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Germinal defects of pseudohypoxia pathway genes in patients with isolated pituitary adenoma.

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Attention PA, Pas and 3PAs

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Abstract (250)

The "3PAs" syndrome, associating pituitary adenoma (PA) and pheochromocytoma/paraganglioma (PPGL), is sometimes associated with mutations in PPGL-predisposing genes such as *SDHx* or *MAX*. In "3PAs" syndrome, PAs can occur before PPGL suggesting a new gateway into *SDHx/MAX*-related diseases.

Objective: determine the *SDHx/MAX* mutations prevalence in patients with isolated PA and characterize the PA of *SDHx/MAX*-mutated patients.

Design: Genes involved in PAs (*AIP/MEN1/CDKN1B*) or PPGLs (*SDHx/MAX*) were sequenced in patients with isolated PA. Next, we conducted a review of cases of PAs in the setting of "3PAs" syndrome.

Results: 263 patients were recruited. Seven (likely) pathogenic variants were found in *AIP*, 2 in *MEN1*, 2 in *SDHA*, and 1 in *SDHC*. The prevalence of *SDHx* mutations reached 1.1% (3/263). Among the 31 reported patients with PA harbouring *SDHx/MAX* mutations (28 from literature and these 3 cases), 6/31 (19%) developed PA before PPGL, and 8/31 (26%) had isolated PA. The age of onset is older than in *AIP/MEN1*-mutated patients. The PAs were mainly macroprolactinomas and a feature of intracytoplasmic vacuoles can be observed by histological study.

Conclusions: For the first time, we found *SDHx* mutations in patients bearing PA with no family or personal history of PPGL. For the moment, data are missing to determine the benefit of *SDHx/MAX* genetic screening in these patients. Meanwhile we recommend that patients with isolated PA must be carefully examined on family history of PPGLs. A family history of PPGL, as well as the presence of intracytoplasmic vacuoles in PA, requires *SDHx/MAX* genetic testing of patients.

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INTRODUCTION

Although Pituitary adenomas (PAs) are benign tumors, they could be responsible for clinical features due to hormonal disturbances and compression symptoms that are secondary to local invasion and that can lead to hypopituitarism. The reported prevalence of symptomatic PAs is up to 1 in 1000 (1). PAs are most frequently sporadic diseases, but are inherited in approximately 5 cases in 100. In these cases, PAs can be isolated as in familial isolated pituitary adenomas (FIPAs) due to *AIP* mutation (AIP; OMIM 605555); or occur in syndromic association such as 1) multiple endocrine neoplasia 1 (MEN1; OMIM 131100), predisposing patients mainly to primary hyperparathyroidism, endocrine duodeno-pancreatic tumors, and PA; and more rarely 2) multiple endocrine neoplasia 4 (MEN4; OMIM 610755), which represents a MEN1-like syndrome; or 3) Carney complex (CNC; OMIM 160980) with cutaneous manifestations, acromegaly, Cushing syndrome, myxoma and schwannoma (2). In syndromic forms or familial cases, patients can benefit from genetic screening to propose specific monitoring and genetic counselling.

The association between PA and pheochomocytoma/paraganglioma (PPGL) was first described by Iversen in 1952 (3). This association can occur during *MEN1* or independently (4, 5, 6, 7, 8). This condition, called "3PAs" syndrome (for pituitary adenoma/ pheochromocytoma/ paraganglioma association) by Xekouki can be described as the co-occurrence of PA and PPGL without other features of MEN1 syndrome (4, 5, 9). This association is rare, with less than 100 cases published in 2019. The "3PAs" syndrome can be associated with germline mutations in genes responsible for predisposition to PPGL as genes encoding for SDH subunits or *MAX* (5, 6, 7, 8).

The objective of this study is to assess the involvement of the main PPGL-predisposed genes in patients with isolated PA and to study the PA characteristics of patients with *SDHx/MAX* mutations. For this purpose, (1) we determined the prevalence of germline mutations in *MEN1*, *CDKN1B*, and *AIP*, and in *SDHA*, *SDHB*, *SDHC*, *SDHD*, *SDHAF2* (herein called *SDHx* genes) and *MAX* genes in a large series of patients for which genetic testing was performed for sporadic or familial isolated PA. (2) We reviewed the literature for published cases of PA in the setting of "3PAs" syndrome to determine if patients with PA and genetic mutation in PPGL-predisposed genes have phenotypic singularities compared to

patients with *AIP*, *MEN1*, *PRKAR1A*, or *CDKN1B* mutation and those with non-genetically determined PA.

PATIENTS AND METHODS

Subjects

All patients who underwent genetic testing in the context of an isolated PA without other endocrine lesions at the molecular laboratory of Marseille Conception Hospital between November 2016 and December 2018 were included. Written informed consent of all patients for genetics analysis was obtained during one-on-one genetic counselling. The ethics committee of Aix-Marseille University approved this study (approval number: 2018-13-12-004).

Next-generation sequencing (NGS)

Genomic DNA was extracted with a QiaSymphony DS DNA Midi Kit (Qiagen, Courtaboeuf, France) from blood lymphocytes (standard EDTA samples). Exons and 20 bp flanking introns of *AIP* (NM_003977.2), *MEN1* (NM_130799.2), *CDKN1B* (NM_004064), *SDHA* (NM_004168.2), *SDHB* (NM_003000.2), *SDHC (NM_003001.3)*, *SDHD* (NM_003002.2), *SDHAF2* (NM-017841.1), and *MAX* (NM_002382.3) were sequenced by next-generation sequencing (NGS) using the QiaSeq library (Qiargen, Courtaboeuf, France) according to the manufacturer's instructions. Libraries were sequenced on MiSeqDX (Illumina). The alignment and variant calling were performed using the Biomedical Genomics Workbench 5.0.1 (Qiagen). Annotation was done using VariantStudio v2.2 (Illumina), according to the HGVS guidelines (10).

Each variant was classified according to the guidelines of the American College of Medical Genetics and Genomics (ACMG) in one of the five following classes (11):

Class 1: benign variant (BV)

Class 2: likely benign variant (LBV)

Class 3: variant of uncertain significance (VUS)

Class 4: likely pathogenic variant (LPV)

Class 5: pathogenic variant (PV)

In silico predictions were performed using Alamut Visual software (Interactive Biosoftware, Rouen, France), including the conservation level, SIFT, PolyPhen-2, and the study of the splicing impact. The population data from population database (gnomAD database, https://gnomad.broadinstitute.org/ last visit november 2019) and from inherited disease

databases: ClinVar, LOVD, and HGMD were collected. The variants with a frequency above 5% in the population were not retained. All PVs and LPVs were confirmed by Sanger sequencing (the primers and protocols are available upon request).

Explorations of *SDHx* mutations in pituitary adenomas

To specify the role of the *SDHx* germinal mutation in the PA, both a SDH immunohistochemistry (IHC) and a research of Loss of heterozygosity (LOH) were done on the formalin-fixed paraffin-embedded (FFPE) slide achieved throughout surgical removal of the pituitary adenoma, if available.

SDH IHC analysis

The investigation of the loss of protein SDH expression in neoplastic cells was performed using commercially available polyclonal antibody against SDHB (Sigma Aldrich, reference HPA002868, dilution of 1 in 150). If any component of the SDH complex was damaged, then the entire SDH complex became unstable, releasing the SDHB subunit into the cytoplasm where it degraded rapidly (12). The staining protocol (XT UltraView DAB v3, Benchmark IHC/ISH module) included pre-treatment with cell conditioner 1, incubation with antibody at 37 °C, and incubation with Prep Kit 517 solution for 32 minutes, followed by counterstaining with haematoxylin for 8 minutes.

Sanger sequencing for research of LOH in tumors

DNA was extracted from samples using a QIAamp DNA FFPE tissue kit (Qiagen). Using the AmpliTaq Gold 360 Master Mix (ThermoFisher Scientific, Waltham, MA, USA), DNA was amplified by PCR targeting exon 5 of *SDHC* or exon 13 of *SDHA* (primers available upon request). After purification the PCR products were sequenced using the Sanger method on a AB3500XLDX (ThermoFisher Scientific).

Comparison of patients with "3PAs" syndrome and "non-3PAs" syndrome based on the literature data.

The characteristics of patients presented with PA and *SDHx*/*MAX* (likely)pathogenic variant were compared to patients with *AIP, MEN1, PRKAR1A, CDKN1B*-related PA and to patients with non-genetically determined PA. The patients with non-genetically determined PA came from the cohort reported by Daly et al. from a Belgian population (1). The *AIP* cases

corresponded to 64 published cases with their phenotype (a list of references is available upon request). The *MEN1* cases were extracted from the UMD-MEN1 Database (13), the Carney complex cases were from a literature review published by Cuny et al. (14), and the MEN4 cases were from reviews conducted by Alrezk et al. and Fredericksen et al. (15, 16).

Statistical analyses

Statistical analyses were performed using Prism v6.0 (GraphPad Software, La Jolla, CA, USA). The patients' characteristics were compared using the two-tailed Fisher's exact test for the qualitative variables and the non-paired non-parametric Mann-Whitney test for the quantitative values.

RESULTS

A total of 263 patients were included (Table 1 and Supplemental Table 1). The mean age at PA diagnosis was 29.3 years (8-78), and the mean age at genetic screening was 36.1 years (8-79). The occurrence of PA was sporadic in 227 patients (86.3%), while 36 patients presented with a familial history of PA (13.7%). By NGS sequencing, we found 295 variants, among which 7 variants were classified as pathogenic, 5 as likely pathogenic, and 7 as VUS (Table 1, Figure 1, Supplemental Table2, and Supplemental Table 3). Five PV and LPV were found in patients with a familial history of PA out of 36, and 7 in patients with sporadic PA out of 227. The odds ratio of harbouring a (likely)pathogenic variant in cases of family history of PA against no history is then 5.069 (95% CI: 1.69 to 15.79, p=0.014). Among the sporadic cases, 5 mutations occurred in patients younger than 30 years (5/133), and 2 occurred in patients older than 30 years. Among the 12 PVs and LPVs, we found 7 variants in *AIP*, 2 in *MEN1*, 2 in *SDHA*, and 1 in *SDHC* (Figure 1, and Supplemental Table 2). The medical histories of the 3 *SDHx*-mutated patients are described as follows and in the Table 2.

Case presentation

Case 1: A previously healthy male patient aged 17 presented with a recent history of severe right visual loss, clinically objectified on the Monoyer scale (right eye: 4/10, left eye: 10/10). The MRI revealed a large (>10 mm) pituitary mass, and his prolactin level was measured at 91 µg/L (reference range: 4.1-15.34 µg/L). The patient underwent a transsphenoidal surgery debulking. The post-operative examination was without complications and demonstrated a marked improvement in vision. The post-operative prolactin secretion was measured at

104µg/L. Cabergoline was initiated at a dose of 0.5 mg weekly; allowing prolactin normalisation (3µg/L at 3 months).

A *SDHC*: c.405+1G>T, p.(?) heterozygous pathogenic variant was demonstrated. This variant was present in population database at a weak allele frequency (Minor Allele Frequency – MAF <0.001%). It was reported as causing the deletion of exon 5 and a shift in the reading frame (17). The SDH IHC analysis of PA was positive (Table 2). The search for LOH in the tumor was negative. A whole body CT and investigation of serum and urinary catecholamine did not show signs of PPGL. The *SDHC* variant was absent in his mother.

Case 2: A male patient aged 42, with no personal and family history of endocrine disease, consulted for an 18-month history of erection disorder and decreased libido. The primary hormonal assessment found a low testosterone rate (0.64 ng/mL) and a pituitary profile with FSH at 1.3 IU/L, LH at 2.7 IU/L and prolactin at 84µg/L supporting a central origin. The cranial MRI showed a pituitary macroadenoma with a moderate suprasellar extension but no visual pathway compression. In this clinical context, cabergoline at a dose of 0.5 mg weekly was initiated with a good medical and biological response. The analysis of *SDHA* revealed a heterozygous frameshift variant on exon 6 of the gene (c.757_758del, p.(Val253Cys*67)), resulting in a premature stop codon. This variant was absent from gnomAD. So it was classified as likely pathogenic.

Case 3: This male was diagnosed with isolated microprolactinoma at 37-year-old with a notable family history of PA with macroprolactinoma in his family but without any PPGL history. His brother died during the surgery of a massive pituitary adenoma. Prolactin secretion was well controlled under cabergoline (PRL <10 µg/L), but secondary to side effects a change to bromocriptine was made. Subsequently, prolactin levels were poorly controlled due to poor adherence to therapy (16.6 to 40 µg/L). A neurosurgical removal was performed. The analysis of PPGL genes found a heterozygous missense variant in exon 13 of *SDHA* (c.1753C>T, p.(Arg585Trp)). This variant is present in population database (gnomAD MAF: 0.0025%), but the *in silico* analysis predicted a deleterious impact and studies reported this variant as pathogenic (18). So, the variant was classified as likely pathogenic. The SDH IHC analysis was positive (Table 1), and the search for LOH by Sanger sequencing was negative. Family members were not available to conduct a genetic family survey.

Literature review and phenotypic features in "3PAs" syndrome

After reclassification of the variants using ACMG criteria (11) and excluding patients with VUS or (likely)benign variants, we found 23 published cases of "3PAs" syndrome with *SDHx* or *MAX* (likely)pathogenic variants (Table 3) (4, 5, 6, 7, 8, 19, 20, 21, 22, 23, 24, 25, 26, 27). Among the 23 cases, 19 patients had *SDHx* (likely) pathogenic variants: 2 in *SDHA*, 9 in *SDHB* (39%, 9/23), 2 in *SDHC*, and 6 in *SDHD* (26%, 6/23); 4 patients had *MAX* pathogenic variants. PA occurred before PPGL in 6 cases (6/23, 26%). Moreover, we also found 5 published cases with an isolated PA and a mutation in *SDHx* (1 in *SDHA* and 4 in *SDHB*), all of them had a family history of PPGLs (Table 4) (5, 28, 29, 30).

Overall, the *SDHx/MAX* patients with PA included those harbouring PPGL (n=23, Table3), those with a family history of PPGL (n=6, Table 4), those without PPGL context (n=3 from this study). Among these 31 *SDHx/MAX* patients, 4 had a family history of PA (Table 3 and our case 3), among them only one without PPGL history (our case 3). They included 16 females and 14 males (sex ratio: 1.1/1, the sex is not specified for one patient). The diagnosis of PA was on average at 42.4 years old (range: 16-72). There were 23 macroPAs, representing 74% of cases, and 4 microPAs (13%). The 2 most frequent types were prolactinoma (19/31, 61%) and GH-secreting adenoma (5/31, 16%) (Table 5).

Then, we compared these *SDHx/MAX* patients with those from published cases of genetically determined PAs (i.e. due to mutations in *AIP, MEN1, CDKN1B* or *PRKAR1A)* and non-genetically determined PAs. The age of occurrence of PAs in the *SDHx/MAX* patients (mean 42.4 years, range: 16 to 72) was older than in the *AIP* patients (mean 25.9 years, range: 10-60, $p<0.001$), in the *MEN1* patients (mean 34.2 years, range: 7-82, $p=0.024$), and in the *PRKAR1A* patients (mean 31 years, range: 16-55, $p=0.007$) (Figure 2 and Table 5).

The proportion of prolactinomas was identical in the *SDHx/MAX* patients and in the control population from the cohort published by Daly et al. (61% versus 66%) (Table 5) (1). However, the PAs of the 31 *SDHx/MAX* patients were larger (23/31 macroadenomas versus 29/68 in control population $p=0.002$). In fact, macroprolactinoma was more frequent in *SDHx/MAX* population (15/31 versus 11/68 $p=0.0013$). The *SDHx/MAX* patients also presented a disposition to have older age at PA diagnosis than in the control population (42.4 years versus 34.5 years) but not statistically significant ($p=0.08$).

DISCUSSION

Even if mutations in *MEN1*, *AIP*, *CDKN1B*, and *PRKAR1A* genes are identified as causal factors in family PA, in most cases, no genetic cause was found. For example, *AIP*

mutations are found in only 20% of FIPA cases (31). In France, according to the TENGEN guidelines (Oncogenetic Network in Neuroendocrine Tumors), mutations in *AIP*, *MEN1*, and *CDKN1B* genes are investigated in patients with PA with (i) family presentation, (ii) syndromic association, or (iii) isolated and sporadic pituitary macroadenoma occurred before age 30. *PRKAR1A* is investigated only in patients with typical syndromic association. Of note, in our cohort, several patients aged of more than 30 years with sporadic and isolated PA had genetic testing on express request of the endocrinologist for notably aggressive or drug-resistant PAs, which are features of *MEN1*- and *AIP*-related PAs. In our cohort, the prevalence of mutations in *AIP* and *MEN1* was consistent with prevalence reported in literature in patients with PAs with similar inclusion criteria (32, 33, 34, 35, 36, 37).

Recently, a novel syndromic association called "3PAs", and involving PAs and PPGLs was described, sometimes associated with germline mutations in *SDHx/MAX* genes (4, 5, 6, 8, 20). A literature review about *SDHx/MAX*-mutated patients with "3PAs" syndrome found 6 patients in which the PAs were prevalent to the PPGLs, and 5 patients having an isolated PA (Tables 3 and 4), suggesting a new gateway into *SDHx/MAX*-related diseases. These data raise several issues regarding: (i) the incidence of *SDHx/MAX* mutations in patients with isolated PAs, (ii) the characteristics of patients with isolated PA harbouring these mutations, and consequently (iii) whether *SDHx/MAX* genes might be tested in patients with isolated PA.

Herein, we demonstrated for the first time the presence of *SDHx/MAX* germline mutations in patients with isolated PA without PPGL history. In our study, among 263 patients with isolated PA, we found 3 (likely) pathogenic variants in *SDHx* genes: 2 in sporadic cases of PA, one in a patient with a strong family history of PAs. The mutation prevalence rate was 1.1%. In 2015, Xekouki et al. studied the prevalence of *SDHx* germline mutations in a cohort of 168 patients with PA, including 143 patients with isolated and sporadic PA, 3 patients with sporadic "3PAs" syndrome, and 22 patients with family "3PAs" syndrome (4). Three *SDHx* mutations were identified in patients with family "3PAs" syndrome. No mutation was found in the patients presenting with isolated PA, probably because the cohort included a high proportion of patients with ACTH-secreting PAs (118/168, 70%).

It should be noted that the presence of *SDHx* (likely) pathogenic variants in patients with PA might be due also to a fortuitous association. In a study of Hoekstra et al., nonsense *SDHA* mutations have been found at a frequency of 0.5% in healthy population (38). Moreover, potentially (likely) pathogenic variants in *SDHx/MAX* genes, as loss of function

variants, are registered in gnomAD. However, we did not identify any *SDHx/MAX* (likely)pathogenic variants in a cohort of 239 patients presented with hyperparathyroidism and without a personal or family history of PPGL and PA (personal data). This datum is in favour of a non-random association between *SDHx/MAX* (likely) pathogenic variants and the PAs.

It is also true that the involvement of *SDHx/MAX* genes in PA tumorigenesis remains unclear. We have shown here that the age of onset of PA in the *SDHx/MAX*-mutated patients is higher than that of other tumour suppressor genes and is equivalent to that of controls. The loss of expression of SDH within tumor tissue is not established in all patients as in the case 1 and 3 of our study. In the literature, among the 24 patients with PA and germline mutations in *SDHx*, SDH IHC and LOH testing in tumor samples was conducted for 10, but a loss of staining/LOH was not found in 2, which doesn't support the hypothesis that Knudson's double hit in these cases. Nevertheless, the *Sdhb* +/- murine model is consistent with the involvement of *SDHx* mutation in pituitary tumorigenesis (4). At 12 months old, the *Sdhb* +/- mice developed hyperplastic adenohypophysis, as classically found in *AIP* deficient mice (39) and in human PA due to *AIP*, *PRKAR1A* mutations or *Xq26* microduplication (40, 41, 42). The adenohypophyseal cells of *Sdhb*+/- mice showed several intranuclear abnormalities and strong HIF-α cytoplasmic and nuclear staining consistent with the activation of the pseudohypoxia pathway of *SDHx*-mutated tumors (4, 8, 43, 44, 45). Nevertheless in humans, data on the over-risk of PA in *SDHx/MAX*-mutated patients is required to conclude on the need of pituitary gland monitoring in symptomatic and asymptomatic careers of *SDHx/MAX* mutations.

On the other hand, *SDHx/MAX* genetic testing for patients with PA should be decided also in considering (i) the low penetrance of *SDHx*-related manifestations, (ii) the possible anxiety generated by this information for the patient and his family (iii) the exposure to ionizing radiation related to lifetime monitoring, and (iv) the cost of the clinical follow-up.

Among the 3 variants identified in patients with isolated PA, 2 occurred in *SDHA*, and 1 in *SDHC*. In PPGL population, *SDHx* germline mutations are accounted for approximately 15% of all cases and for the half of the family cases. *SDHB* and *SDHD* mutations are the most common, *SDHA* and *SDHC* mutations are less frequent (46). On a large series of *SDHA*-PPGL, the penetrance was calculated at 10% at 70 years (47), while Benn et al. and Maniam et al., using a Bayesian statistical approach, indicated an overall penetrance of 1.7% (95% CI:

0.8% to 3.8%) and 0.1%-4.9%, respectively (48, 49). In the same study, Benn et al. calculated the *SDHC* penetrance at 8.3% (95% CI: 3.5% to 18.5%) (48). Consequently, the absence of PPGL in our patients with *SDHA* or *SDHC* mutations and their families is not unexpected since the penetrance of *SDHA/SDHC*-related PPGL is low and the age of disease onset is late.

To the current state of our knowledge, it seems obvious that the presence of *SDHx/MAX* (likely) pathogenic variants in patients with isolated PA justifies a screening for PPGLs via careful clinical examination, full body imaging, and the measurement of urinary catecholamine levels (38)**.** However data are missing to determine if the over-risk of PPGL in *SDHx*-mutated family without family history of PPGL is equal to that of family with history of PPGL, and if these patients require the same level of monitoring, especially for *SDHA* asymptomatic career. The monitoring data of patients with *SDHB*, *SDHC*, *SDHD*, and *SDHAF2* mutations as secondary findings in clinical exome and genome sequencing from the ACMG will certainly provide some answers (50). In any case, patients with PA must be carefully examined not only for their family history of PA but also of PPGL, particularly for patients bearing macroprolactinomas. Considering literature data, in case of family history of PPGL, a genetic screening of *SDHx/MAX* is absolutely required for a family member bearing an isolated PA. For patients with isolated PA and any family history of PPGL the benefits of *SDHx/MAX* genetic testing remains to be assessed.

A rare condition requesting *SDHx/MAX* genetic testing in patients with PA seems to be the presence of tumoral intracytoplasmic vacuoles, a particular histological phenotype reported in *SDHx*-related PAs (7, table 3). These vacuoles seem not to be mitochondrial or endoplasmic reticulum parts (5) and should represent autophagic bodies, due to the pseudohypoxia (45, 51, 52). Like in renal carcinoma (46), vacuolisation of the cytoplasm should lead to perform SDHB (+/-SDHA) IHC and *SDHx* genetic analysis.

In conclusion, we found for the first time *SDHx* mutations in patients bearing PA without any family or personal history of PPGL. The prevalence rate of 1.1% is similar to those of *MEN1* in this indication, leading the question whether *SDHx/MAX* systematic genetic screening is required for such patients. Data are missing to determine the benefit of *SDHx/MAX* genetic testing of patients with isolated PA and any family history of PPGL. *Vice-versa*, data on the over-risk of PA is needed to conclude on the monitoring pituitary gland in symptomatic and asymptomatic careers of *SDHx/MAX* mutations. Meanwhile we

recommend a careful examination of patients with isolated PA not only on family history of PAs but also of PPGLs. A family history of PPGL, as well as the presence of intracytoplasmic vacuoles in PA, requires *SDHx/MAX* genetic testing for PA patients.

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Figure 1. Repartition of VUS, LPV, and PV by gene in this study

VUS: variant of unknown significance, LPV: likely pathogenic variant, PV: pathogenic variant.

Figure 2. Comparison of the age of occurrence of PAs in genetic syndromes and controls

Controls: Patients with non-genetically determined PA selected as reference from the patients described by Daly et al. from a Belgian population (10). *AIP* cases: 57 published cases (list available upon request). *MEN1*: MEN1 cases harbouring pathogenic or likely pathogenic variants extracted from the UMD-MEN1 Database (11). *PRKAR1A*: Carney complex published cases listed by Cuny et al.(12). *CDKN1B*: 11 MEN4 cases listed in the reviews by Alrezk et al. and Fredericksen et al. (13-14).

*p<0.05, **p<0.01****p<0.0001.

Table 1. Clinical characteristics of the patients included in this study

F: female, M: male, Yrs: years, NA: not available, PRL: prolactin, GH: growth hormone, ACTH: adrenocorticotropic hormone, NFPA: non-functional pituitary adenoma, LH: luteinising hormone, FSH: follicular-stimulating hormone, TSH: thyroid-stimulating hormone, VUS: variant of uncertain significance, LPV: likely pathogenic variant, PV: pathologic variant, Macroadenoma is defined by a diameter >10 mm, microadenoma is defined by a diameter <10 mm.

Table 2: Characteristics of patients harboring isolated pituitary adenoma and a *SDHx/MAX* likely pathogenic or pathogenic variant in this study, genetics exploration and functional analysis.

Table 3. Patients with "3PA" syndrome with personal PPGL bearing SDHx/MAX mutations in the literature

F: female, M: male, PRL: prolactin, GH: growth hormone, PPGL: pheochromocytoma/paraganglioma, P: pheochromocytoma, PGL: paraganglioma, HNPGL: head and neck paraganglioma, LOH: loss of heterozygosity, IHC: immunohistochemical analysis, MEN1: multiple endocrine neoplasia type 1, pNET: pancreatic neuroendocrine tumor, MTC: medullar thyroid carcinoma, NFPA: non-functional pituitary adenoma, PTC: papillary thyroid carcinoma, GIST: gastro-intestinal stromal tumor, HPTH: hyperparathyroidism, NA: not available, NP: not performed, LPV: likely pathogenic variant, PV: pathologic variant, VUS: variant of uncertain significance. *Classification using ACMG guidelines for classification of sequence variants (11).

Table 4. Patients with "3PA" syndrome with isolated PA and familial PPGL bearing SDHx mutations in the literature.

For Review Only

F: female, M: male, yrs: years, PA: pituitary adenoma, PRL: prolactin, P: pheochromocytoma, PGL: paraganglioma, NFPA: non-functional pituitary adenoma, LOH: loss of heterozygosity, IHC: immunohistochemical analysis, PV: pathologic variant, NA: not available, *Classification using ACMG guidelines for classification of sequence variants (13).

Table 5. Characteristics of patients with pituitary adenoma in genetic and sporadic conditions

NA: not available; M: male; F: female, PA: pituitary adenoma, PRL: prolactinome, macroPRL: macroprolactinoma, GH: somatotropinoma, NFPA: non-functional pituitary adenoma, ACTH: adrenocorticotropic hormone, LH: luteinising hormone, FSH: follicular-stimulating hormone, TSH: thyroid-stimulating hormone. *percentages are calculated from available data, patients whose data are unavailable are excluded.

AIP, *MEN1*, *PRKAR1A*, *CDKN1B* cases and control cases are from published cases with an individual description of the cases. Non-genetically determined PA selected as reference are from the patients described by Daly et al. from a Belgian population (1). The *AIP* cases were 57 published cases (a list of references is available upon request). The *MEN1* cases were extracted from the UMD-MEN1 Database (13), the Carney complex cases were from a literature review published by Cuny et al. (14), and the MEN4 (*CDKN1B*) cases were from reviews conducted by Alrezk et al. and Fredericksen et al. (15, 16).

Supplemental Table 1. Clinical characteristics of the patients included in this study

F: female, M: male, NA: not available, PA: pituitary adenoma, PRL: prolactinoma, ACTH: corticotropinoma, GH: somatotropinoma, NFPA: non-functional pituitary adenoma

Supplemental Table 2. Clinical and genetic characteristics of the patients harbouring pathogenic or likely pathogenic variant in this study.

F: female, M: male, Yrs: years, PRL: prolactin, GH: growth hormone, ACTH: adrenocorticotropic hormone, NFPA: non-functional pituitary adenoma, PA: pituitary adenoma, PV: pathologic variant, LPV: likely pathogenic variant; macroadenoma is defined by a diameter >10 mm, microadenoma is defined by a diameter <10 mm. *Classification using ACMG guidelines for classification of sequence variants (11).

Supplemental Table 3. Clinical and genetic characteristics of the patients with variants of uncertain significance* in this study

F: female, M: male, NFPA: non-functional pituitary adenoma, yrs: years

*Classification using ACMG guidelines for classification of sequence variants (11).

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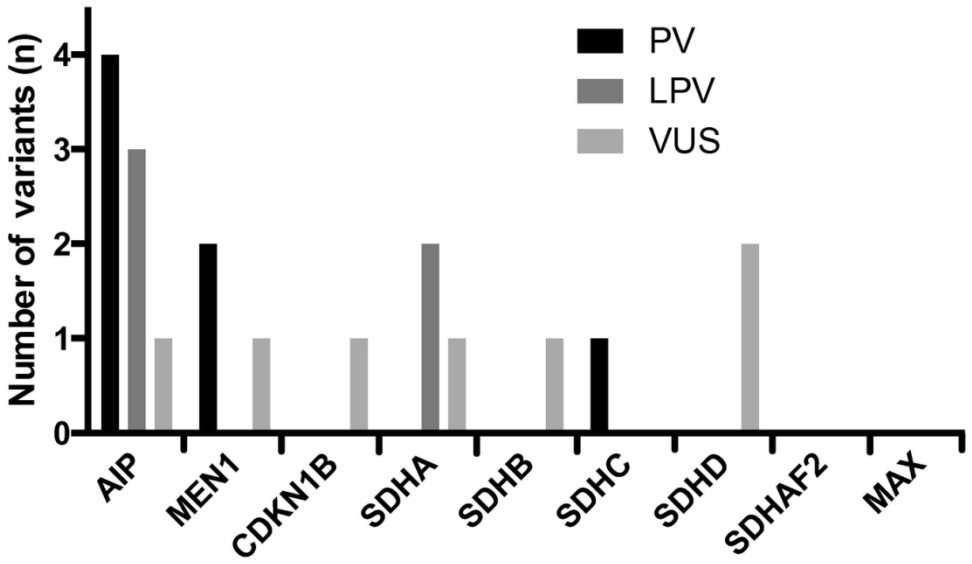


Figure 1. Repartition of VUS, LPV, and PV by gene in this study
VUS: variant of unknown significance, LPV: likely pathogenic variant, PV: pathogenic variant.

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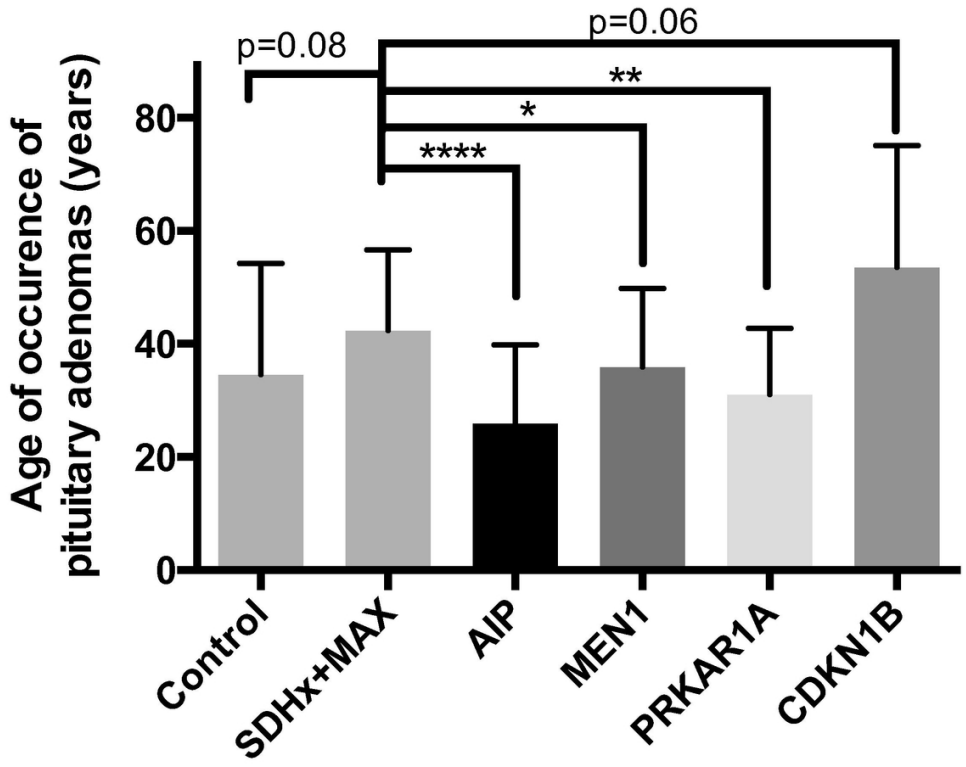


Figure 2. Comparison of the age of occurrence of PAs in genetic syndromes and controls
Controls: Patients with non-genetically determined PA selected as reference from the patients described by Daly et al. from a Belgian population (10). AIP cases: 57 published cases (list available upon request). MEN1: MEN1 cases harbouring pathogenic or likely pathogenic variants extracted from the UMD-MEN1 Database (11). PRKAR1A: Carney complex published cases listed by Cuny et al.(12). CDKN1B: 11 MEN4 cases listed in the reviews by Alrezk et al. and Fredericksen et al. (13-14).
*p<0.05, **p<0.01****p<0.0001.

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Table 1. Clinical characteristics of the patients included in this study

	Sporadic cases		Familial cases	Total
	<30 yrs	>30 yrs		
Number of patients (n=)	133	94	36	263
Age at diagnosis (yrs) mean (range)	22.2 (9-30)	39.4 (31-78)	32.3 (8-77)	29.3 (8-78)
Sex ratio (male/female)	58 M/75 F (0.77)	62 M/32 F (1.9)	17 M/19 F (0,9)	135 M/128 F (1.05)
Size of pituitary adenoma				
Macro adenoma	95	70	22	187 (71.1%)
Micro adenoma	15	4	6	25 (9.5%)
NA	23	20	8	51 (19.4%)
Secretion of pituitary adenoma (n (%))				
PRL	52	20	14	86 (32.7%)
GH	31	36	5	72 (27.4%)
NFPA	8	6	8	22 (8.4%)
ACTH	21	3	2	26 (9.9%)
Mixed	4	8	1	13 (4.2%)
LH/FSH	1	4	0	5 (1.9%)
TSH	1	1	0	2 (0.8%)
NA	15	16	6	37 (14,1%)
Number of variants, all genes (n=)	148	115	32	295
VUS	4	3	0	7
LPV	0	2	3	5
PV	5	0	2	7

F: female, M: male, Yrs: years, NA: not available, PRL: prolactin, GH: growth hormone, ACTH: adrenocorticotropic hormone, NFPA: non-functional pituitary adenoma, LH: luteinising hormone, FSH: follicular-stimulating hormone, TSH: thyroid-stimulating hormone, VUS: variant of uncertain significance, LPV: likely pathogenic variant, PV: pathologic variant, Macroadenoma is defined by a diameter >10 mm, microadenoma is defined by a diameter <10 mm.

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Table 2: Characteristics of patients harboring isolated pituitary adenoma and a *SDHx/MAX* likely pathogenic or pathogenic variant in this study, genetics exploration and functional analysis.

	Case 1	Case 2	Case 3
Sex	M	M	M
Age at PA diagnosis	17	42	37
Size of PA	macro	macro	micro
Secretion of PA	PRL	PRL	PRL
Familial PA	no	no	Father and brother: macroPRL nephew: microPRL
Familial PPGL	no	no	no
PRL at diagnosis (µg/L)	91	84	55
Medical treatment	cabergoline (post operative)	carbergoline	cabergoline, bromocriptine
Surgery	yes	no	yes
Results of AIP, MEN1, CDKN1B genetic testing	normal	normal	normal
Gene	*SDHC*	*SDHA*	*SDHA*
Variant	c.405+1G>T, p.(?)	c.757_758del, p.(Val253Cys*67)	c.1753C>T, p.(Arg585Trp)
Classification$	PV	LPV	LPV
Histopathological examination	yes	no	yes
hormonal status	PRL+		PRL+
ki67	5%		<1%
% of P53-positive cells	10%		<1%
IHC SDH	positive	-	positive
LOH	negative	-	negative

M: Male; PRL: prolactin; PV: pathogenic variant, LPV: likely pathogenic variant; macro: macroadenoma, defined by a diameter >10 mm, micro: micro adenoma, defined by a diameter <10 mm; IHC SDH: immuohistochesmtry SDH, LOH: Loss of heterozygosity
*Classification using ACMG guidelines for classification of sequence variants (11)

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Table 3. Patients with "3PA" syndrome with personal PPGL bearing SDHx/MAX mutations in the literature

Patient no.	Sex	Age at PA diagnosis (yrs)	Size of PA	Secretion of PA	PPGL	Age at PPGL diagnosis	Mutation identified	Class of mutation*	LOH/IHC in PA	Familial endocrine features	References
1	M	49	Micro	PRL	P	32	*MAX* del exon 3	PV	NP	No	Daly et al. 2018
2	F	26	Macro	GH	Bilateral P	35	*MAX* del exons 1-3	PV	NP	No	Daly et al. 2018
3	M	16	Macro	GH	Metastatic bilateral P	22	*MAX* del exon 4	PV	NP	No	Daly et al. 2018
4	F	49	Macro	PRL	bilateral P	49	*MAX* c.296-1G>T, p.(?)	PV	NP	No	Roszko et al. 2017
5	F	35	Macro	NA	HNPGL, mediastinal PGL	38	*SDHB* (no info about the variant)	PV	NP	Brother: PGL and positive for mutation, mother and sister: mutation carriers	Gorospe et al. 2017
6	F	38	Macro	PRL	HNPGL, abdominal PGL	38	*SDHB* del exon 1	PV	NP	Brother: PGL, mother and sister: mutation carriers	Guerrero Perez et al. 2016
7	M	29	Macro	NFPA	P, HNPGL, abdominal	10	*SDHD* c.315-?_480+?del	PV	NP	Father and 2 brothers: mutation	Lemelin et al. 2019

					PGL					carriers	
8	F	27	NA	PRL	P	NA	*SDHA* c.91C>T, p.(Arg31*) *VHL* c.589G>A p.(Asp197Asn)	PV VUS	NP	No	Dénes J et al. 2015
9	F	49	Macro	PRL	Bilateral HNPGL	49	*SDHA* c.91C>T, p.(Arg31*)	PV	p.D38V; somatic mutation as a second hit of biallelic inactivation/SDHA and SDHB IHC negative	NA	Niemeijer et al. 2015
10	F	53	Macro	NFPA	HNPGL	28	*SDHB* c.587G>A, p.(Cys196Tyr)	PV	LOH at SDHB locus/SDHB staining: diffuse/intracytoplasmic vacuoles	No	Dénes J et al. 2015
11	M	33	Macro	PRL	HNPGL	33	*SDHB* c.298T>C, p.(Ser100Pro)	PV	LOH at SDHB locus/intracytoplasmic vacuoles	Son of patient no. 3 in Table 4	Dénes J et al. 2015
12	F	60	Macro	PRL	HNPGL	60	*SDHB*	PV	NP	NA	Dénes J et al.

							c.423+1G>A, p.(?)				2015
13	F	50	Micro	NFPA	P	50	*SDHB* c.770dupT, p.(Asn258Glufs*17)	LPV	NP	NA	Dénes J et al. 2015
14	M	72	NA	GH	HNPGL bilateral	70	*SDHB* c.689G>A, p.(Arg230His)	PV	NP	Sister: bilateral HNPGL, brother and niece: PA	Xekouki et al. 2015
15	F	50	Micro	PRL	metastatic PGL	47	*SDHB* c.642+1G>A, p.(?)	PV	NP	Brother: HNPGL, grandmother: GIST	Xekouki et al. 2015
16	M	53	Macro	PRL	HNPGL	38	*SDHC* c.380A>G, p.(His127Arg)	LPV	NP	Brother: PGL, cousin: PA	Dénes J et al. 2015
17	M	60	Macro	PRL	HNGPL	60	*SDHC* c256-257insTTT, p.(Phe85dup)	LPV	NP	No	Lopez Jimenez et al. 2008
18	F	23	Macro	PRL	Bilateral HNPGL	32	*SDHD* c.242C>T, p.(Pro81Leu)	PV	NP	Sister: bilateral HNPGL, sister, aunt and grandmother:	Xekouki et al. 2015

										PA	
19	M	60	Macro	PRL	HNPGL, P	62	*SDHD* c.274G>T, p.(Asp92Tyr)	PV	LOH at SDHD locus/SDHB IHC negative/SDHA IHC positive	NA	Papathomas TG et al. 2014
20	F	56	Macro	GH	HNPGL	56	*SDHD* c.274G>T, p.(Asp92Tyr)	PV	No LOH at SDHD locus/SDHA and SDHb IHC positive	Father and 2 sisters: HNPGL, sister: GIST	Papathomas TG et al. 2014
21	F	33	Macro	PRL	Bilateral HNPGL	39	*SDHD* c.242C>T, p.(Pro81Leu)	PV	NP	Brother, uncle, and aunt: HNPGL	Varsavsky et al. 2012
22	M	37	Macro	GH	HNPGL, abdominal PGL, bilateral P	37	*SDHD* c.298_301del, p.(Thr100Phefs*34)	PV	LOH at SDHD locus/SDHB IHC diffuse but patchy	Sister and paternal uncle: HNPGL	Xekouki et al. 2012
23	M	45	NA	NFPA	PGL	40	*SDHB* c.166_170del p.(Pro56Tyrfs*5)	PV	NP	Brother : metastatic pheo	Guerrero-Perez et al. 2019

F: female, M: male, PRL: prolactin, GH: growth hormone, PPGL: pheochromocytoma/paraganglioma, P: pheochromocytoma, PGL: paraganglioma, HNPGL: head and neck paraganglioma, LOH: loss of heterozygosity, IHC: immunohistochemical analysis, MEN1: multiple endocrine neoplasia type 1, pNET: pancreatic

neuroendocrine tumour, MTC: medullar thyroid carcinoma, NFPA: non-functional pituitary adenoma, PTC: papillary thyroid carcinoma, GIST: gastro-intestinal stromal tumour, HPTH: hyperparathyroidism, NA: not available, NP: not performed, LPV: likely pathogenic variant, PV: pathologic variant, VUS: variant of uncertain significance. *Classification using ACMG guidelines for classification of sequence variants (13).

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Table 4. Patients with "3PA" syndrome with isolated PA and familial PPGL bearing SDHx mutations in the literature

Patient no.	Sex	Age at PA diagnosis (yrs)	Size of PA	Secretion of PA	Mutation	Class of mutation*	LOH/IHC in PA and cytoplasmic vacuoles	Familial features	References
1	M	30	Macro	NFPA	*SDHA* c.1873C>T, p.(His625Tyr)	PV	Negative	Mother: P and mutation carrier	Dwight T et al. 2013
2	F	56	Macro	PRL	*SDHB* c.298T>C, p.(Ser100Pro)	PV	Negative	Father: bilateral P and mutation carrier	Maher A et al. 2018
3	NA	15	NA	NA	*SDHB* c.761dup, p.(Lys255*)	PV	NA	Familial P	Benn et al. 2006
4	F	35	Macro	PRL	*SDHB* c.298T>C, p.(Ser100Pro)	PV	Positive	Mother of patient n°10 of table 3	Denes et al. 2015
5	F	31	Macro	PRL	*SDHB* deletion of exon 6 to 8	PV	LOH at SDHB locus/ SDHB IHC negative	Grandmother's first cousin: PGL	Denes et al. 2015

F: female, M: male, yrs: years, PA: pituitary adenoma, PRL: prolactin, P: pheochromocytoma, PGL: paraganglioma, NFPA: non-functional pituitary adenoma, LOH: loss of heterozygosity, IHC: immunohistochemical analysis, PV: pathologic variant, NA: not available, *Classification using ACMG guidelines for classification of sequence variants (13).

Table 5. Characteristics of patients with pituitary adenoma in genetic and sporadic conditions

	SDHx-mutated	*SDHx -/MAX*-mutated	*MEN1*-mutated	*AIP*-mutated	*PRKAR1A*-mutated	*CDKN1B*-mutated	Patients with not genetically determined PA
Number of patients (n=)	26	31	405	64	19	17	68
Sex (M/F)	11 M/14 F (1 NA)	14 M/16 F (1 NA)	155 M/250 F	39 M/25 F	8 M/11 F	2 M/12 F (3 NA)	22 M/46 F
Age at diagnosis (yrs) mean (range)	43.6 (17-72)	42.4 (16-72)	34.2 (7-82)	25.9 (10-60)	31 (16-55)	53.5 (6-79)	34.5 (1-68)
Size of PA (n, %)*							
Macro	20 (87%)	23 (85%)	190 (85%)	59 (92%)	5 (31%)	1 (17%)	29 (42%)
Micro	3 (13%)	4 (15%)	34 (15%)	5 (8%)	11 (69%)	5 (83%)	39 (58%)
NA	3	4	181	0	3	10	0
Type of secretion (n, %)*							
PRL	17 (71%)	19 (66%)	175 (78%)	7 (11%)	1 (6%)	1 (6%)	45 (66%)
macroPRL	14 (58%)	15 (52%)	136 (61%)	6 (9%)	0	NA	11 (16%)
GH	3 (12.5%)	5 (17%)	12 (5%)	51 (80%)	16 (94%)	8 (50%)	9 (13%)
NFPA	4 (16.5%)	5 (17%)	11 (5%)	3 (5%)	0	5 (31%)	10 (15%)
Mixed GH-PRL	0	0	6 (3%)	3 (5%)	0	0	0

Other (ACTH, LH/FSH, TSH)	0	0	20 (10%)	0	0	2 (ACTH-secreting, 13%)	4 (ACTH-secreting, 6%)
NA	2	2	181	0	2	1	0

NA: not available; M: male; F: female, PA: pituitary adenoma, PRL: prolactinome, macroPRL: macroprolactinoma, GH: somatotropinoma, NFPA: non-functional pituitary adenoma, ACTH: adrenocorticotropic hormone, LH: luteinising hormone, FSH: follicular-stimulating hormone, TSH: thyroid-stimulating hormone. *percentages are calculated from available data, patients whose data are unavailable are excluded.

AIP, MEN1, PRKAR1A, CDKN1B cases and control cases are from published cases with an individual description of the cases. Non-genetically determined PA selected as reference are from the patients described by Daly et al. from a Belgian population (1). The *AIP* cases were 57 published cases (a list of references is available upon request). The *MEN1* cases were extracted from the UMD-MEN1 Database (15), the Carney complex cases were from a literature review published by Cuny et al. (16), and the MEN4 cases were from reviews conducted by Alrezk et al. and Fredericksen et al. (17, 18).

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Supplemental Table 1. Clinical characteristics of the patients included in this study

Patient no.	Sex	Age at diagnosis (yrs)	Size of PA	Type of PA	Other	Familial feature
1	F	17	NA	PRL		Sister: PA
2	F	39	NA	NA		Familial history of PA
3	M	22	Macro	ACTH	Aggressive, recurrence	Sporadic
4	F	13	Macro	PRL		Niece: PA
5	M	33	Macro	PRL		Sporadic
6	M	23	Macro	PRL	Recurrence	Sporadic
7	F	25	NA	ACTH		Sporadic
8	M	40	Macro	NFPA		Sporadic
9	M	8	NA	ACTH		Mother: prolactinoma Great uncle: PA
10	F	30	NA	GH		Sporadic
11	F	19	Macro	NFPA		Sporadic
12	M	16	Micro	PRL		Sporadic
13	F	18	Micro	ACTH		Sporadic
14	M	45	NA	PRL		Sporadic
15	M	31	Macro	PRL		Sporadic
16	F	49	NA			Sporadic
17	M	30	Macro	PRL		Sporadic
18	M	40	NA	GH	Aggressive and resistant to treatment	Sporadic
19	M	34	NA	GH		Sporadic
20	M	19	Macro	PRL		Sporadic
21	F	25	NA	ACTH		Sporadic
22	F	24	Macro	PRL		Sporadic
23	F	17	Micro	PRL		Sporadic
24	M	16	Macro	PRL		Sporadic
25	F	27	Macro	NA		Sporadic
26	M	39	Macro	PRL		Sporadic
27	M	24	NA	GH	Apoplexy	Sporadic
28	M	33	Macro	GH	Aggressive	Sporadic
29	F	27	NA	ACTH		Sporadic
30	F	21	Micro	ACTH		Sporadic
31	M	50	Micro	PRL		Father: macroprolactinoma
32	F	53	NA	GH		Sporadic
33	M	26	Macro	ACTH		Sporadic
34	M	47	Macro	PRL		Sporadic
35	F	19	Macro	NA		Sporadic
36	F	25	NA	GH		Sporadic
37	M	23	Macro	PRL	Recurrence	Sporadic
38	F	34	NA	GH		Sporadic
39	F	16	Micro	PRL		Brother: macroprolactinoma
40	M	27	NA	ACTH		Sporadic
41	M	18	Micro	ACTH		Sporadic
42	M	28	NA	NA		Father: acromegaly Brother: NFPA
43	M	36	Macro	ACTH		Sporadic

44	M	31	Macro	PRL		Sporadic
45	M	24	Macro	PRL		Sporadic
46	F	17	Macro	GH		Sporadic
47	M	30	Macro	NA		Sporadic
48	M	54	NA	NA		Sporadic
49	F	17	Macro	NFPA		Sporadic
50	F	33	Macro	NA		Sporadic
51	F	26	Micro	PRL		Sporadic
52	F	16	Macro	ACTH		Sporadic
53	M	41	Macro	GH	Resistant to treatment	Sporadic
54	M	18	NA	PRL		Grandfather: PA
55	F	22	Macro	ACTH		Sporadic
56	F	34	Macro	NA		Sporadic
57	F	29	Macro	NA		Sporadic
58	M	21	Macro	PRL		Aunt: PA
59	M	14	Micro	ACTH		Sporadic
60	F	20	Macro	GH		Sporadic
61	F	21	Macro	NA		Niece: acromegaly
62	M	19	Macro	PRL		Sporadic
63	F	37	Micro	GH		Sporadic
64	M	22	Macro	PRL		Sporadic
65	F	37	Macro	GH		Sporadic
66	F	28	Macro	GH		Sporadic
67	M	27	Macro	Mixed PRL-ACTH		Sporadic
68	F	38	Macro	LH-FSH		Sporadic
69	F	21	Macro	PRL		Sporadic
70	F	19	Micro	PRL	Resistant to treatment	Sporadic
71	M	29	Macro	GH		Sporadic
72	M	33	Macro	PRL		Sporadic
73	M	64	Macro	LH-FSH		Sporadic
74	F	31	Macro	GH		Sporadic
75	M	14	Micro	ACTH		Sporadic
76	M	33	Macro	NFPA	Panhypopituitarism	Sporadic
77	M	34	Macro	Mixed PRL-GH	Aggressive	Sporadic
78	M	29	Macro	PRL		Sporadic
79	M	35	Macro	NA		Sporadic
80	M	31	Macro	mixed PRL-GH		Sporadic
81	F	44	Macro	PRL		Sporadic
82	M	28	Macro	NFPA		Sporadic
83	M	25	Macro	GH	Invasive	Sporadic
84	M	39	NA	NA		Sporadic
85	F	37	Macro	NFPA		Sporadic
86	M	41	Macro	GH		Sporadic
87	F	60	Micro	NFPA		Mother: acromegaly
88	M	34	NA	GH		Sporadic

89	F	16	Macro	PRL		Aunt and 3 cousins: PA
90	F	25	Macro	PRL		Sporadic
91	F	48	NA	NA		Sporadic
92	F	21	Macro	PRL		Sporadic
93	M	40	Macro	NFPA		Brother: PA
94	M	40	Macro	NFPA		Brother: PA
95	F	20	Macro	PRL		Sporadic
96	M	28	Macro	PRL		Sporadic
97	M	17	Macro	PRL		Sporadic
98	F	35	Macro	GH		Sporadic
99	M	39	Macro	GH		Sporadic
100	F	25	Micro	GH		Sister: prolactinoma
101	F	27	Micro	NFPA		Mother: microprolactinoma
102	M	11	Macro	NFPA		Father: somatotropinoma
103	F	30	Macro	NFPA		Sporadic
104	F	33	Macro	GH		Sporadic
105	F	15	NA	PRL		Sporadic
106	F	30	Micro	ACTH		Sporadic
107	F	22	Macro	GH		Sporadic
108	M	30	Macro	NFPA	Recurrence	Sporadic
109	M	31	Macro	GH		Sporadic
110	F	35	Macro	GH		Sporadic
111	F	27	Macro	mixed GH-LH/FSH		Sporadic
112	M	35	Macro	GH		Sporadic
113	F	29	NA	NA		Sporadic
114	M	67	NA	NA	Apoplexy	Brother: acromegaly
115	F	20	Micro	PRL		Sporadic
116	F	33	Macro	NFPA	Recurrence	Sporadic
117	M	28	Macro	GH		Sporadic
118	F	16	Macro	PRL		Grandmother: PA
119	M	39	Macro	GH		Sporadic
120	F	17	Macro	GH		Sporadic
121	F	30	NA	ACTH	Recurrence	Sporadic
122	M	28	Macro	LH		Sporadic
123	F	15	Macro	PRL		Sporadic
124	F	34	Macro	GH		Sporadic
125	M	35	Macro	Mixed PRL-GH		Sporadic
126	F	27	Macro	PRL		Sporadic
127	M	16	Macro	GH		Sporadic
128	M	16	Macro	PRL		Mother and cousin: PA
129	M	15	NA	PRL		Sporadic
130	F	20	Macro	NFPA		Great aunt: PA
131	M	37	Micro	PRL		Father and brother: macroprolactinoma Nephew: macroprolactina

132	F	16	Macro	PRL		Sporadic
133	M	15	NA	ACTH		Sporadic
134	F	15	Macro	GH		Sporadic
135	M	24	Macro	NA		Sporadic
136	M	26	Macro	NA	Invasive, recurrence	Sporadic
137	F	24	Macro	Mixed PRL-GH		Sporadic
138	F	11	Macro	PRL		Sporadic
139	F	18	NA	GH		Sporadic
140	F	27	NA	GH		Sporadic
141	M	38	Macro	PRL		Sporadic
142	F	16	Macro	GH		Sporadic
143	M	20	Macro	NA		Mother: ACTH-secreting PA
144	F	30	Macro	Mixed PRL-GH		Son: Macro PA
145	F	15	NA	PRL		Sporadic
146	M	22	Macro	PRL		Sporadic
147	F	12	Macro	PRL		Sporadic
148	F	20	Macro	NA		Sporadic
149	F	20	Macro	NFPA		Sporadic
150	M	31	Macro	NA		Sporadic
151	M	25	Macro	ACTH		Sporadic
152	M	18	Macro	PRL		Sporadic
153	M	24	Macro	NA		Sporadic
154	F	33	Macro	ACTH		Sporadic
155	F	31	Macro	NFPA		Sporadic
156	F	32	Macro	NA		Sporadic
157	F	31	Macro	GH	Resistant to treatment	Sporadic
158	M	64	NA	GH		Sporadic
159	F	26	Macro	PRL		Sporadic
160	M	23	Macro	GH		Sporadic
161	F	31	Micro	Mixed PRL-GH		Sporadic
162	M	46	NA	GH		Sporadic
163	M	17	Macro	PRL		Sporadic
164	M	34	Macro	GH		Sporadic
165	F	36	Macro	NA		Sporadic
166	M	27	Macro	PRL		Sporadic
167	M	26	micro	ACTH		Sporadic
168	F	50	Macro	NA		Sister: prolactinome
169	F	46	Macro	GH		Sporadic
170	M	52	Macro	GH		Sporadic
171	M	18	Macro	PRL		Sporadic
172	M	24	Macro	NA	Alpha sub-unit PA	Sporadic
173	F	12	NA	NA		Sporadic
174	M	19	Macro	PRL		Sporadic
175	F	50	NA	NFPA		Brother: PA
176	M	35	Macro	Mixed PRL-GH		Sporadic
177	F	25	Macro	PRL		Sporadic
178	M	45	NA	GH		Sporadic
179	M	42	Macro	Mixed		Sporadic

				PRL-GH		
180	F	38	NA	NA		Sporadic
181	F	21	Macro	NFPA		Sporadic
182	H	45	Macro	PRL		Sporadic
183	M	29	Macro	PRL		Sporadic
184	M	22	Macro	GH		Sporadic
185	M	62	NA	NA		Sporadic
186	M	28	Macro	GH		Sporadic
187	M	44	Macro	GH		Sporadic
188	M	78	Macro	ACTH		Sporadic
189	F	53	NA	GH		Sister: acromegaly
190	F	24	NA	TSH		Sporadic
191	F	61	NA	GH		Sporadic
192	M	36	NA	GH		Sporadic
193	F	23	Macro	ACTH		Father: NFPA
194	F	29	NA	NA		Sporadic
195	F	12	NA	PRL		Sporadic
196	F	36	Macro	GH		Sporadic
197	F	21	Macro	GH		Sporadic
198	M	49	Macro	GH		Cousin: GH-secreting macroadenoma
199	F	14	Micro	PRL		Sporadic
200	M	42	Macro	NA		Sporadic
201	M	19	Macro	GH		Sporadic
202	F	25	Macro	GH		Sporadic
203	F	29	Macro	GH		Sporadic
204	F	37	Macro	GH		Sporadic
205	F	30	Macro	PRL		Cousin: PA
206	F	13	Macro	PRL		Mother: macroprolactinoma
207	F	28	Macro	GH	Apoplexy and panhypopituitarism	Sporadic
208	F	17	NA	PRL		Sporadic
209	F	29	NA	NA		Sporadic
210	M	23	Macro	PRL		Sporadic
211	F	16	Macro	PRL		Sporadic
212	M	15	NA	ACTH		Sporadic
213	F	47	Macro	mixed GH-ACTH		Sporadic
214	M	36	Macro	PRL		Sporadic
215	M	74	Macro	NFPA		Son: NFPA
216	M	21	Macro	PRL		Sporadic
217	M	33	Macro	PRL		Sporadic
218	M	32	Macro	NA		Sporadic
219	M	38	Macro	PRL		Sporadic
220	M	42	Macro	PRL		Sporadic
221	M	30	Macro	PRL		Sporadic
222	M	38	Micro	GH		Sporadic
223	M	31	Macro	PRL		Sporadic
224	F	21	Micro	PRL		Sporadic
225	M	31	NA	NA		Sporadic

226	F	15	Macro	GH		Sporadic
227	M	34	Macro	LH-FSH		Sporadic
228	F	30	Macro	NA		Sporadic
229	M	41	Macro	TSH		Sporadic
230	F	77	Macro	GH		Niece: mixed macroadenoma (GH-PRL)
231	M	37	Macro	GH		Sporadic
232	M	22	Macro	PRL		Cousin: PA
233	M	36	Macro	PRL		Sporadic
234	F	30	Macro	PRL	Recurrence	Sporadic
235	M	33	Macro	PRL		Sporadic
236	F	26	NA	GH		Sporadic
237	M	34	Macro	PRL		Sister: PA
238	M	23	Macro	NA		Sporadic
239	M	57	Macro	GH		Sporadic
240	M	21	Macro	PRL		Sporadic
241	M	30	Macro	GH		Sporadic
242	F	16	Macro	NFPA		Sporadic
243	M	23	Macro	GH		Sporadic
244	F	36	NA	GH		Sporadic
245	F	36	NA	NA		Sporadic
246	F	16	Macro	PRL		Sporadic
247	M	35	Macro	FSH		Sporadic
248	F	22	Macro	GH		Sporadic
249	F	48	Micro	NFPA		Sporadic
250	F	28	Macro	ACTH		Sporadic
251	M	31	Macro	GH		Sister: prolactinoma
252	M	33	Macro	PRL		Sporadic
253	F	9	Macro	PRL		Sporadic
254	M	29	Macro	Mixed GH-TSH		Sporadic
255	F	27	Macro	GH		Sporadic
256	F	21	Micro	PRL		Sporadic
257	M	37	Macro	PRL		Sporadic
258	M	55	Macro	GH		Sporadic
259	M	14	Macro	ACTH		Sporadic
260	M	44	Macro	PRL		Sporadic
261	F	29	Macro	PRL		Sporadic
262	M	25	Macro	GH		Sporadic
263	M	40	Macro	Mixed PRL-FSH		Sporadic

F: female, M: male, NA: not available, PA: pituitary adenoma, PRL: prolactinoma, ACTH: corticotropinoma, GH: somatotropinoma, NFPA: non-functional pituitary adenoma

Supplemental table 2. Clinical and genetic characteristics of the patients harbouring a pathogenic or likely pathogenic variants in this study

Patient no.	Sex	Age at diagnosis (yrs)	Phenotype of pituitary adenoma	Familial feature	Gene	HGVSc	HGVSp	Genotype	Consequence	Classification*
1	M	17	Macroprolactinoma	Sporadic	*SDHC*	c.405+1G>T	p.(?)	Heterozygous	Splicing variant	PV
2	M	42	Macroprolactinoma	Sporadic	*SDHA*	c.757_758del	p.(Val253Cys*67)	Heterozygous	Frameshift variant	PV
3	M	37	Microprolactinoma	Father and brother: macroprolactinoma nephew: microprolactinoma	*SDHA*	c.1753C>T	p.(Arg585Trp)	Heterozygous	Missense variant	LPV
4	F	19	Macroadenoma NFPA	Sporadic	*AIP*	c.350delG	p.(Gly117Ala*39)	Heterozygous	Frameshift variant	PV
5	M	21	Macroprolactinoma	Aunt: PA	*AIP*	c.805_825dup	p.(Phe269_His275dup)	Heterozygous	In-frame insertion	LPV
6	M	23	GH-producing macroadenoma	Sporadic	*AIP*	c.601A>T	p.(Lys201*)	Heterozygous	Nonsense variant	PV
7	M	22	Macroprolactinoma	Cousin: PA	*AIP*	c.55C>T	p.(Gln19*)	Heterozygous	Nonsense variant	PV
8	M	22	GH-producing macroadenoma	Sporadic	*AIP*	c.853C>T	p.(Gln285*)	Heterozygous	Nonsense variant	PV
9	F	30	Mixed	Son: Macro PA	*AIP*	c.911G>A	p.(Arg304G	Heterozy	Missense	LPV

			Macroadenoma (PRL-GH)					ln)	gous	variant	
10	F	47	Mixed macroadenoma (GH-ACTH)	Sporadic		*AIP*	c.865G>A	p.(Ala289Thr)	Heterozygous	Missense variant	LPV
11	F	20	Macroadenoma NFPA	Maternal PA	great-aunt:	*MEN1*	c.1314delC	p.(Val441Cys*4)	Heterozygous	Frameshift variant	PV
12	F	11	Macroprolactinoma	Sporadic		*MEN1*	c.574C>T	p.(Gln192*)	Heterozygous	Nonsensevariant	PV

*Classification using ACMG guidelines for classification of sequence variants (11).

F: female, M: male, Yrs: years, PRL: prolactin, GH: growth hormone, ACTH: adrenocorticotropic hormone, NFPA: non-functional pituitary adenoma, PA: pituitary adenoma, PV: pathologic variant, LPV: likely pathogenic variant, . Macroadenoma is defined by a diameter >10 mm, microadenoma is defined by a diameter <10 mm.

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Supplemental Table 3. Clinical and genetic characteristics of the patients with variants of uncertain significance* in this study

Patient no.	Sex	Age at diagnosis (yrs)	Phenotype of pituitary adenoma	Familial feature	Gene	HGVSc	HGVSp	Genotype	Consequence
1	F	53	Somatotropinoma	Sporadic	*AIP*	c.326C>T	p.(Ala109Val)	Heterozygous	Missense variant
2	M	15	Prolactinoma	Sporadic	*CDKN1B*	c.586C>T	p.(Arg196Cys)	Heterozygous	Missense variant
3	M	31	Macroprolactinoma	Sporadic	*MEN1*	c.903C>T	p.(=)	Heterozygous	Synonymous variant
4	F	26	Somatotropinoma	Sporadic	*SDHA*	c.512G>A	p.(Arg171His)	Heterozygous	Missense variant
5	M	33	Macroadenoma NFPA	Sporadic	*SDHB*	c.312C>T	p.(=)	Heterozygous	Synonymous variant
6	F	25	Macroprolactinoma	Sporadic	*SDHD*	c.110A>G	p.(Asp37Gly)	Heterozygous	Missense variant
7	F	29	Macroprolactinoma	Sporadic	*SDHD*	c.158C>T	p.(Pro53Leu)	Heterozygous	Missense variant

F: female, M: male, NFPA: non-functional pituitary adenoma, yrs: years

*Classification using ACMG guidelines for classification of sequence variants (11).

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