele frequency | Clinvar | ACMG classification |
|---|---|---|---|---|---|---|---|---|
| c.231_265del | ex1 | p.Ala78Argfs*13 | Disordered region | Somatic | 17 | Absent | NR | Likely Pathogenetic (PVS1-PM2) |
| c.682C > T | ex3 | p.Gln228Ter | ARM3 domain | Germline—somatic | 61-23 | Absent | NR | Pathogenetic (PVS1-PM2-PP5) |
| c.1123del | ex3 | p.Met375Trpfs*86 | ARM6 domain | Germline | 6 | Absent | NR | Likely Pathogenetic (PVS1-PM2) |
| c.1586dup | ex4 | p.Ser530Valfs*8 | Common region | Germline | 31 | Absent | NR | Likely Pathogenetic (PVS1-PM2) |
| c.2192C > G | ex6 | p.Pro731Arg | Low complexity region | Germline | 43-71 | 0.00171 | Uncertain significance | Benign (BS1-BP4-BP1-PP %) |
| c.2436del | ex6 | p.Cys813Valfs*104 | BTB/POZ domain | Germline—somatic | 17 | absent | Pathogenetic | Pathogenetic (PVS1-PM2-PP5) |
| c.2486G > A | ex6 | p.Gly829Asp | Low complexity region | Somatic | 29 | absent | NR | Likely Benign (BP1-BP3-PM2) |
| c.2635C > T | ex6 | p.Arg879Trp | Low complexity region | Germline | 34 | 0.00000583 | NR | Uncertain Significance (PM2-BP1) |

Abbreviations: ACGM, American College of Medical Genetics and Genomics; ex, exon; PM2, absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium; PP2, missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease; PP3, multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.); PP5, reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation; Pt ID, patient identification number; PVS1, null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where LOF is a known mechanism of disease; NR, not reported in the ClinVar database.

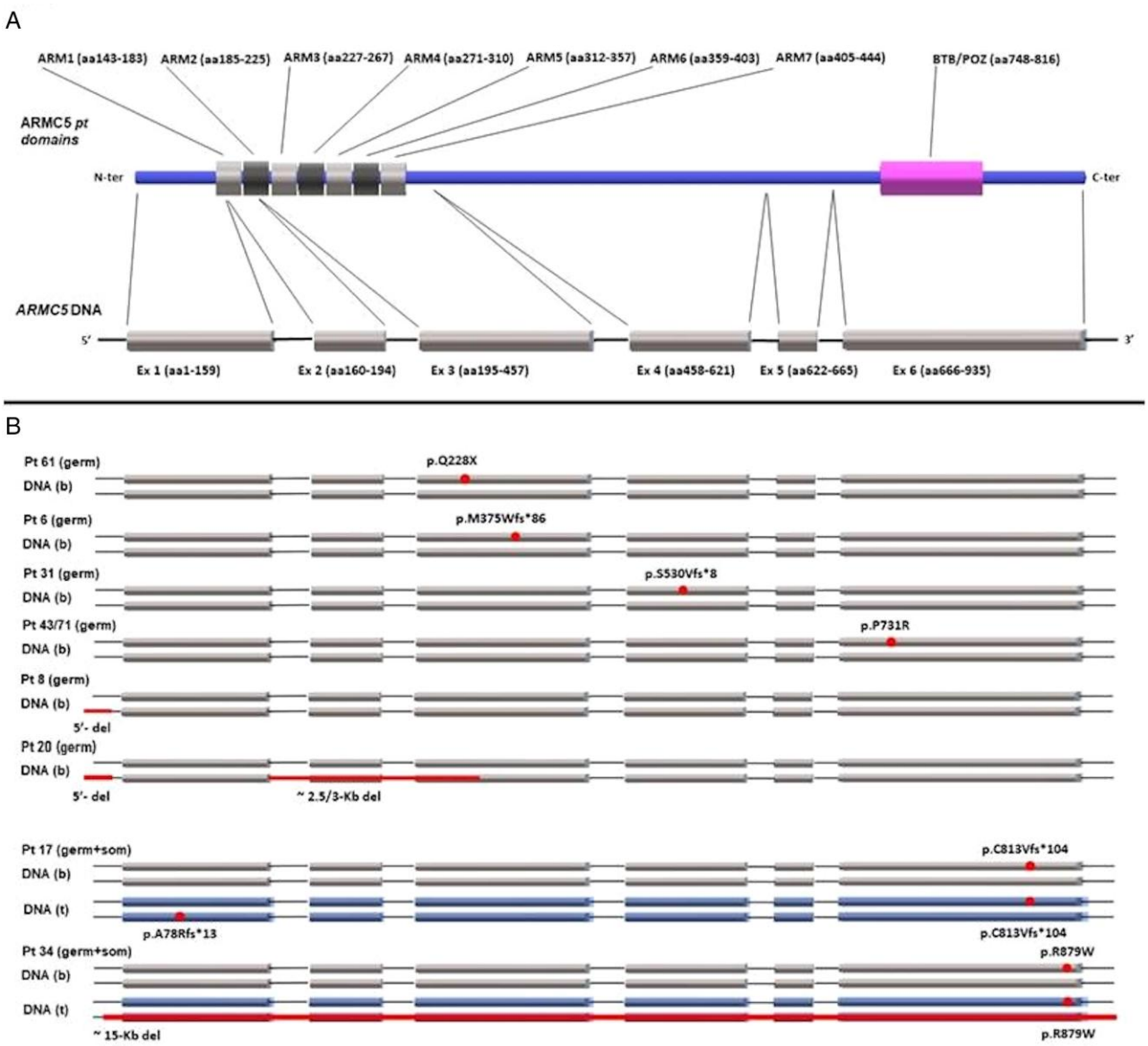


**Figure 2.** Picture that summarizes the localization of *ARMC5* germline SNVs and CNVs detected in our cohort of patients according to the *Homo sapiens* (human) genome assembly GRCh38 (hg38) from Genome Reference Consortium. This figure also shows the position of CNV TaqMan assays, all genetic alterations with respect to coding regions of all four investigated *ARMC5* isoforms (NM_001105247, NM_001288767, NM_024742, and NM_001301820) and to protein domains of the commonly studied NM_001105247 isoform. For patients 17 and 34, also the localization of *ARMC5* somatic SNVs and CNVs is reported. Single nucleotide variants and CNVs are highlighted in red. *ARMC5*, armadillo repeat-containing 5; CNV, copy number variant; DNA (b), blood; DNA (t), tissue; SNV, single nucleotide variant.

Larger genetic defects, or rather CNVs affecting part or all the sequence causing loss of heterozygosity (LOH) of the *ARMC5* gene, were identified in nine patients, two (P8 and P20) at germline, and seven (P1, P11, P16, P23, P29, P34, and P37) at somatic level (Table 3 and Figure 2). P8 and P20 shared the same small germline deletion of about 4 kb in the 5′ upstream region, affecting exon 1 of the long isoform NM_001288767 and possibly regulatory regions for the other isoforms, while P20 also had a second loss of material of about 2.5-3 kb that removed exons encoding from the first amino acid residue up to the ARM3 functional domain. Another partial *ARMC5* deletion was detected in P1, and it was a somatic defect of about 3 kb, which eliminates the sequence for the second half of the protein, including the BTB/POZ domain. The additional six somatic LOHs were expanded deletions removing the whole *ARMC5* sequence. Deletions identified in P34 and in P37 extended for about 15 kb, while those in P11, P16, P23, and P29 measured more than 18 kb. Although we did not characterize breakpoints, we were able to predict the extension of deletions, using the location of CNV TaqMan assays and informative markers, like heterozygous single nucleotide polymorphisms, and to hypothesize the presence of specific genomic structures able to favor recurrent structural rearrangement mechanisms.[18]

Our findings confirmed that most loss-of-function *ARMC5* variants are frameshift (22%), nonsense (11%), or large deletions (50%), while missense variants were approximately 17%. When considered separately, germline and somatic alterations presented a different pattern of frequency of *ARMC5* variants (frameshift 37% vs 10%, nonsense 13%

**Table 3.** Clinical and biochemical characteristics of patients with large somatic mutations on adrenal tissue.

| pt ID | Gender | Age | Genetic alteration | Diam R + L(cm) | F-1mgDST (ug/dL) | ACTH (ng/L) | hUFC | AH | DM | DL | OP | OB | TND | Medical history | Reason for surgery | Surgery (histology and hormonal outcome) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| P1 | F | 71 | Ex4-6 del | 4.2 + 2.7 | 2.3 | 7.6 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | Primary hyperparathyroidism | L nodule enlargement | Adenoma (with hemorrhagic necrosis) |
| P 11 | M | 74 | Whole gene del. | 4.5 + 1.7 | 3.5 | 6.9 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | Macroprolactinoma; Colorectal polyposis | R nodule dimension | PBMAH |
| P 16 | F | 52 | Whole gene del. | 3 + 2.5 | 19.3 | 5 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | Bipolar disorder | Hypercortisolism | Adenoma |
| P 23 | M | 66 | Whole gene del. | 2.5 + 1 | 2.6 | 15.6 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | Prostate cancer | Hypercortisolism | PBMAH |
| P 29 | F | 74 | Whole gene del. | 2.5 + 5 | 3.5 | 9 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | None | L nodule dimension | Adenoma |
| P 34 | F | 78 | Whole gene del. | 1.7 + 5 | 2.6 | 12,1 | 0 | 1 | 1 | 1 | 0 | 1 | n.a. | Hyperandrogenism | L nodule dimension | PBMAH |
| P 37 | M | 76 | Whole gene del. | 2.3 + 2.7 | 3.1 | 25 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | Bladder cancer | Hyperaldosteronism | Adenoma |

Abbreviations: hUFC, high urinary free cortisol; IPMN, intraductal papillary mucinous neoplasm; L, left; n.a., not available; PBMAH, primary bilateral macronodular adrenal hyperplasia; R, right; TND, thyroid nodular disease.

vs 10%, large deletions 25% vs 70%, and missense 25% vs 10%, respectively).

In five patients (P1, P22, P51, P63, and P69) who presented additional clinical features that could suggest a possible syndromic form of PBMAH, we also performed the screening for pathogenic variants at the *MEN1* gene, but we did not discover any genetic defect.

## Genotype–phenotype analysis

The prevalence of germline pathogenic variants (MUT) among patients with MACS (MACS+) and BAI was 18.8% (8 out of 48 patients). No pathogenic variants were found among the 24 patients without cortisol autonomous secretion (MACS−).

Clinical and biochemical characteristics and comorbidities of MUT patients are summarized in Table 4. In P17 and P34, a second somatic *ARMC5* mutation was also found in adrenal samples, whereas for the remaining two operated MUT patients (P6 and P43), adrenal tissue samples were unfortunately not available. The 24 MACS− patients, 39 WT-MACS+, and 9 MUT-MACS+ patients were comparable in age, gender, and BMI (Table 5). As expected, MACS− patients showed higher ACTH levels but also had smaller adrenal lesions than MACS+ patients. In MUT-MACS+ patients, F-1mgDST levels were higher than that observed in WT patients (4.4 vs 2.2 µg/dL, $P = .013$). Receiver operating characteristic curve analysis showed that F-1mgDST levels >5 µg/dL have a 33% sensitivity (SE) and a 90.5% SP in identifying the presence of *ARMC5* germline pathogenic variants. The total diameter was significantly higher in MUT-MACS+ when compared with WT-MACS− patients ($5.8 \pm 2.6$ vs $3.4 \pm 1.2$, $P = .021$), but not statistically different between WT-MACS+ and MUT-MACS+ patients (Table 5).

Regarding comorbidities, the prevalence of AH, DL, and DM was similar when comparing WT and MUT patients, regardless of the MACS presence (AH, 76.2% vs 77.8%; DL, 49.2 vs 66.7%; and DM, 31.7% vs 33.3%, $P > .50$). The prevalence of DL was significantly higher in MACS+ than in MACS− (60.4 vs 33.3 $P = .04$). In addition to metabolic complications, in MUT patients, we observed that hematological disorders and infections were the most frequently associated conditions (Table 3). The prevalence of OP was 77.8% in MUT patients and 42.9% in WT patients ($P = .075$), showing a positive trend passing from MACS− to WT-MACS+ and MUT-MACS+. Similarly, the history of CVE, PHPT and unrelated cancer, was comparable among the three groups (Table 5). Thyroid nodular disease prevalence was similar among the three groups (Table 5). In particular, no thyroid malignancies were observed among MUT-MACS+ patients.

In patients with somatic pathogenic variants (Table 3), we observed one case of PHPT due to a parathyroid adenoma (P1, *MEN1* mutation absent), two patients with a medical history of urinary tract neoplasms, and one patient with macroprolactinoma and colorectal polyposis (*MEN1* mutation absent; not evaluated the presence of *APC* gene mutations).

In four (P1, P11, P23, and P29) out of six patients with the somatic mutation alone, we observed a normalization of cortisol secretion after surgery (minimum follow-up of 4 years). On the other hand, P16 and P37 have persistence of MACS after surgery (Table 3). Considering MUT patients undergoing unilateral adrenalectomy, P43 and P34 normalized cortisol secretion (minimum follow-up of 4 years after surgery), while P17 has a persistence of MACS (Table 4).

**Table 4.** Clinical and biochemical characteristics of patients with germline mutations.

| Pt ID | Gender | Age (yrs) | Pathogenic variants | Diameter R + L (cm) | F-1mgDST (μg/dL) | ACTH (ng/L) | hUFC | AH | DM | DL | OP | OB | TND | Medical history | Surgery (yes/no, type, histology, hormonal outcome) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **P 61** | M | 74 | ex3A c.682C > T p.Q228X | 2.1 + 4.1 | 9.1 | 14 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | Hodgkin lymphoma | No |
| **P 17** [a] | M | 60 | ex6B c.2436del p.C813Vfs*104 | 1.0 + 5.0 | 13.9 | 5 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | Hypogammaglobulinemia, relapsing HSV infections | Yes MAx PBMAH |
| **P 31** | F | 77 | ex4A c.1586dup p.S530Vfs*8 | 1.3 + 1.5 | 4.4 | 8 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | Ischemic heart disease, HCV-related cirrhosis | No |
| **P 43** | M | 76 | ex5/6A c.2192C > G p.P731R | 3.9 + 1.0 | 3.9 | 9.4 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | Multiple myeloma | Yes MAx adenoma |
| **P 71** | M | 58 | ex5/6A c.2192C > G p.P731R | 3.1 + 1.0 | 2.2 | 12.5 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | No |
| **P 6** | M | 75 | ex3B c.1123del p.M375Wfs*86 | 5.7 + 6.3 | 20.5 | 10 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | Yes BAx PBMAH |
| **P 34** [a] | F | 78 | Ex6B c.2635C > T—p.R879W | 1.7 + 5.0 | 2.6 | 12.1 | 0 | 1 | 1 | 1 | 0 | 1 | n.a | IPMN, chronic HCV-related hepatitis | Yes MAx PBMAH |
| **P 8** | F | 83 | 5′UTR del | 2.4 + 3.0 | 3 | 13 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | No |
| **P 20** | F | 78 | 5′UTR del + Ex1-3 del | 2.8 + 1.1 | 4.4 | 11.5 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | No |

Abbreviations: BAx, bilateral adrenalectomy; IPMN, intraductal papillary mucinous neoplasm; MAx, monolateral adrenalectomy; n.a., not available; PBMAH, primary bilateral macronodular adrenal hyperplasia; TND, thyroid nodular disease.
[a]Patients with both germline and somatic mutations.

**Table 5.** Clinical and biochemical characteristics of patients with and without mild autonomous cortisol secretion and germline *ARMC5* mutations.

| | MACS− | MACS+ | |
|---|---|---|---|
| | WT (24) | WT (39) | MUT (9) |
| Age, years | 69 (61-77) | 71 (66-77) | 78 (76-83) |
| Gender, % (*n*) | M 45.8 (11)<br>F 54.2 (13) | M 38.5 (15)<br>F 61.5 (24) | M 55.6 (5)<br>F 44.4 (4) |
| Total diameter, cm | 3.25 (2.5-4.1)[a,b,c] | 4.9 (3.6-6.1) | 5.4 (4-6.45) |
| BMI, kg/m$^2$ | 27.6 (26.1-31.2) | 27.2 (23.9-31) | 25.3 (24.3-28.8) |
| F-1mgDST, µg/dL | 1.3 (1-1.6)[a,b,c] | 3.1 (2.3-3.7) | 4.4 (2.8-11.5) |
| ACTH, ng/L | 15.9 (9.7-21.3)[b,c] | 8.1 (5.7-18) | 11.5 (8.7-12.3) |
| High UFC (%) | 4.2 (1) | 20.5 (8) | 11.1 (1) |
| AH, % (*n*) | 75 (18) | 76.9 (30) | 77.8 (7) |
| DM, % (*n*) | 20.8 (5) | 38.5 (15) | 33.3 (3) |
| DL, % (*n*) | 33.3 (8)[c] | 58.9 (23) | 66.7 (6) |
| CVE, %(*n*) | 29.2 (7) | 25.6 (10) | 11.1 (1) |
| Npl, % (*n*) | 50.0 (12) | 43.6 (17) | 22.2 (2) |
| PHPT, % (*n*) | 12.5 (3) | 17.9 (7) | 0 (0) |
| OP, % (*n*) | 33.3 (8) | 48.7 (19) | 77.8 (7) |
| TND, % (*n*) | 58.3 (14) | 53.8 (21) | 50.0 (4) |

Data are expressed as median (interquartile range) or percentages (number).
Abbreviations: AH, arterial hypertension; BMI, body mass index; CVE, cardiovascular events; DL, dyslipidemia; DM, diabetes mellitus; F-1mgDST, cortisol levels after 1 mg overnight dexamethasone suppression; MACS−, patients without MACS; MACS+, patients with mild autonomous cortisol secretion; MUT, positive for ARMC5 germline mutation; Npl, history of unrelated malignancy; OP, osteoporosis; PHPT, primary hyperparathyroidism; TND, thyroid nodular disease; WT, wild type for germline *ARMC5* mutations.
[a]$P < .05$ MACS− vs MUT-MACS+.
[b]$P < .05$ MACS− vs WT-MACS+.
[c]$P < .05$ MACS− vs MACS+.

## Discussion

The present study investigated the prevalence of pathogenic *ARMC5* genetic defects in a cohort of BAI focusing on the possible existence of genotype–phenotype correlations.

In our cohort of BAI patients, the prevalence of germline ARMC5 pathogenic variants among patients with possible autonomous cortisol secretion was 18.8% consistent with the prevalence reported in a large series of nonselected AI patients.[35] The prevalence of *ARMC5* mutation remains higher (12.5%) even after the exclusion of three patients with histologically proven PBMAH. No germline pathogenic variants were detected in patients MACS−, accordingly to recent findings.[35] In addition to previous studies, we used a double technical approach based on direct sequencing and real-time polymerase chain reaction for CNV, which allowed us to identify both SNV and/or CNV located in the coding region of the *ARMC5* gene. This procedure led to easier detection of possible cases of LOH, with or without associated point mutations, which means that we were able to perform a deeper evaluation of both alleles for different types of genetic defects in each tested patient increasing the probability of detecting both pathogenic variants associated with the clinical phenotype. Moreover, for the first time to our knowledge, we searched for *ARMC5* genetic defects common to all the four expressed isoforms, intending to identify hypothetical transcript-specific alterations, that could help in the definition of transcript and/or tissue-specific functions, as well as justifying the proportion of patients still without a genetic diagnosis and/or without the identification of both pathogenic variants in the *ARMC5* gene.

MUT-MACS+ patients, accordingly to literature data, showed higher F-1mgDST levels than WT patients.[13,15,17,18,35] Moreover, we found that F-1mgDST levels >5 µg/dL have poor sensitivity but a 90.5% SP in identifying the presence of *ARMC5* germline pathogenic variants.

Unfortunately, from a clinical point of view, we did not find any parameter predictive of the *ARMC5* mutation presence. A worst metabolic profile (DL prevalence significantly higher) was found in MACS+ patients when compared with MACS − patients, as expected, regardless of the presence of an *ARMC5* mutation. The lack of difference in AH and DM prevalence among groups could be due to the limited number of patients. Similarly, despite the expression of the *ARMC5* gene has been described in tissues other than adrenal, we found neither a different prevalence of NTD nor of unrelated cancer among mutated and nonmutated patients.[23,24,25] Among malignant lesions, breast cancer, lymphoproliferative diseases (lymphoma, myeloma, and polycythemia), and neuroendocrine tumors were the most frequent (8.3%, 8.3%, and 5.6% respectively). In particular, hematological disorders and infections were the most frequently associated conditions in MUT patients. A possible bias can be hypothesized regarding the higher presence of hematological disorders and neuroendocrine tumors in our study cohort as Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico is a referral center for hematological and neuroendocrine diseases. However, a possible link connecting lymphoproliferative diseases and *ARMC5* pathogenic variants has been previously described.[34]

The analysis of 10 adrenal tissue samples of operated patients confirmed the presence of a second somatic mutation in two patients with a germline mutation. Moreover, somatic pathogenic variants, mainly large deletions, were also found in six BAI patients without germline *ARMC5* variants. To the best of our knowledge, no studies have reported somatic pathogenic variants in patients with BAI without germline *ARMC5* pathogenic variants, even though somatic pathogenic variants have also been described in tumors other than adrenal adenomas.[24] The role of these pathogenic variants remains to be clarified by more detailed pathological studies (ie, immunohistochemical staining for *ARMC5*)

also given the different clinical responses of patients to unilateral adrenalectomy.

Our study has some limitations, starting from its cross-sectional design. Moreover, we did not evaluate the possible different expression of aberrant receptors and the presence of *KDM1A* mutations in BAI WT-MACS+ patients. On a morphological level, a significant difference between the total diameters of the MUT MACS+ and WT MACS+ patients has not been found, contrary to what was expected; we can only hypothesize that comparing other parameters, such as the number of nodules or the overall size of the adrenal glands, could lead to a statistical significance.

As far as comorbidities are concerned, the presence of a possible meningioma has been excluded by targeted magnetic resonance imaging only in three MUT patients and we do not yet have the clinical and genetic data of the first-degree relatives of the MUT patients.

In conclusion, in MACS+ BAI patients, the presence of *ARMC5* mutation should be evaluated, even in the absence of typical signs of CS. Further studies are needed to elucidate the pathophysiological role of somatic *ARMC5* somatic pathogenic variants on adrenal tumor development in otherwise WT patients.

## Supplementary material

Supplementary material is available at *European Journal of Endocrinology* online.

## Funding

The work was partially supported by Ricerca Corrente Funds from the Italian Ministry of Health to Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico.

*Conflict of interest:* None declared.

## Data availability

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## References

1. Mete O, Erickson LA, Christofer JC, *et al.* Overview of the 2022 WHO classification of adrenal cortical tumors. *Endocr Pathol*. 2022;33(1):155-196. https://doi.org/10.1007/s12022-022-09710-8
2. Bertherat J, Bourdeau I, Bouys L, Chasseloup F, Kamenicky P, Lacroix A. Clinical, pathophysiologic, genetic and therapeutic progress in primary bilateral macronodular adrenal hyperplasia. *Endocr Rev*. 2023;44(4):567-628. https://doi.org/10.1210/endrev/bnac034
3. Hsiao HP, Kirschner LS, Bourdeau I, *et al.* Clinical and genetic heterogeneity, overlap with other tumor syndromes, and atypical glucocorticoid hormone secretion in adrenocorticotropin-independent macronodular adrenal hyperplasia compared with other adrenocortical tumors. *J Clin Endocrinol Metab*. 2009;94(8):2930. https://doi.org/10.1210/JC.2009-0516
4. Libé R, Coste J, Guignat L, *et al.* Aberrant cortisol regulations in bilateral macronodular adrenal hyperplasia: a frequent finding in a prospective study of 32 patients with overt or subclinical Cushing's syndrome. *Eur J Endocrinol*. 2010;163(1):129-138. https://doi.org/10.1530/EJE-10-0195
5. Lacroix A. ACTH-independent macronodular adrenal hyperplasia. *Best Pract Res Clin Endocrinol Metab*. 2009;23(2):245-259. https://doi.org/10.1016/J.BEEM.2008.10.011
6. Di Dalmazi G. The landscape of bilateral adrenal incidentalomas associated with subclinical hypercortisolism. *Endocrine*. 2016;53(3):621-623. https://doi.org/10.1007/s12020-016-1004-8
7. Bouys L, Chiodini I, Wiebke A, Reincke M, Bertherat J. Update on primary bilateral macronodular adrenal hyperplasia (PBMAH). *Endocrine*. 2020;71(3):595-603. https://doi.org/10.1007/s12020-021-02645-w
8. Vassiliadi DA, Tsagarakis S. Diagnosis and management of primary bilateral macronodular adrenal hyperplasia. *Endocr Relat Cancer*. 2019;26(10):R567-R581. https://doi.org/10.1530/ERC-19-0240
9. Vezzosi D, Libé R, Baudry C, *et al.* Phosphodiesterase 11A (*PDE11A*) gene defects in patients with ACTH-Independent Macronodular Adrenal Hyperplasia (AIMAH): functional variants may contribute to genetic susceptibility of bilateral adrenal tumors. *J Clin Endocrinol Metab*. 2012;97(11):2063-2069. https://doi.org/10.1210/jc.2012-2275
10. Lacroix A, Bourdeau I, Lampron A, Mazzuco TL, Tremblay J, Hamet P. Aberrant G-protein coupled receptor expression in relation to adrenocortical overfunction. *Clin Endocrinol (Oxf)*. 2010;73(1):1-15. https://doi.org/10.1111/j.1365-2265.2009.03689.x
11. Cavalcante IP, Berthon A, Fragoso MC, *et al.* Primary bilateral macronodular adrenal hyperplasia: definitely a genetic disease. *Nat Rev Endocrinol*. 2022;18(11):699-711. https://doi.org/10.1038/s41574-022-00718-y
12. Vaczlavik A, Bouys L, Violon F, *et al.* KDM1A Inactivation causes hereditary food-dependent Cushing syndrome. *Genet Med*. 2022;24(2):374-383. https://doi.org/10.1016/j.gim.2021.09.018
13. Chasseloup F, Bourdeau I, Tabarin A, *et al.* Loss of KDM1A in GIP-dependent primary bilateral macronodular adrenal hyperplasia with Cushing's syndrome: a multicentre, retrospective, cohort study. *Lancet Diabetes Endocrinol*. 2021;9(12):813-824. https://doi.org/10.1016/S2213-8587(21)00236-9
14. Fragoso MC, Alencar GA, Lerario AM, *et al.* Genetics of primary macronodular adrenal hyperplasia. *J Endocrinol*. 2015;224(1):R31-R43. https://doi.org/10.1530/JOE-14-0568
15. Araujo-Castro M, Marazuela M. Cushing's syndrome due to bilateral adrenal cortical disease: bilateral macronodular adrenal cortical disease and bilateral micronodular adrenal cortical disease. *Front Endocrinol (Lausanne)*. 2022;13:913253. https://doi.org/10.3389/fendo.2022.913253
16. Mariani BMP, Nishi MY, Wanichi IQ, *et al.* Allelic variants of ARMC5 in patients with adrenal incidentalomas and in patients with Cushing's syndrome associated with bilateral adrenal nodules. *Front Endocrinol (Lausanne)*. 2020;11:36. https://doi.org/10.3389/fendo.2020.00036
17. Zilbermint M, Xekouki P, Faucz FR, *et al.* Primary aldosteronism and ARMC5 variants. *J Clin Endocrinol Metab*. 2015;100(6):E900-E909. https://doi.org/10.1210/JC.2014-4167
18. Assié G, Libé R, Espiard S, *et al.* ARMC5 mutations in macronodular adrenal hyperplasia with Cushing's syndrome. *N Engl J Med*. 2013;369(22):2105-2114. https://doi.org/10.1056/NEJMoa1304603
19. Espiard S, Ragazzon B, Bertherat J. Protein kinase A alterations in adrenocortical tumors. *Horm Metab Res*. 2014;46(12):869-875. https://doi.org/10.1055/S-0034-1385908
20. Gagliardi L, Schreiber AW, Hahn CN, *et al.* ARMC5 mutations are common in familial bilateral macronodular adrenal hyperplasia. *J Clin Endocrinol Metab*. 2014;99(9):E1784-E1792. https://doi.org/10.1210/jc.2014-1265
21. Albiger NM, Regazzo D, Rubin B, *et al.* A multicenter experience on the prevalence of ARMC5 mutations in patients with primary bilateral macronodular adrenal hyperplasia: from genetic characterization to clinical phenotype. *Endocrine*. 2017;55(3):959-968. https://doi.org/10.1007/s12020-016-0956-z
22. Elbelt U, Trovato A, Kloth M, *et al.* Molecular and clinical evidence for an ARMC5 tumor syndrome: concurrent inactivating germline and somatic mutations are associated with both primary macronodular adrenal hyperplasia and meningioma. *J Clin Endocrinol*

*Metab*. 2015;100(1):E119-E128. https://doi.org/10.1210/jc.2014-2648
23. Berthon A, Faucz F, Bertherat J, Stratakis CA. Analysis of ARMC5 expression in human tissues. *Mol Cell Endocrinol*. 2017;441:140-145. https://doi.org/10.1016/J.MCE.2016.08.018
24. Damjanovic SS, Antic JA, Elezovic-Kovacevic VI, *et al*. ARMC5 alterations in patients with sporadic neuroendocrine tumors and multiple endocrine neoplasia type 1 (*MEN1*). *J Clin Endocrinol Metab*. 2020;105(12):E4531-E4542. https://doi.org/10.1210/clinem/dgaa631
25. Cavalcante IP, Rizk-Rabin M, Ribes C, *et al*. Tumor suppressor gene ARMC5 controls adrenal redox state through NRF1 turnover. *Endocr Relat Cancer*. 2022;29(11):615-624. https://doi.org/10.1530/ERC-22-0099
26. Williams B, Mancia G, Spiering W, *et al*.. 2018 ESC/ESH guidelines for the management of arterial hypertension. *Eur Heart J*. 2018;39(33):3021-3104. https://doi.org/10.1093/EURHEARTJ/EHY339
27. American Diabetes Association. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes-2021. *Diabetes Care*. 2021;44(Suppl 1):S15-S33. https://doi.org/10.2337/dc21-S002
28. Cleeman JI. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III). *J Am Med Assoc*. 2001;285(19):2486-2497. https://doi.org/10.1001/jama.285.19.2486
29. Kanis JA, Melton LJ, Christiansen C, Johnston CC, Khaltaev N. The diagnosis of osteoporosis. *J Bone Miner Res*. 1994;9(8):1137-1141. https://doi.org/10.1002/JBMR.5650090802
30. Bengoa-Rojano N, Fernández-Argüeso M, Botella-Carretero JI, Pascual-Corrales E, Araujo-Castro M. Prevalence and phenotype of primary bilateral macronodular adrenal hyperplasia with autonomous cortisol secretion: a study of 98 patients. *Revista Clínica Española (English Edition)*. 2022;222(8):458-467. https://doi.org/10.1016/j.rceng.2022.01.003
31. Russ G, Bonnema SJ, Erdogan MF, Durante C, Ngu R, Leenhardt L. European Thyroid Association guidelines for ultrasound malignancy risk stratification of thyroid nodules in adults: the EU-TIRADS. *Eur Thyroid J*. 2017;6(5):225-237. https://doi.org/10.1159/000478927
32. Eastell R, Arnold A, Brandi ML, *et al*. Diagnosis of asymptomatic primary hyperparathyroidism: proceedings of the third international workshop. *J Clin Endocrinol Metab*. 2009;94(2):340-350. https://doi.org/10.1210/jc.2008-1758
33. Jin P, Janjua MU, Zhang Q, Dong CS, Yang Y, Mo ZH. Extensive ARMC5 genetic variance in primary bilateral macronodular adrenal hyperplasia that started with exophthalmos: a case report. *J Med Case Rep*. 2018;12(1):13. https://doi.org/10.1186/s13256-017-1529-3
34. Vena W, Morelli V, Carrabba M, *et al*. Case report: a novel ARMC5 germline mutation in a patient with primary bilateral macronodular adrenal hyperplasia and hypogammaglobulinemia. *Front Genet*. 2022;13:834067. https://doi.org/10.3389/fgene.2022.834067
35. Bouys L, Vaczlavik A, Jouinot A, *et al*. Identification of predictive criteria for pathogenic variants of primary bilateral macronodular adrenal hyperplasia (PBMAH) gene ARMC5 in 352 unselected patients. *Eur J Endocrinol*. 2022;187(1):123-134. https://doi.org/10.1530/eje-21-1032