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Germinal defects of SDHx genes in patients with isolated pituitary adenoma

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1 **Germinal defects of pseudohypoxia pathway genes**
2 **in patients with isolated pituitary adenoma.**
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4

5 **Authors**

6 Grégory Mougel¹, Arnaud Lagarde¹, Frédérique Albarel², Wassim Essamet³, Perrine Luigi⁴,
7 Céline Mouly⁵, Magaly Vialon⁵, Thomas Cuny⁶, Frédéric Castinetti⁶, Alexandru Saveanu¹,
8 Thierry Brue⁶, Anne Barlier¹, Pauline Romanet¹

9
10 **Affiliations**

11 ¹Aix Marseille Univ, INSERM, MMG, Laboratory of Molecular Biology Hospital La
12 Conception, Marseille, France

13 ²Department of Endocrinology, Hospital La Conception, APHM, Marseille, France

14 ³Department of Pathology, Hospital La Timone, APHM, Marseille, France

15 ⁴Department of Endocrinology, Hospital Lapeyronie, CHU Montpellier, France

16 ⁵Department of Endocrinology, Hospital Larrey, CHU Toulouse, France

17 ⁶Aix Marseille Univ, INSERM, MMG, Department of Endocrinology, Hospital La
18 Conception, Marseille, France

19
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21
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24
25 **Corresponding author**

26 Pr Anne Barlier, MD, PhD

27 Aix Marseille University, INSERM, MMG, UMR 1251

28 Faculté de Médecine La TIMONE, 27, Boulevard Jean Moulin 13385 Marseille Cedex 5,
29 France

30 Tel.: +33 491 69 87 89

31 Fax: +33 491 69 89 20

32 Email: anne.barlier@univ-amu.fr

33 **Attention PA, Pas and 3PAs**

34

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37

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39

40 **Abstract (250)**

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

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

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

50 **Results:** 263 patients were recruited. Seven (likely) pathogenic variants were found in *AIP*, 2
51 in *MEN1*, 2 in *SDHA*, and 1 in *SDHC*. The prevalence of *SDHx* mutations reached 1.1%
52 (3/263). Among the 31 reported patients with PA harbouring *SDHx/MAX* mutations (28 from
53 literature and these 3 cases), 6/31 (19%) developed PA before PPGL, and 8/31 (26%) had
54 isolated PA. The age of onset is older than in *AIP/MEN1*-mutated patients. The PAs were
55 mainly macroprolactinomas and a feature of intracytoplasmic vacuoles can be observed by
56 histological study.

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

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Clinical Study

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67 **Word count: 3527**

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INTRODUCTION

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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.

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The association between PA and pheochromocytoma/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).

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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

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

101

102 **PATIENTS AND METHODS**

103 **Subjects**

104 All patients who underwent genetic testing in the context of an isolated PA without other
105 endocrine lesions at the molecular laboratory of Marseille Conception Hospital between
106 November 2016 and December 2018 were included. Written informed consent of all patients
107 for genetics analysis was obtained during one-on-one genetic counselling. The ethics
108 committee of Aix-Marseille University approved this study (approval number: 2018-13-12-
109 004).

110

111 **Next-generation sequencing (NGS)**

112 Genomic DNA was extracted with a QiaSymphony DS DNA Midi Kit (Qiagen, Courtaboeuf,
113 France) from blood lymphocytes (standard EDTA samples). Exons and 20 bp flanking introns
114 of *AIP* (NM_003977.2), *MEN1* (NM_130799.2), *CDKN1B* (NM_004064), *SDHA*
115 (NM_004168.2), *SDHB* (NM_003000.2), *SDHC* (NM_003001.3), *SDHD* (NM_003002.2),
116 *SDHAF2* (NM-017841.1), and *MAX* (NM_002382.3) were sequenced by next-generation
117 sequencing (NGS) using the QiaSeq library (Qiagen, Courtaboeuf, France) according to the
118 manufacturer's instructions. Libraries were sequenced on MiSeqDX (Illumina). The
119 alignment and variant calling were performed using the Biomedical Genomics Workbench
120 5.0.1 (Qiagen). Annotation was done using VariantStudio v2.2 (Illumina), according to the
121 HGVS guidelines (10).

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

124 Class 1: benign variant (BV)

125 Class 2: likely benign variant (LBV)

126 Class 3: variant of uncertain significance (VUS)

127 Class 4: likely pathogenic variant (LPV)

128 Class 5: pathogenic variant (PV)

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

133 databases: ClinVar, LOVD, and HGMD were collected. The variants with a frequency above
134 5% in the population were not retained. All PVs and LPVs were confirmed by Sanger
135 sequencing (the primers and protocols are available upon request).

136

137 **Explorations of *SDHx* mutations in pituitary adenomas**

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

142

143 SDH IHC analysis

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

152

153 Sanger sequencing for research of LOH in tumors

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

159

160 **Comparison of patients with “3PAs” syndrome and “non-3PAs” syndrome based on the 161 literature data.**

162

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

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

171

172 **Statistical analyses**

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

177

178 **RESULTS**

179 A total of 263 patients were included (Table 1 and Supplemental Table 1). The mean age at
180 PA diagnosis was 29.3 years (8-78), and the mean age at genetic screening was 36.1 years (8-
181 79). The occurrence of PA was sporadic in 227 patients (86.3%), while 36 patients presented
182 with a familial history of PA (13.7%). By NGS sequencing, we found 295 variants, among
183 which 7 variants were classified as pathogenic, 5 as likely pathogenic, and 7 as VUS (Table 1,
184 Figure 1, Supplemental Table2, and Supplemental Table 3). Five PV and LPV were found in
185 patients with a familial history of PA out of 36, and 7 in patients with sporadic PA out of 227.
186 The odds ratio of harbouring a (likely)pathogenic variant in cases of family history of PA
187 against no history is then 5.069 (95% CI: 1.69 to 15.79, p=0.014). Among the sporadic cases,
188 5 mutations occurred in patients younger than 30 years (5/133), and 2 occurred in patients
189 older than 30 years. Among the 12 PVs and LPVs, we found 7 variants in *AIP*, 2 in *MEN1*, 2
190 in *SDHA*, and 1 in *SDHC* (Figure 1, and Supplemental Table 2). The medical histories of the
191 3 *SDHx*-mutated patients are described as follows and in the Table 2.

192

193 **Case presentation**

194 Case 1: A previously healthy male patient aged 17 presented with a recent history of severe
195 right visual loss, clinically objectified on the Monoyer scale (right eye: 4/10, left eye: 10/10).
196 The MRI revealed a large (>10 mm) pituitary mass, and his prolactin level was measured at
197 91 µg/L (reference range: 4.1-15.34 µg/L). The patient underwent a transsphenoidal surgery
198 debulking. The post-operative examination was without complications and demonstrated a
199 marked improvement in vision. The post-operative prolactin secretion was measured at

200 104µg/L. Cabergoline was initiated at a dose of 0.5 mg weekly; allowing prolactin
201 normalisation (3µg/L at 3 months).

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

208

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

219

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

232

233 Literature review and phenotypic features in “3PAs” syndrome

234 After reclassification of the variants using ACMG criteria (11) and excluding patients
235 with VUS or (likely)benign variants, we found 23 published cases of “3PAs” syndrome with
236 *SDHx* or *MAX* (likely)pathogenic variants (Table 3) (4, 5, 6, 7, 8, 19, 20, 21, 22, 23, 24, 25,
237 26, 27). Among the 23 cases, 19 patients had *SDHx* (likely) pathogenic variants: 2 in *SDHA*, 9
238 in *SDHB* (39%, 9/23), 2 in *SDHC*, and 6 in *SDHD* (26%, 6/23); 4 patients had *MAX*
239 pathogenic variants. PA occurred before PPGL in 6 cases (6/23, 26%). Moreover, we also
240 found 5 published cases with an isolated PA and a mutation in *SDHx* (1 in *SDHA* and 4 in
241 *SDHB*), all of them had a family history of PPGLs (Table 4) (5, 28, 29, 30).

242 Overall, the *SDHx/MAX* patients with PA included those harbouring PPGL (n=23, Table3),
243 those with a family history of PPGL (n=6, Table 4), those without PPGL context (n=3 from
244 this study). Among these 31 *SDHx/MAX* patients, 4 had a family history of PA (Table 3 and
245 our case 3), among them only one without PPGL history (our case 3). They included 16
246 females and 14 males (sex ratio: 1.1/1, the sex is not specified for one patient). The diagnosis
247 of PA was on average at 42.4 years old (range: 16-72). There were 23 macroPAs, representing
248 74% of cases, and 4 microPAs (13%). The 2 most frequent types were prolactinoma (19/31,
249 61%) and GH-secreting adenoma (5/31, 16%) (Table 5).

250 Then, we compared these *SDHx/MAX* patients with those from published cases of
251 genetically determined PAs (i.e. due to mutations in *AIP*, *MEN1*, *CDKN1B* or *PRKARIA*) and
252 non-genetically determined PAs. The age of occurrence of PAs in the *SDHx/MAX* patients
253 (mean 42.4 years, range: 16 to 72) was older than in the *AIP* patients (mean 25.9 years, range:
254 10-60, p<0.001), in the *MEN1* patients (mean 34.2 years, range: 7-82, p=0.024), and in the
255 *PRKARIA* patients (mean 31 years, range: 16-55, p=0.007) (Figure 2 and Table 5).

256 The proportion of prolactinomas was identical in the *SDHx/MAX* patients and in the
257 control population from the cohort published by Daly et al. (61% versus 66%) (Table 5) (1).
258 However, the PAs of the 31 *SDHx/MAX* patients were larger (23/31 macroadenomas versus
259 29/68 in control population p=0.002). In fact, macroprolactinoma was more frequent in
260 *SDHx/MAX* population (15/31 versus 11/68 p=0.0013). The *SDHx/MAX* patients also
261 presented a disposition to have older age at PA diagnosis than in the control population (42.4
262 years versus 34.5 years) but not statistically significant (p=0.08).

263

264 DISCUSSION

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

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

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

285

286 Herein, we demonstrated for the first time the presence of *SDHx/MAX* germline
287 mutations in patients with isolated PA without PPGL history. In our study, among 263
288 patients with isolated PA, we found 3 (likely) pathogenic variants in *SDHx* genes: 2 in
289 sporadic cases of PA, one in a patient with a strong family history of PAs. The mutation
290 prevalence rate was 1.1%. In 2015, Xekouki et al. studied the prevalence of *SDHx* germline
291 mutations in a cohort of 168 patients with PA, including 143 patients with isolated and
292 sporadic PA, 3 patients with sporadic “3PAs” syndrome, and 22 patients with family “3PAs”
293 syndrome (4). Three *SDHx* mutations were identified in patients with family “3PAs”
294 syndrome. No mutation was found in the patients presenting with isolated PA, probably
295 because the cohort included a high proportion of patients with ACTH-secreting PAs (118/168,
296 70%).

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

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

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

323

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

328

329 Among the 3 variants identified in patients with isolated PA, 2 occurred in *SDHA*, and
330 1 in *SDHC*. In PPGL population, *SDHx* germline mutations are accounted for approximately
331 15% of all cases and for the half of the family cases. *SDHB* and *SDHD* mutations are the most
332 common, *SDHA* and *SDHC* mutations are less frequent (46). On a large series of *SDHA*-
333 PPGL, the penetrance was calculated at 10% at 70 years (47), while Benn et al. and Maniam
334 et al., using a Bayesian statistical approach, indicated an overall penetrance of 1.7% (95% CI:

335 0.8% to 3.8%) and 0.1%-4.9%, respectively (48, 49). In the same study, Benn et al. calculated
336 the *SDHC* penetrance at 8.3% (95% CI: 3.5% to 18.5%) (48). Consequently, the absence of
337 PPGL in our patients with *SDHA* or *SDHC* mutations and their families is not unexpected
338 since the penetrance of *SDHA/SDHC*-related PPGL is low and the age of disease onset is late.

339

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

354

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

361

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

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

372

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- 621

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

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

624

625

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

627 Controls: Patients with non-genetically determined PA selected as reference from the patients
628 described by Daly et al. from a Belgian population (10). *AIP* cases: 57 published cases (list available
629 upon request). *MEN1*: MEN1 cases harbouring pathogenic or likely pathogenic variants extracted
630 from the UMD-MEN1 Database (11). *PRKARIA*: Carney complex published cases listed by Cuny et
631 al.(12). *CDKN1B*: 11 MEN4 cases listed in the reviews by Alrezk et al. and Fredericksen et al. (13-
632 14).

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

634

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

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

641

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

644

645 **Table 3.** Patients with “3PA” syndrome with personal PPGL bearing *SDHx/MAX* mutations in the
646 literature

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

655

656 **Table 4.** Patients with “3PA” syndrome with isolated PA and familial PPGL bearing *SDHx* mutations
657 in the literature.

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

662

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

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

669 *AIP*, *MEN1*, *PRKARIA*, *CDKN1B* cases and control cases are from published cases with an individual
670 description of the cases. Non-genetically determined PA selected as reference are from the patients
671 described by Daly et al. from a Belgian population (1). The *AIP* cases were 57 published cases (a list
672 of references is available upon request). The *MEN1* cases were extracted from the UMD-MEN1
673 Database (13), the Carney complex cases were from a literature review published by Cuny et al. (14),
674 and the MEN4 (*CDKN1B*) cases were from reviews conducted by Alrezk et al. and Fredericksen et al.
675 (15, 16).

676

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

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

680

681

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

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

689

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

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

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

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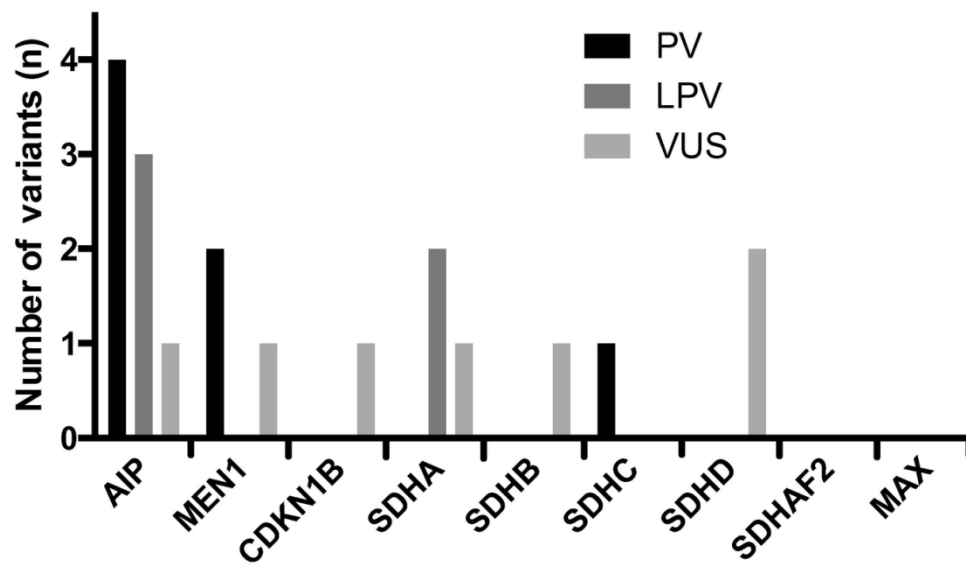


Figure 1. Repartition of VUS, LPV, and PV by gene in this study
 VUS: variant of unknown significance, LPV: likely pathogenic variant, PV: pathogenic variant.

119x72mm (300 x 300 DPI)

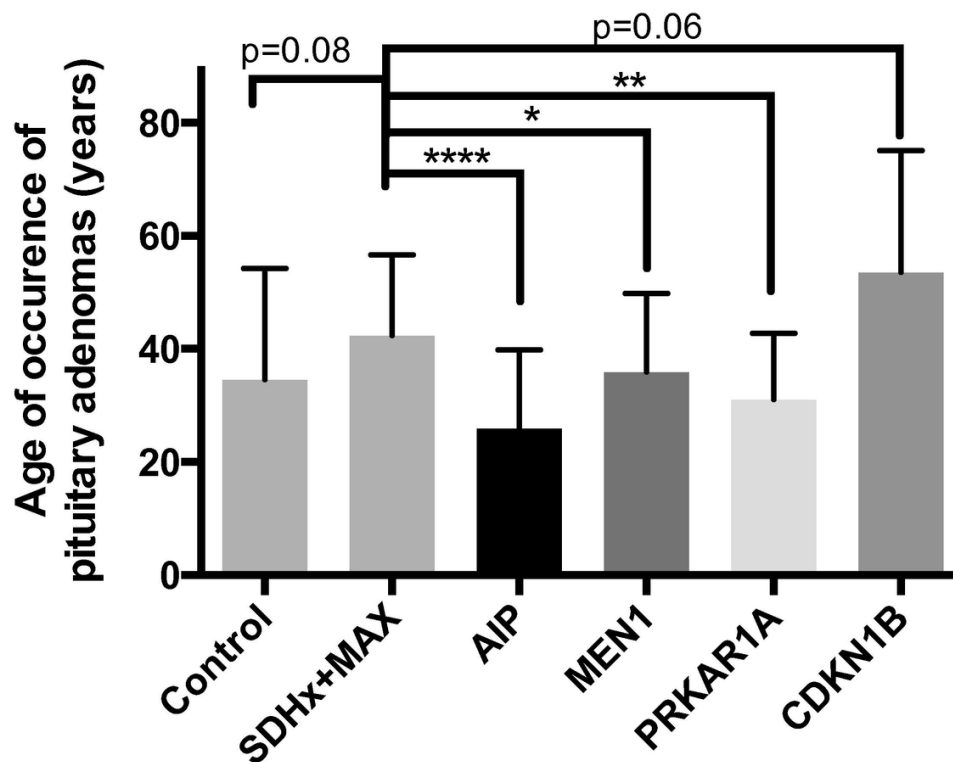


Figure 2. Comparison of the age of occurrence of PAs in genetic syndromes and controls
 Controls: Patients with non-genetically determined PA selected as reference from the patients described by Daly et al. from a Belgian population (10). AIP cases: 57 published cases (list available upon request).
 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).
 * $p < 0.05$, ** $p < 0.01$ **** $p < 0.0001$.

99x79mm (300 x 300 DPI)

1

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: adrenocorticotrophic 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.

2

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 | <i>SDHC</i> | <i>SDHA</i> c.757_758del, p.(Val253Cys* | <i>SDHA</i> c.1753C>T, p.(Arg585Trp) |
| Variant | c.405+1G>T, p.(?) | 67) | |
| 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: immunohistochemistry SDH, LOH: Loss of heterozygosity
*Classification using ACMG guidelines for classification of sequence variants (11)

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 | <i>MAX</i> del exon 3 | PV | NP | No | Daly et al. 2018 |
| 2 | F | 26 | Macro | GH | Bilateral P | 35 | <i>MAX</i> del exons 1-3 | PV | NP | No | Daly et al. 2018 |
| 3 | M | 16 | Macro | GH | Metastatic bilateral P | 22 | <i>MAX</i> del exon 4 | PV | NP | No | Daly et al. 2018 |
| 4 | F | 49 | Macro | PRL | bilateral P | 49 | <i>MAX</i> c.296-1G>T, p.(?) | PV | NP | No | Roszko et al. 2017 |
| 5 | F | 35 | Macro | NA | HNPGL, mediastinal PGL | 38 | <i>SDHB</i> (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 | <i>SDHB</i> 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 | <i>SDHD</i> c.315-?_480+?del | PV | NP | Father and 2 brothers: mutation | Lemelin et al. 2019 |

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

| | | | | | | | | | | | |
|----|---|----|-------|------|--------------------|----|---|-----|----|--|------------------------------|
| | | | | | | | c.423+1G>A, p.(?) | | | | 2015 |
| 13 | F | 50 | Micro | NFPA | P | 50 | <i>SDHB</i> c.770dupT, p.(Asn258Glu fs*17) | LPV | NP | NA | Dénes J et al. 2015 |
| 14 | M | 72 | NA | GH | HNPGL bilateral | 70 | <i>SDHB</i> 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 | <i>SDHB</i> c.642+1G>A, p.(?) | PV | NP | Brother: HNPGL, grandmother: GIST | Xekouki et al. 2015 |
| 16 | M | 53 | Macro | PRL | HNPGL | 38 | <i>SDHC</i> c.380A>G, p.(His127Arg) | LPV | NP | Brother: PGL, cousin: PA | Dénes J et al. 2015 |
| 17 | M | 60 | Macro | PRL | HNGPL | 60 | <i>SDHC</i> c256- 257insTTT, p.(Phe85dup) | LPV | NP | No | Lopez Jimenez et al. 2008 |
| 18 | F | 23 | Macro | PRL | Bilateral HNPGL | 32 | <i>SDHD</i> 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 | <i>SDHD</i> c.274G>T, p.(Asp92Tyr) | PV | LOH at <i>SDHD</i> locus/ <i>SDHB</i> IHC negative/ <i>SDHA</i> IHC positive | NA | Papathomas TG et al. 2014 |
| 20 | F | 56 | Macro | GH | HNPGL | 56 | <i>SDHD</i> c.274G>T, p.(Asp92Tyr) | PV | No LOH at <i>SDHD</i> locus/ <i>SDHA</i> and <i>SDHb</i> IHC positive | Father and 2 sisters: HNPGL, sister: GIST | Papathomas TG et al. 2014 |
| 21 | F | 33 | Macro | PRL | Bilateral HNPGL | 39 | <i>SDHD</i> 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 | <i>SDHD</i> c.298_301del, p.(Thr100Phe fs*34) | PV | LOH at <i>SDHD</i> locus/ <i>SDHB</i> IHC diffuse but patchy | Sister and paternal uncle: HNPGL | Xekouki et al. 2012 |
| 23 | M | 45 | NA | NFPA | PGL | 40 | <i>SDHB</i> 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 | <i>SDHA</i> c.1873C>T, p.(His625Tyr) | PV | Negative | Mother: P and mutation carrier | Dwight T et al. 2013 |
| 2 | F | 56 | Macro | PRL | <i>SDHB</i> c.298T>C, p.(Ser100Pro) | PV | Negative | Father: bilateral P and mutation carrier | Maher A et al. 2018 |
| 3 | NA | 15 | NA | NA | <i>SDHB</i> c.761dup, p.(Lys255*) | PV | NA | Familial P | Benn et al. 2006 |
| 4 | F | 35 | Macro | PRL | <i>SDHB</i> c.298T>C, p.(Ser100Pro) | PV | Positive | Mother of patient n°10 of table 3 | Denes et al. 2015 |
| 5 | F | 31 | Macro | PRL | <i>SDHB</i> deletion of exon 6 to 8 | PV | LOH at <i>SDHB</i> locus/ <i>SDHB</i> 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

| | <i>SDHx</i> -mutated | <i>SDHx</i> -/ <i>MAX</i> -mutated | <i>MEN1</i> -mutated | <i>AIP</i> -mutated | <i>PRKARIA</i> -mutated | <i>CDKN1B</i> -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, PRKARIA, 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).

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 | <i>SD</i> <i>HC</i> | c.405+1G >T | p.(?) | Heterozygous | Splicing variant | PV |
| 2 | M | 42 | Macroprolactinoma | Sporadic | <i>SD</i> <i>HA</i> | c.757_75 8del | p.(Val253Cys*67) | Heterozygous | Frameshift variant | PV |
| 3 | M | 37 | Microprolactinoma | Father and brother: macroprolactinoma nephew: microprolactinoma | <i>SD</i> <i>HA</i> | c.1753C> T | p.(Arg585Trp) | Heterozygous | Missense variant | LPV |
| 4 | F | 19 | Macroadenoma NFPA | Sporadic | <i>AIP</i> | c.350del G | p.(Gly117Ala*39) | Heterozygous | Frameshift variant | PV |
| 5 | M | 21 | Macroprolactinoma | Aunt: PA | <i>AIP</i> | c.805_82 5dup | p.(Phe269_His275dup) | Heterozygous | In-frame insertion | LPV |
| 6 | M | 23 | GH-producing macroadenoma | Sporadic | <i>AIP</i> | c.601A> T | p.(Lys201*) | Heterozygous | Nonsense variant | PV |
| 7 | M | 22 | Macroprolactinoma | Cousin: PA | <i>AIP</i> | c.55C>T | p.(Gln19*) | Heterozygous | Nonsense variant | PV |
| 8 | M | 22 | GH-producing macroadenoma | Sporadic | <i>AIP</i> | c.853C>T | p.(Gln285*) | Heterozygous | Nonsense variant | PV |
| 9 | F | 30 | Mixed | Son: Macro PA | <i>AIP</i> | c.911G>A | p.(Arg304G) | Heterozygous | Missense | LPV |

| | | | | | | | | | | |
|----|---|----|-------------------------------------|----------------|---------------------------------------|------------|----------------------|------------------|-----------------------|-----|
| | | | Macroadenoma (PRL-GH) | | | | ln) | gous | variant | |
| 10 | F | 47 | Mixed macroadenoma (GH- ACTH) | Sporadic | <i>AIP</i> | c.865G>A | p.(Ala289T hr) | Heterozy gous | Missense variant | LPV |
| 11 | F | 20 | Macroadenoma NFPA | Maternal PA | great-aunt: <i>ME</i> <i>NI</i> | c.1314delC | p.(Val441C ys*4) | Heterozy gous | Frameshift variant | PV |
| 12 | F | 11 | Macroprolactinoma | Sporadic | <i>ME</i> <i>NI</i> | c.574C>T | p.(Gln192*) | Heterozy gous | Nonsenseva riant | PV |

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

F: female, M: male, Yrs: years, PRL: prolactin, GH: growth hormone, ACTH: adrenocorticotrophic 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.

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 | <i>AIP</i> | c.326C>T | p.(Ala109Val) | Heterozygous | Missense variant |
| 2 | M | 15 | Prolactinoma | Sporadic | <i>CDKN1B</i> | c.586C>T | p.(Arg196Cys) | Heterozygous | Missense variant |
| 3 | M | 31 | Macroprolactinoma | Sporadic | <i>MEN1</i> | c.903C>T | p.(=) | Heterozygous | Synonymous variant |
| 4 | F | 26 | Somatotropinoma | Sporadic | <i>SDHA</i> | c.512G>A | p.(Arg171His) | Heterozygous | Missense variant |
| 5 | M | 33 | Macroadenoma NFPA | Sporadic | <i>SDHB</i> | c.312C>T | p.(=) | Heterozygous | Synonymous variant |
| 6 | F | 25 | Macroprolactinoma | Sporadic | <i>SDHD</i> | c.110A>G | p.(Asp37Gly) | Heterozygous | Missense variant |
| 7 | F | 29 | Macroprolactinoma | Sporadic | <i>SDHD</i> | 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).