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Original Article

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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Abstract

Context: Primary macronodular adrenal hyperplasia (PMAH) is a rare cause of Cushing's syndrome (CS), 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 analysed for accompanying somatic mutations in the identified target genes.

Results: PMAH presenting either as overt or subclinical CS 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.

Adrenocorticotropin-independent macronodular adrenal hyperplasia (AIMAH) is a rare cause (less than 2%) of endogenous Cushing's syndrome (CS). It is characterised 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 due to 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 (MAS) due to an activating mutation in the *Gsα-(stimulatory G protein α subunit)* gene leading to an activation of the cAMP signalling pathway (4-6). In earlier adulthood, AIMAH may be associated with multiple endocrine neoplasia type 1 (MEN 1) (7-9), familial adenomatous polyposis (FAP) (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 MAS were first described by Fragoso et al. (13). However, the majority of patients is diagnosed in their fifth to seventh decade (with subtle signs of CS preceding the diagnosis by several years) and is not part of an established multiple tumor syndrome (14). While 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), luteinizing hormone/human chorionic gonadotrophin (25), and interleukin-1 via type I interleukin-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 signalling 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 analysed the whole genome in 16 members of a family with PMAH aiming to identify the underlying pathogenic germline mutation. Whilst undertaking this research, heterozygous germline mutations in the *armadillo repeat containing 5 (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 adrenalectomised 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 out 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.

Case vignette

A 34-year-old female patient (F1 VII, 153 cm, 80 kg, BMI 34.2 kg/m²) was admitted to a psychiatric clinic after the delivery of a healthy girl. She presented with post partum depression, severe back pain and poor wound healing. Clinical signs of CS were truncal obesity, moon-like face, facial acne, and broad purple striae. An MRI of the lumbar spine showed recent osteoporotic fractures (vertebral bodies of Th11, 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) following a 2 mg overnight dexamethasone suppression test. In addition, 24-h urinary free cortisol excretion was increased to 576 nmol/24h (reference range: 11.8-485.6 nmol/24h) and salivary cortisol levels showed loss of diurnal variation with 16.8 nmol/L at 12 am (reference range: 2.2-15.7 nmol/L), 15.7 nmol/L at 6 pm (reference range: 1.9-12.1 nmol/L), and 5.8 nmol/L at 12 pm (reference range: 0.8-9.1 nmol/L). Computed tomography (CT) scans (Fig. 1) showed bilaterally enlarged adrenal glands with multiple nodules (up to 3.0 cm on the right side) with little and inhomogeneous enhancement following the administration of a contrast agent. Overt CS caused by PMAH was diagnosed.

Screening for aberrant adrenal receptors (2)) showed a 71% increase of cortisol (from 276 to 473 nmol/L) in the posture test, while a standard mixed meal, and sequential administration of GnRH (100 µg) and TRH (200 µg) intravenously as well as glucagon (1 mg) intramuscularly did not induce significant changes in cortisol levels. However, a 323% increase of cortisol (from 363 to 1174 nmol/L) was measured after ACTH administration (250 µg intravenously). The patient underwent simultaneous bilateral adrenalectomy. The size of the left (right) adrenal was 9.2x4.6x3.5 (8.3x4.6x2.2) cm with a weight of 51 (54) g. Histology of the removed adrenals showed diffuse as well as a nodular hyperplasia without hemorrhage or infarction. After bilateral adrenalectomy the patient 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, whilst our index patient underwent her work-up, her older sister (F1 II, 49-year-old) was also diagnosed with overt CS due to PMAH and underwent simultaneous bilateral adrenalectomy.

Patients and Methods

Clinical characterisation 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 characterised. 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 Schnjo3). A pedigree chart of the family is given in Fig. 2. All adult (>18 years) 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 work up 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 am, 12 am, 6 pm, and 12 pm (reference ranges are given in Table 1).

Furthermore, 24-h 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 re-assessed 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 following administration of 1 mg dexamethasone (≥55 nmol/L), increased 24-h 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 imaging was offered to patients with clinical or subclinical CS.

Whole genome sequencing

DNA from blood leukocytes was obtained from 16 family members including the three adrenalectomised patients with confirmed PMAH: P1, 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 (CG) (Complete Genomics Inc., Mountain View, CA, USA) (39). The samples were processed through the CG Standard Sequencing Pipeline for WGS, versions 2.2.0.26 and 2.4.0.43 (PII). For detailed description of WGS, data processing, and in silico analysis of pathogenicity of variants see Supplemental Materials and Methods and Supplemental Fig. 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 adrenalectomised 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 of 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 *ITGAX* (*Integrin, alpha X*) 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 (PI).

Results

Clinical and biochemical characterisation 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 characterised (Table 1). The clinical and biochemical assessment was carried out a blinded fashion, i.e. at the time of phenotyping we did not have knowledge of the presence of *AMRC5* 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-h free cortisol excretion was documented as normal while 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-months 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

Employing 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 millions variant positions, 7.9 millions variants remained after strict quality control filtering (Supplemental Fig. 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 Fig. 1 and Supplemental Table 1). Among the variants considered we found a heterozygous frameshift mutation in *AMRC5* at 16p11.2 (A110fs*9). The variant co-segregated with an *ITGAX* variant (T3341C) and a *TOX3* variant (C370T/C385T) both on chromosome 16 in affected individuals only and not in controls (Supplemental Fig. 2 and Table 2). The latter variants were identified as single nucleotide polymorphism (SNPs) that occur in databases of known variants at low allele frequencies (dbSNP build 138 rs201752610 and rs145367964 and frequency cataloged in Exome Sequencing Project (ESP) 6500 database: at 0.000996 and 0.0000154 for *TOX3* and *ITGAX* respectively).

Analysis of tumor samples

Next, we assessed adrenal tumor samples of the three adrenalectomised 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 signalling 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 frameshift 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 frameshift mutations that were all at very early positions in the gene (p.A55fs, p.S102fs, p.A106fs), suggesting deleterious effects. Furthermore, we found a LOH status twice in adrenal nodules at p.A110fs*9, and two novel nonsense mutations. The positions of the germline and somatic variants in *ARMC5* are given in Fig. 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 II, and intracranial meningothelial meningioma

WHO grade I, P I). In the meningioma we found a somatic frameshift mutation in *ARMC5* (p.R502fs) (see Table 3) but no somatic mutation in *TOX3 and ITGAX*. As 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 MutationTaster (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 frameshift 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 Assié et al. (36) in a cohort of French patients (18 out of 33 PMAH patients) and has recently been confirmed in an US cohort with 15 of 34 PMAH patients displaying a germline *ARMC5* mutation (37).

Since familial clustering of PMAH may be underestimated due to subclinical disease (e.g. 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 that underwent adrenalectomy the germline mutation was associated with somatic mutations in tumor tissue supporting the hypothesis that germline mutations in association with 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 (out 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 following the low-dose overnight dexamethasone suppression test and an ACTH level of ≤10 pg/mL. Interestingly, the diagnostic utility of 24-h 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 (32) who were diagnosed by insufficient suppression of cortisol following overnight dexamethasone and demonstration of adrenal enlargement. In their series 24-h urinary free (or total) cortisol excretion was above the reference range in only two of 14 diagnosed PMAH patients and similarly, late-night salivary cortisol was only increased in 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 (MC2R) 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-h free cortisol excretion is not present in early stages of the disease and only occurs if a sufficiently large adrenal mass is reached in the course of disease progression. This view is supported by the markedly higher mean adrenal weight of patients with mutated *ARMC5* (106 g for both sides) compared to 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 adrenalectomised patients.

Familial screening for *ARMC5* gene mutations in 11 supposed 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, the findings from Brazilian (32) and Australian families (46) together with our findings favour early genetic testing of families of PMAH

affected patients with germline *ARMC5* mutations, as 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, since *ARMC5* is expressed in many organs, a concern of potential proliferative consequences of germline mutations for extra-adrenal tissues has been raised (31). We, therefore, assessed the occurrence of further neoplasias in our PMAH affected family. Further tumors (eleven intracranial meningiomas in the mother of our index patient, P I; pancreatic serous microcystic adenoma, F1 II; pinealoma, F1 IV; intracranial meningioma, F1 VII) were found in some affected family members but none in non-affected members. Analysis of the meningioma (histopathology: WHO grade I, meningothelial subtype) resulted in a somatic *ARMC5* variant with a frameshift (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 is 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 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 somatic *ARMC5* mutation in an intracranial menigioma 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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Figures and Legends

Figure 1. **Macronodular hyperplasia of the right (Panel A) and left adrenal (Panel B) on abdominal CT in the index patient (F1 VII).**

Figure 2. **Pedigree chart of the PMAH affected family.** Squares indicate male family members, circles female family members.

Figure 3. **Schematic representation of the ARMC5 protein showing germline (grey) and somatic (red) mutations found in the PMAH family.** Ensembl protein identification ENSP00000268314 (UniProt peptide Q96C12, 935 aa).

Abbreviations: ARM, Armadillo Repeats; BTB, BTB(POZ) domain