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

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Context: Primary macronodular adrenal hyperplasia (PMAH) is a rare cause of Cushing’s syndrome, which may present in the context of different familial multitumor syndromes. Heterozygous inactivating germline mutations of armadillo repeat containing 5 (ARMC5) have very recently been described as cause for sporadic PMAH. Whether this genetic condition also causes familial PMAH in association with other neoplasias is unclear.

Objective: The aim of the present study was to delineate the molecular cause in a large family with PMAH and other neoplasias.

Patients and Methods: Whole-genome sequencing and comprehensive clinical and biochemical phenotyping was performed in members of a PMAH affected family. Nodules derived from adrenal surgery and pancreatic and meningeal tumor tissue were analyzed for accompanying somatic mutations in the identified target genes.

Results: PMAH presenting either as overt or subclinical Cushing’s syndrome was accompanied by a heterozygous germline mutation in ARMC5 (p.A110fs*9) located on chromosome 16. Analysis of tumor tissue showed different somatic ARMC5 mutations in adrenal nodules supporting a second hit hypothesis with inactivation of a tumor suppressor gene. A damaging somatic ARMC5 mutation was also found in a concomitant meningioma (p.R502fs) but not in a pancreatic tumor, suggesting biallelic inactivation of ARMC5 as causal also for the intracranial meningioma.

Conclusions: Our analysis further confirms inherited inactivating ARMC5 mutations as a cause of familial PMAH and suggests an additional role for the development of concomitant intracranial meningiomas. (J Clin Endocrinol Metab 100: E119–E128, 2015)
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drenocorticotropic-independent macronodular adrenal hyperplasia (AIMAH) is a rare cause (<2%) of endogenous Cushing’s syndrome (CS). It is characterized by massive bilateral adrenal enlargement with hypersecretion of cortisol and consecutive suppression of ACTH release from the pituitary gland, resulting in low plasma levels of ACTH (1, 2). However, the prevalence of AIMAH might be underestimated because of mild disease and the challenge of diagnosing patients with subclinical CS (3).

In rare cases AIMAH occurs in infancy associated with the McCune-Albright syndrome due to an activating mutation in the Gsα-(stimulatory G protein α subunit) gene leading to an activation of the cAMP signaling pathway (4–6). In earlier adulthood, AIMAH may be associated with multiple endocrine neoplasia type 1 (7–9), familial adenomatous polyposis (9–11), hereditary leiomyomatosis, or renal cancer syndrome (fumarate hydratase gene mutation) (12). In addition, activating somatic mutations in the Gsα gene in female adults with CS due to AIMAH without features of the McCune-Albright syndrome were first described by Fragoso et al (13). However, most patients are diagnosed in their fifth to seventh decade (with subtle signs of CS preceding the diagnosis by several years), and in these patients AIMAH is not part of an established multiple tumor syndrome (14). Although most cases of AIMAH in later adulthood appear to be sporadic familial clustering has been reported (15–21).

Increased cortisol secretion of hyperplastic adrenal glands in AIMAH often involves stimulation of ectopic membrane receptors (22, 23). These primarily aberrant G protein-coupled receptors showing hyperactivity or paradoxical stimulation include ectopic receptors for glucose-dependent insulinotropic peptide (22, 23), catecholamines (24), LH/human chorionic gonadotrophin (25), and IL-1 via type I IL-1 receptors (26) as well as eutopic receptors for vasopressin type 1a (27), serotonin type 4 (25, 28), and possibly leptin (29). Very recently a paracrine regulation of cortisol secretion in macronodular adrenal hyperplasia tissue was described with the release of ectopic adrenal ACTH triggered by ligands of aberrant membrane receptors (30). Thus, Lacroix (31) judged the term, ACTH-independent macronodular adrenal hyperplasia, to be no longer appropriate. Therefore, this term will be replaced here by the term, primary macronodular adrenal hyperplasia (PMAH), as suggested by Alencar et al (32).

In addition, with increasing awareness of familial clustering genetic defects associated with PMAH were found in the cAMP signaling pathway with increased levels of cAMP (33–35). Recently a first mutation underlying familial PMAH has been reported (21). By using whole-exome sequencing of tumor tissue DNA, a mutation of the endothelin receptor type A (EDNRA) gene was identified in two members of a Chinese family affected by PMAH and in one patient with sporadic PMAH (21); however, functional assays proving a causative role of the EDNRA variant in the pathogenesis of PMAH are lacking.

To further elucidate the pathophysiology of PMAH, we analyzed the whole genome in 16 members of a family with PMAH aiming to identify the underlying pathogenic germline mutation. While undertaking this research, heterozygous germline mutations in the ARMC5 gene locus at 16p11.2, resulting in decreased ARMC5 protein levels, were described in 55% of a series of 33 patients with PMAH, mostly sporadic cases (36). Analysis of adrenal nodules of adrenalectomized patients showed additional nodule-specific somatic ARMC5 mutations or loss of heterozygosity (LOH) as a second hit in all cases resulting in biallelic inactivation of ARMC5 (36). Follow-up studies confirmed ARMC5 germline mutations in the context of PMAH with CS (37), and the simultaneous occurrence of germline and somatic ARMC5 mutations in a large Brazilian family further substantiated the role of this putative tumor suppressor gene for the pathogenesis of PMAH (32). Interestingly, the occurrence of intracranial meningiomas together with PMAH was described in three of seven members of the Brazilian family (32), suggesting a possible role of ARMC5 for the development of further neoplasias. However, this has never been tested directly. Here we had the opportunity to also include two nonadrenal tumors in our molecular analyses.

Patients and Methods

Case vignette

A 34-year-old female patient (F1 VII, 153 cm, 80 kg, body mass index 34.2 kg/m²) was admitted to a psychiatric clinic after the delivery of a healthy girl. She presented with postpartum depression, severe back pain, and poor wound healing. Clinical signs of CS were truncal obesity, moon-like face, facial acne, and broad purple striae. A magnetic resonance imaging scan of the lumbar spine showed recent osteoporotic fractures (vertebral bodies of T11, L2, and L3). The patient was admitted to the endocrine clinic for suspected CS. Laboratory examination showed hypokalemia (potassium 3.2 mmol/L, reference range 3.4–5.2 mmol/L), mild leukocytosis (white blood cell count 12.3/nL, reference range 4.5–11.0/nL), mild thrombocytosis (platelet count 464/nL, reference range 150–400/nL), undetectable plasma ACTH (<5 pg/mL, reference range <46 pg/mL), and an insufficient suppression of serum cortisol (337 nmol/L, reference range <55 nmol/L) after a 2-mg overnight dexamethasone suppression test. In addition, 24-hour urinary free cortisol excretion was increased to 576 nmol per 24 hours (reference range 11.8–485.6 nmol per 24 hours), and salivary cortisol levels showed a loss of diurnal variation with 16.8 nmol/L at 12:00 AM (reference range 2.2–15.7 nmol/L), 15.7 nmol/L at 6:00 PM.
received replacement therapy with hydrocortisone and fludrocortisone and her health improved markedly.

Importantly, a detailed family history indicated further CS cases within the patient’s family. The mother of the patient (P I) had undergone sequential bilateral adrenalectomy due to PMAH and overt CS at the age of 66 years. Furthermore, at the same time our index patient underwent her workup, her older sister (F1 II, 49 y old) was also diagnosed with overt CS due to PMAH and underwent simultaneous bilateral adrenalectomy.

Clinical characterization of the PMAH family

All participants (n = 17) gave written informed consent for clinical evaluation and genetic analysis of tumor and leukocyte DNA [one participant (F2 VII) later withdrew his consent for genetic testing]. Thus, a total of 16 family members were characterized. Clinical phenotyping, whole-genome sequencing (WGS), and genetic analysis of tumor tissue was approved by the Institutional Review Board of the Charité-Universitätsmedizin Berlin (EA1/169/08 and EA1/031/12) and by the Ethics Review Panel of the University of Luxembourg (12–001–12 Schano3). A pedigree chart of the family is given in Figure 2. All adult (>18 y) family members were invited for endocrine evaluation and with only one exception participated in our examination.

A comprehensive history with a special focus on symptoms of CS and neoplasias was obtained and all participants underwent a complete physical examination with a focus on symptoms and signs of CS. Laboratory workup was done in all participants including full blood counts, blood glucose, serum electrolytes, urea, creatinine, liver function tests, and paired serum cortisol, and plasma ACTH. In addition, in all participants a low-dose, overnight, 1-mg dexamethasone suppression test was performed and salivary diurnal cortisol profile was collected with samples at 6:00 AM, 12:00 AM, 6:00 PM, and 12:00 PM (reference ranges are given in Table 1).

Furthermore, 24-hour urine samples were collected for detailed assessment of glucocorticoid production by gas chromatography/mass spectrometry as previously described (38); this included measurement of free cortisol and the total sum of glucocorticoid metabolites (free cortisol, tetrahydrocortisol, 5α-tetrahydrocortisol, α-cortol, β-cortol, tetrahydrocortisone, α-cortolone, and β-cortolone). Additionally, blood was drawn for whole-genome sequencing.

Adrenal imaging was carried out in the first instance employing ultrasound to avoid radiation exposure; only in case of suspected adrenal enlargement subsequent CT scans were performed. Participants suspected to suffer from subclinical CS were invited to be reassessed in follow-up visits.

The diagnosis of ACTH-independent CS was based on a combination of biochemical test results including suppressed plasma ACTH levels (≥10 pg/mL), insufficient suppression of serum cortisol after the administration of 1 mg dexamethasone (≥55 nmol/L), increased 24-hour urinary free cortisol excretion, and altered salivary cortisol diurnal profiles as well as clinical signs of cortisol excess. Family members were classified as overt CS if they had abnormal biochemical test results together with typical clinical signs of CS. Family members with no clinical signs but at least two abnormal test results or with subtle clinical signs (apart from truncal obesity) in combination with at least one abnormal biochemical finding were classified as having subclinical CS.

During follow-up visits, patients were asked whether they had undergone cerebral imaging ever before. In addition, cerebral

Figure 1. Macronodular hyperplasia of the right (panel A) and left adrenal (panel B) on abdominal CT in the index patient (F1 VII).
imaging was offered to the patients with clinical or subclinical CS.

Whole-genome sequencing

DNA from blood leukocytes was obtained from 16 family members including the following: three adrenalectomized patients with confirmed PMAH (P I, F1 II, F1 VII); the five newly diagnosed patients with overt/subclinical CS (F1 I, F1 IV, F1 VIII, F2 IV, F2 IX); and the eight patients without any evidence of overt or subclinical CS (PII, F1 III, F1 VI, F2 V, F2 VI, F2 VIII, F2 XIV, F2 XV). DNA samples were sequenced by Complete Genomics (Complete Genomics Inc) (39). The samples were processed through the Complete Genomics Standard Sequencing Pipeline for WGS, versions 2.2.0.26 and 2.4.0.43 (P II). For a detailed description of WGS, data processing, and in silico analysis of pathogenicity of variants, see Supplemental Materials and Methods and Supplemental Figure 1.

Sanger sequencing

Validation experiments were performed using Sanger sequencing methodology according to modified versions of previously published protocols and primers (36, 40).

Analysis of tumor samples

Tumor samples of the three adrenalectomized participants (P I, F1 II, F1 VII) were studied for somatic mutations within the different adrenal nodules. In addition, tissue of a pancreatic serous microcystic adenoma (F1 II) and an intracranial meningioma [histopathology: World Health Organization (WHO) grade I, meningothelial subtype] (P I) was examined.

DNA extraction from formalin-fixed paraffin embedded tissue samples and targeted sequencing of ARMC5, TOX3 (TOX high mobility group box family member 3), and ITGA8 (integrin, alpha 8) were performed as described in detail in Supplemental Material and Methods. In addition, targeted sequencing of NF2 (neurofibromatosis type 2) was performed for the intracranial meningioma tissue (P I).

Results

Clinical and biochemical characterization of the PMAH family

Three family members (including the index patient) had already been diagnosed with PMAH and had undergone bilateral adrenalectomy with subsequent remission of CS (P I, F1 II, F1 VII). Thus, familial screening for the presence of PMAH was performed in 14 first- and second-degree relatives of our index patient (F1 VII). With the exception of one brother, all siblings of the index patient and their adult children were clinically characterized (Table 1). The clinical and biochemical assessment was carried out a blinded fashion, ie, at the time of phenotyping, we did not have knowledge of the presence of ARMC5 mutations in the participants. The assessment led to the diagnosis of overt CS and bilateral adrenal enlargement in one further family member (F1 I); interestingly, 24-hour free cortisol excretion was documented as normal, whereas total glucocorticoid metabolite excretion was pathologically increased. Five further family members were classified as subclinical CS (F1 IV, F1 VIII, F2 IV, F2 IX, F2 XIV) with two of them showing bilateral adrenal enlargement upon imaging (F1 IV, F2 IX); notably their urinary cortisol and glucocorticoid metabolite excretion was in the normal range. However, one participant (F2 XIV) showed normal hormonal test results at a 12-month follow-up with the exception of an insufficient suppression of cortisol in the low-dose overnight dexamethasone suppression test, which, however, was performed under oral contraception.

PMAH was present in three consecutive generations, affecting both sexes and transmitted by both sexes. Approximately half of the descendants of affected family
members developed PMAH suggesting an autosomal dominant pattern of inheritance.

**Whole-genome sequencing**

Using WGS, a total of 10,646,574 variant positions were identified at which at least one family member had an allele that varied from the reference genome. Of the 10.6 million variant positions, 7.9 million variants remained after strict quality control filtering (Supplemental Figure 1 and Supplemental Table 1). Due to the pedigree structure, we further filtered for dominant inheritance and shared identity by descent regions between the affected individuals, for which 1831 variants could be identified. To narrow down the list, we screened for presumably rare variants (n = 308) with predicted exonic defects (n = 6) and subsequent functional consequence (n = 3) (Supplemental Figure 1 and Supplemental Table 1). Among the variants considered, we found a heterozygous frame shift mutation in ARMC5 at 16p11.2 (A110fs*9). The variant cosegregated with an ITGAX variant (T3341C) and a TOX3 variant (C3707T/C385T) both on chromosome 16 in affected individuals only and not in controls (Supplemental Figure 2 and Table 2). The latter variants were identified as single-nucleotide polymorphism that occur in databases of known variants at low allele frequencies (database single-nucleotide polymorphism build 138 rs201752610 and rs145367964 and frequency catalogued in the Exome Sequencing Project 6500 database: at 0.000096 and 0.0000154 for TOX3 and ITGAX, respectively).

**Analysis of tumor samples**

Next, we assessed adrenal tumor samples of the three adrenalectomized participants (P I, F1 II, F1 VII) in the PMAH-affected family for additional somatic mutations in the genes for ARMC5, TOX3, and ITGAX. We found various somatic mutations and LOHs in ARMC5 (see Table 3). TOX3 variants have been described within the context of breast cancer susceptibility and disease progression (41) and have been reported to affect the cAMP signaling pathway (42). However, we did not find any additional somatic mutations in TOX3 in the adrenal tumor tissue and a careful history of further neoplasias did not indicate an increased incidence of breast cancer in our PMAH family. In addition, no concurrent somatic mutation in adrenal tumor tissue was found in the ITGAX gene. The ARMC5 mutations found in tumor tissue DNA were novel somatic variants (Table 3) with the exception of a frame shift mutation at position 104 of the mature protein (p.A104fs) that had been published previously (36). Among the new mutations presented here, we found three frame shift mutations that were all at very early positions in the gene (p.A55fs, p.S102fs, and p.A106fs), suggesting deleterious effects. Furthermore, we found a LOH status twice in adrenal nodules at p.A110fs*9 and also two novel nonsense mutations. The positions of the germline and somatic variants in ARMC5 are given in Figure 3, Table 3, and Supplemental Table 2.

We screened for additional somatic mutations also in other tumors from affected individuals in our family (pancreatic serous microcystic adenoma, F1 I, and intracranial meningothelial meningioma WHO grade I, P I). In the meningioma we found a somatic frame shift mutation in ARMC5 (p.R50fs) (see Table 3) but no somatic mutation in TOX3 and ITGAX. Because a biallelic loss of NF2 can cause familial occurrence of meningioma (43), we screened the meningioma for NF2 mutations, which we did not find. Moreover, we did not find somatic mutations of either ARMC5, TOX3, or ITGAX in the pancreatic tumor. We have tested the functional impact of all somatic and germline mutations found in ARMC5 with Mutation Taster (http://www.mutationtaster.org) (44). All but one somatic mutation were predicted as disease causing (Supplemental Table 2).

**Discussion**

Here we report a new heterozygous germline ARMC5 variant with a frame shift mutation in the genomic region 16p11.2 (c.323_324insC), leading to the protein variant p.A110fs*9 in affected members of our PMAH family. In addition, different second somatic mutational events or LOHs of the ARMC5 gene were found in macronodular tissue derived from adrenalectomy, supporting a second hit hypothesis of the inactivation of a tumor suppressor gene. Biallelic ARMC5 inactivation by a germline and somatic mutations as a causative factor for PMAH leading to CS was initially reported by Assie et al (36) in a cohort of French patients (18 of 33 PMAH patients) and has recently been confirmed in a US cohort with 15 of 34 PMAH patients displaying a germline ARMC5 mutation (37).

Because familial clustering of PMAH may be underestimated due to subclinical disease (eg, references 15–17), the question arises whether ARMC5 gene mutations are also causative for familial PMAH. In our PMAH-affected family, the germline ARMC5 mutation was identified in all members with confirmed PMAH as well as in members with newly diagnosed overt or subclinical CS in contrast to family members without CS. In the affected subjects who underwent adrenalectomy, the germline mutation was associated with somatic mutations in tumor tissue, supporting the hypothesis that germline mutations in as-
somatic mutations of ARMC5 are indeed causative for familial PMAH occurrence (second hit). Another heterozygous germline variant in the ARMC5 gene (c.1094T>C; p.Leu365Pro) was identified very recently in all 16 PMAH-affected family members (of 47 family members evaluated for the presence of PMAH) in a large Brazilian family (32). In accordance with our findings, analysis of the Brazilian family pedigree suggested an autosomal dominant inheritance pattern (32).

Until now, little is known about the functional consequences of the ARMC5 deletion. Altered transcriptomes of tumors with ARMC5 gene mutations and increased apoptosis after overexpression of ARMC5 in H295R and HeLa cells suggest a tumor-suppressor function of the gene product (36). Alencar et al (32) discuss a potential role of ARMC5 in the canonical Wnt pathway, which plays a well-documented role in adrenal tumorigenesis (45). However, the precise pathomechanism of ARMC5 inactivation for the development of nodular hyperplasia remains to be determined.

In our cohort, screening of the family identified five members suffering from previously not recognized overt (F1 I) or subclinical CS (F1 IV, F1 VIII, F2 IV, F2 IX). However, clinical signs were subtle in most affected patients. The most consistent laboratory abnormalities were an insufficient suppression of cortisol after the low-dose overnight dexamethasone suppression test and an ACTH level of 10 pg/mL or less. Interestingly, the diagnostic util-

### Table 1. Clinical Characteristics and Endocrine Evaluation of the Affected PMAH Family

| Participants, n | Sex, f/m | Age, y | BMI, kg/m² | ACTH, pg/mL (reference range <46)ᵃ | Cortisol After 1 mg of Dexamethasone, nmol/L (regular suppression <55) | Urinary Free Cortisol Excretion, μg per 24 h (reference range 25–116) | Total Urinary Glucocorticoid Metabolites, μg per 24 h (reference range 3294–15 827) |
|----------------|----------|--------|------------|-----------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Adrenalectomized family members with confirmed PMAH | | | | |
| P I | F | 69 | 25.8 | | | | |
| F I | F | 49 | 21.1 | | | | |
| F VII | F | 35 | 35.9 | | | | |
| Family members with newly diagnosed overt or subclinical CS | | | | |
| F I I | M | 50 | 35.3 | N.D. | | | |
| F I IV | M | 45 | 22.8 | <5 | | | |
| 24-month follow-upᵇ | | | 22.8 | <5 | | | |
| F I VII | F | 32 | 36.1 | 10 | | | |
| 12-month follow-upᶜ | | | 40.1 | 11 | | | |
| F II IV | M | 27 | 26.6 | 6 | | | |
| F II IX | M | 23 | 27.1 | 7 | | | |
| 24-month follow-upᵈ | | | 28.4 | 9.6 | | | |
| Family members without CS | | | | |
| P II | M | 73 | 24.3 | N.D. | N.D. | N.D. | N.D. |
| F I III | F | 47 | 24.2 | 13 | 19 | 39 | 6194 |
| F II V | F | 40 | 32.0 | 9 | 22 | 40 | 6955 |
| F II VI | F | 25 | 18.8 | 14 | 41 | 67 | 4195 |
| F II VII | F | 27 | 20.3 | 9 | 44 | 40 | 3049 |
| F II VIII | M | 25 | 29.0 | 6 | 20 | 105 | 14 809 |
| F II XIV | F | 21 | 22.2 | 8 | 29 | 12 | 395 |
| 12-month follow-upᶜ | | | 26.2 | 11 | 75 | 90 | 117 |
| F II XV | F | 19 | 22.9 | 17 | 33 | 49 | 117 |

Abbreviations: F, female; L, left; M, male; R, right.

ᵃ Suppressed was defined as plasma ACTH of 10 pg/mL or less.
ᵇ Reference ranges: 6:00 AM, 5.2–40.3 nmol/L; 12:00 AM, 2.2–15.7 nmol/L; 6:00 PM, 1.9–12.1 nmol/L; and 12:00 PM, 0.8–9.1 nmol/L.
ᶜ For adrenalectomized patients, adrenal weight is given.
ᵈ Evaluation by Praxisgemeinschaft an der Kaisereiche, Berlin, Germany.
ᵉ Different reference range: 12.8–82.5 μg per 24 hours.
ᶠ Low-dose overnight dexamethasone suppression test with 1 mg dexamethasone performed under oral contraceptive treatment.
ᵍ Different reference range: 36.0–137.0 μg per 24 hours.
ity of 24-hour urinary cortisol excretion was far lower, with none of the patients demonstrating increased excretion of free cortisol at initial evaluation, with increased total glucocorticoid metabolite excretion only in the patient with newly diagnosed overt CS (F1 I). These findings are in line with the results of the Brazilian family members (32), who were diagnosed by insufficient suppression of cortisol following overnight dexamethasone and demonstration of adrenal enlargement. In their series 24-hour urinary free (or total) cortisol excretion was above the reference range in only 2 of 14 diagnosed PMAH patients and similarly, late-night salivary cortisol was increased in only 4 of 15 patients with PMAH (32).

Inactivation of ARMC5 has been associated with decreased steroidogenesis and reduced mRNA levels of genes encoding the steroidogenic enzymes cytochrome P450 17A1 (CYP17A1) and cytochrome P450 21A2 (CYP21A2) as well as reduced mRNA levels of the gene encoding adrenal steroidogenic factor 1 (NR5A1) and melanocortin 2 receptor in cell-culture models (36). The reduced cortisol synthesis in an ARMC5 gene inactivated cell-culture model (36) may serve as an explanation for the observation that cortisol excess with increased 24-hour free cortisol excretion is not present in early stages of the disease and occurs only if a sufficiently large adrenal mass is reached in the course of disease progression. This view is supported by the markedly higher

Table 2. Variants With Predicted Impact on Protein Function

| Gene Symbol | Genomic Variant | Cytoband | Transcript/Protein Variant | Function Prediction |
|-------------|-----------------|----------|-----------------------------|---------------------|
| ARMC5       | chr16: 31471168:insC | p11.2 | NM_001105247.1:NM_024742.2:c.323_324insC:p.A110fs*9 | Likely pathogenic |
| TOX3        | chr16: 52497869:G>A | q12.1 | NM_001080430.2:c.370C>T:p.L124F NM_001146188.1:c.385C>T:p.L129F | Damaging |
| ITGAX       | chr16: 31392282:T>C | p11.2 | NM_0008837:3:c.341T>C:p.I114T | Damaging |

Abbreviations: chr, chromosome; ITGAX, Integrin, αX; TOX3, TOX high mobility group box family member 3. Function predictions are either from SIFT (damaging) or Ingenuity (likely pathogenic).
mean adrenal weight of patients with mutated ARMC5 (106 g for both sides) compared with the weight of adrenals from PMAH patients not carrying the ARMC5 mutation (55 g for both sides) (36, 37) and is in line with a mean total adrenal weight of 97 g in our adrenalectomized patients.

Familial screening for ARMC5 gene mutations in 11 supposedly healthy first-degree relatives of seven index patients of the French cohort revealed ARMC5 germline mutation in six and adrenal nodular hyperplasia in five of these subjects (36). These results and the findings from Brazilian (32) and Australian families (46) together with our findings favor early genetic testing of families of PMAH-affected patients with germline ARMC5 mutations because early detection of family members affected by overt or subclinical disease becomes feasible and may avoid clinical complications of CS.

Up to now, PMAH has been suggested to be a benign process (2), and the development of a malignant adrenal tumor has, to the best of our knowledge, not been described so far. However, because ARMC5 is expressed in many organs, a concern of potential proliferative consequences of germline mutations for extraadrenal tissues has been raised (31). We therefore assessed the occurrence of further neoplasias in our PMAH affected family. Further tumors (11 intracranial meningiomas in the mother of our index patient, P I; pancreatic serous microcystic adenoma, F I II; pinealoma, F I IV; intracranial meningioma, F I VII) were found in some affected family members but none in nonaffected members. Analysis of the meningioma (histopathology: WHO grade I, meningothelial subtype) resulted in a somatic ARMC5 variant with a frame shift (p.R502fs), suggesting a role of ARMC5 inactivation in the pathogenesis of this tumor. Intriguingly, intracranial meningiomas have also been described in the PMAH affected Brazilian family (32) and had been reported earlier for two sisters with PMAH with ectopic expression of vasopressin receptors leading to clinical CS (19). Familial occurrence of meningiomas is a well-known feature of the dominantly inherited type 2 neurofibromatosis syndrome caused by predisposing mutations in NF2 (43). NF2 acts as a tumor suppressor and tumorigenesis in such cases had been reported to be caused by a biallelic loss of NF2 (47). However, apart from NF2, data on the genetic basis of familial meningiomas are sparse (48). ARMC5 may represent a novel gene responsible for familial meningiomas for which none of the so-far-identified mutations (48) can be found. Based on our observation patients that carrying an ARMC5 germline mutation should be carefully monitored for other tumor entities to delineate the full spectrum of ARMC5 related neoplasias, as a coincidence of PMAH with other neoplasias (including acromegaly and primary hyperparathyroidism), has been noted before (46).

In conclusion, we were able to identify a pathogenic ARMC5 germline mutation in our PMAH family by using WGS. The genetic analysis of adrenal tumor tissue shows second somatic mutational events or LOHs in the ARMC5 gene, further supporting the second hit hypothesis. Importantly, we describe for the first time an additional so-

### Table 3. Clinical Diagnosis and Genetic Evaluation of the Affected PMAH Family

| Participants, n | Sex, F/M | Age, y | Clinical Diagnosis | ARMC5 Germline Variant | Adrenal Tumor | ARMC5 Somatic Variants | Intracranial Meningioma |
|----------------|---------|------|------------------|------------------------|-------------|--------------------------|-------------------------|
| P I            | F       | 69   | Confirmed CS     | p.A110fs*9 c.323_324insC | p.S362fs c.1084C>T | p.A104fs c.311delC, p.A55fs c.164_165insG | p.E433* c.1297C>T p.R502fs c.1506_1507delCA |
| F I II         | F       | 49   | Confirmed CS     | p.A110fs*9 c.323_324insC | p.A106fs c.305_341del | p.A106fs c.315_316insG |                       |
| F I VII        | F       | 35   | Confirmed CS     | p.A110fs*9 c.323_324insC | p.R502fs c.311delC, p.A55fs c.164_165insG | p.R654* c.1960C>T |                       |

Abbreviations: F, female; M, male.

topathology: WHO grade I, meningothelial subtype resulted in a somatic ARMC5 variant with a frame shift (p.R502fs), suggesting a role of ARMC5 inactivation in the pathogenesis of this tumor. Intriguingly, intracranial meningiomas have also been described in the PMAH affected Brazilian family (32) and had been reported earlier for two sisters with PMAH with ectopic expression of vasopressin receptors leading to clinical CS (19). Familial occurrence of meningiomas is a well-known feature of the dominantly inherited type 2 neurofibromatosis syndrome caused by predisposing mutations in NF2 (43). NF2 acts as a tumor suppressor and tumorigenesis in such cases had been reported to be caused by a biallelic loss of NF2 (47). However, apart from NF2, data on the genetic basis of familial meningiomas are sparse (48). ARMC5 may represent a novel gene responsible for familial meningiomas for which none of the so-far-identified mutations (48) can be found. Based on our observation patients that carrying an ARMC5 germline mutation should be carefully monitored for other tumor entities to delineate the full spectrum of ARMC5 related neoplasias, as a coincidence of PMAH with other neoplasias (including acromegaly and primary hyperparathyroidism), has been noted before (46).

In conclusion, we were able to identify a pathogenic ARMC5 germline mutation in our PMAH family by using WGS. The genetic analysis of adrenal tumor tissue shows second somatic mutational events or LOHs in the ARMC5 gene, further supporting the second hit hypothesis. Importantly, we describe for the first time an additional so-

![Figure 3](https://academic.oup.com/jcem/article-abstract/100/1/E119/2812891/27707/10.1210/jc.2014-2361)

Figure 3. Schematic representation of the ARMC5 protein showing germline (gray) and somatic (red) mutations found in the PMAH family. Ensembl protein identification ENSP00000268314 (UniProt peptide Q96C12, 935 amino acids).
matic ARMC5 mutation in an intracranial meningioma corroborating the association of germline ARMC5 mutations with the occurrence of meningiomas. Whether further neoplasias are involved as part of this putative inherited tumor syndrome remains to be elucidated.

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