Very low frequency of germline *GPR101* genetic variation and no biallelic defects with *AIP* in a large cohort of patients with sporadic pituitary adenomas

Anne-Lise Lecoq^{1,2,5,*}, Jérôme Bouligand^{1,3,5,*}, Mirella Hage^{1,2,5}, Laure Cazabat², Sylvie Salenave², Agnès Linglart^{1,4}, Jacques Young^{1,2,5}, Anne Guiochon-Mantel^{1,3,5}, Philippe Chanson^{1,2,5} and Peter Kamenický^{1,2,5}

¹Faculté de Médecine Paris-Sud, Université Paris-Sud, Université Paris-Saclay, UMR-S1185, 94276 Le Kremlin Bicêtre, France, ²Assistance Publique-Hôpitaux de Paris, Hôpital de Bicêtre, Service d'Endocrinologie et des Maladies de la Reproduction, 94275 Le Kremlin-Bicêtre, France, ³Service de Génétique moléculaire, Pharmacogénétique et Hormonologie, 94275 Le Kremlin-Bicêtre, France, ⁴Service d'Endocrinologie Pédiatrique, 94275 Le Kremlin-Bicêtre, France and ⁵Institut National de la Santé et de la Recherche Médicale (INSERM) U1185, 94276 Le Kremlin-Bicêtre, France

^{*}(A-L Lecoq and J Bouligand contributed equally to this work)

Correspondence should be addressed to P Kamenický
Email
peter.kamenicky@u-psud.fr

Abstract

Context: Recently, germline and somatic *GPR101* p.(E308D) mutation was found in patients with isolated acromegaly.

It is not known whether *GPR101* point mutations are associated with other histological types of pituitary adenoma.

Objective: We sought germline *GPR101* mutations in patients with sporadic pituitary adenomas, and compared the phenotypes of *GPR101* mutation carriers and *AIP* mutation carriers.

Design: An observational cohort study performed between 2007 and 2014 in a single referral center.

Participants: This prospective study involved 766 unselected patients (413 women) with sporadic pituitary adenomas of all histotypes.

Methods: Entire *GPR101* and *AIP* coding sequence were screened for germline mutations.

Results: Twelve patients (1.6%) were found to carry the *GPR101* p.(E308D) mutation or rare *GPR101* variants. The minor allele frequency of the *GPR101* mutation and variants was higher in patients with pituitary adenomas than in unaffected individuals included in the Exome Aggregation Consortium database. Three of the six patients with the *GPR101* p.(E308D) mutation had adult-onset acromegaly, two had adrenocorticotropin-secreting adenomas, and one had a nonfunctioning macroadenoma. Six patients carried rare *GPR101* variants. Germline *AIP* mutations or rare *AIP* variants were identified in 32 patients (4.2%). *AIP* mutation carriers were younger at diagnosis than *GPR101* mutation carriers and non carriers. None of the patients harbored mutations in both the *GPR101* and *AIP* genes.

Conclusion: Germline *GPR101* mutations are very rare in patients with sporadic pituitary adenomas of various histotypes. No digenism with *AIP* was identified. Further studies are required to establish whether and how genetic variation in *GPR101* gene contributes to pituitary tumorigenesis.

European Journal of
Endocrinology
(2016) 174, 523–530

Introduction

The molecular mechanisms underlying pituitary tumorigenesis remain elusive. Very few pituitary adenomas occur as a component of hereditary syndromes such as MEN type 1 and 4, the Carney complex or in the absence of syndromic clinical features, familial isolated pituitary adenoma (FIPA). The vast majority of pituitary adenomas are sporadic (1). Recently, microduplications in chromosome region Xq26.3 were shown to cause very-young-onset X-linked acrogigantism (X-LAG) in patients with growth hormone (GH)-secreting pituitary adenomas or hyperplasia (2, 3). The duplicated region in Xq26.3 contained four protein-coding genes, of which only *GPR101* was frankly overexpressed in pituitary tumors from patients with X-LAG. *GPR101* encodes an orphan G-protein-coupled receptor, even though recent *in vitro* data suggest that *GPR101* mediates the biological effects of the GnRH-(1–5) pentapeptide (4). Both somatic and germline activating *GPR101* p.(E308D) mutation was found in a large international cohort of patients with isolated acromegaly (2). In keeping with this finding, we reported in a Letter to the Editor the cases of three patients (1.1%) with germline *GPR101* p.(E308D) mutation, identified among 263 unselected acromegalic patients from our cohort (5).

It is unclear whether *GPR101* mutations are associated with sporadic pituitary adenomas of other histological types. We therefore screened our entire cohort of 766 patients with sporadic pituitary adenomas for germline mutations of *GPR101*. We then analyzed the clinical and genetic characteristics of patients carrying the p.(E308D) mutation or previously unreported rare *GPR101* variants.

Germline mutations in *AIP* (encoding the Aryl-hydrocarbon receptor-Interacting Protein) have been identified in patients with both familial (6, 7, 8, 9, 10) and sporadic pituitary adenomas (10, 11, 12, 13, 14). The penetrance of pituitary adenomas in *AIP* mutation carriers is low, especially in patients with no familial history (1, 10). We thus screened our cohort for *AIP* mutations in order to search for digenism with *GPR101* variants that could account for the variable phenotype of *AIP*-mutated patients and influence the penetrance of pituitary adenomas in *AIP* mutation carriers.

Patients and methods

Patients

The cohort was prospectively constituted to evaluate the prevalence of *AIP* mutations in patients with sporadic

pituitary adenomas. All patients seen in our referral center for pituitary adenomas between June 2007 and December 2014 were asked to participate. Based on clinical, biological and/or histological criteria, the patients were grouped into the following diagnostic categories: GH-secreting adenomas, GH- and prolactin-secreting adenomas, prolactin-secreting adenomas, adrenocorticotrophin (ACTH)-secreting adenomas, nonfunctioning adenomas (including gonadotropin-secreting adenomas) and thyrotrophin-secreting adenomas (including thyrotrophin (TSH)- and GH-secreting adenomas). Gigantism was defined by excessively rapid growth velocity for age in children or adolescents (>97th percentile), or a final height > +2 SD above normal for the French population, together with abnormal insulin-like growth factor 1 values for age and unsuppressed GH after an oral glucose tolerance test. Patients with MEN type 1 and type 4, the Carney complex, the McCune–Albright syndrome or X-LAG were not enrolled, and samples from relatives of *GPR101* or *AIP* mutation-positive *simplex* cases were not studied. Relevant clinical, biological, morphological and histological data were collected for all the patients. Based on pituitary magnetic resonance imaging, the adenomas were divided into microadenomas (larger diameter <10 mm), macroadenomas (larger diameter between 10 and 39 mm), and giant adenomas (larger diameter >40 mm). All the patients or their relatives signed informed consent forms approved by the local ethics committee.

Genetic testing

Genomic DNA was extracted from white blood cells either manually using the high salt method or by automated QIA-symphony SP instrument (Qiagen). We sequenced the entire coding sequence of the *GPR101* gene using the following primers: A-forward GTGTTTCTTTTGTCAC-CAGCATAG and A-reverse GGGTCATCTTGGACGGGTA; B-forward TTAGCCTCACCCACCTGTTC, and B-reverse GCCCCTGGCCTCTACACTAC; C-forward AGGTGAGGT-CAAGGCCAAG, and C-reverse AATGGTCTTGTGCATGTAGCC; D-forward CAAAGCTGCTAAAGTGATCTTCAT, and D-reverse TTAATGAATTGTGGGTCCATTG. The entire *AIP* coding region (exons 1–6) and intron-exon junctions were amplified and sequenced with primers described elsewhere (14). PCR and sequencing products were purified on a Biomek NXP-96 Laboratory Automation Workstation (Beckman Coulter, Villepinte, France) with Agencourt Ampure XP and Agencourt Cleanseq (Beckman

Coulter Genomics, Danvers, MA, USA). Sequencing products were analyzed with an automated capillary sequencer (ABI PRISM 3730S Genetic Analyzer; Applied Biosystems). Electropherogram-derived sequences were compared with NCBI references for the *GPR101* gene (NG_016367.1 RefSeqGene and NM_054021.1) and the *AIP* gene (NG_008969.1 RefSeq- Gene and NM_003977.2 transcript). All genetic abnormalities were confirmed by a second analysis. Screening for large deletion or duplication in *AIP* gene by multiplex ligation-dependent probe amplification (MLPA) was performed in patients with GH-secreting and mixt GH- and prolactin-secreting pituitary adenomas, who were younger than 40 years at diagnosis, using the SALSA MLPA kit P244 (MRC Holland, Amsterdam, The Netherlands). The promoter area of *AIP* was not analyzed. The presence of all new variants was verified in the Exome Aggregation Consortium (ExAC) database (<http://exac.broadinstitute.org/>). Only rare variants with minor allele frequencies (MAF) <1% in unaffected individuals included in the ExAC database were considered for further analysis. *In silico* analysis of *GPR101* and *AIP* variants was performed with various algorithms contained in Alamut Mutation Interpretation Software (Interactive Bio Software, Rouen, France) in order to assess their impact at both the mRNA and protein levels. The new variants were classified as pathogenic, likely pathogenic, variants of uncertain significance (VUS) or likely benign, according to the standards and guidelines for the interpretation of sequence variants (15).

Statistical analysis

Continuous variables are reported as median (range). Allele frequencies and categorical variables were compared using the χ^2 test. The nonparametric Mann–Whitney *U*-test or the Kruskal–Wallis test was used to analyze the quantitative data. Statistical significance was assumed

when the *P* value was <0.05. All statistical analyses were carried out using GraphPad Prism 5 Statistical Software (GraphPad Software Inc., La Jolla, CA, USA).

Results

Study population

Seven hundred seventy seven individuals were screened for germline *GPR101* and *AIP* mutations. Eight patients with pituitary tumors in whom the diagnosis of pituitary adenoma could not be established because of a lack of clinical, biological and/or histological data were excluded from the analysis. Two acromegalic patients who were the members of a kindred with FIPA were also excluded, as was a sporadic giant with X-LAG. The study population thus consisted of 766 patients with sporadic pituitary adenomas, comprising 443 patients previously described in a study of germline *AIP* mutations in apparently sporadic pituitary adenomas (14), and 323 new patients. The characteristics of the cohort are shown in Table 1. Thirty-eight patients were diagnosed in childhood or adolescence (<18 years). Among the patients with acromegaly, 22 patients had non-syndromic gigantism and 241 had adult-onset acromegaly due to pure GH-secreting or GH- and prolactin-cosecreting adenomas. Except for ACTH-secreting adenomas, macroadenomas predominated in patients with other histotypes.

Patients with the *GPR101* p.(E308D) mutation or rare *GPR101* variants

Twelve (1.6%) of the 766 patients with pituitary adenomas were positive for the previously reported germline p.(E308D) mutation or for rare *GPR101* variants. The clinical characteristics of these patients are shown in Table 2. Age at diagnosis did not differ between patients

Table 1 General description of the study population.

Adenoma type	Number of patients	Number of females <i>n</i> (%)	Age at diagnosis median (range)	Macroadenoma <i>n</i> (%)
Total	766	413 (54)	39 (11–87)	563 (73)
GH-secreting	218	100 (46)	42 (15–77)	172 (79)
GH- and prolactin-cosecreting	45	16 (36)	36 (14–66)	44 (98)
Prolactin-secreting	256	164 (64)	29 (13–78)	151 (59)
ACTH-secreting	68	52 (76)	38 (11–73)	24 (35)
Nonfunctioning ^a	165	77 (47)	55 (14–87)	159 (96)
Thyotropin-secreting	14	4 (29)	40 (22–75)	13 (93)

^aNonfunctioning pituitary adenomas include gonadotropin-secreting adenomas.

Clinical Study	A-L Lecoq, J Bouligand and others	<i>GPR101</i> mutations and pituitary adenoma	174:4	526
-----------------------	-----------------------------------	---	--------------	------------

Table 2 Clinical, morphological and genetic characteristics of the patients with the *GPR101* p.(E308D) mutation or rare *GPR101* variants.

Patient No.	Adenoma type	Sex	Age at diagnosis (years)	Symptoms at diagnosis	Adenoma size	Mutation or variant	Protein consequence
1 ^a	GH-secreting	F	48	Acromegaly + HPT	Macro	c.924 G>C	p.(E308D)
2 ^a	GH-secreting	M	45	Acromegaly	Macro	c.924 G>C	p.(E308D)
3 ^a	GH-secreting	F	41	Mild acromegaly	Micro	c.924 G>C	p.(E308D)
4 ^a	GH-secreting	M	44	Acromegaly	Macro	c.1098C>A	p.(D366E)
5	Prolactin-secreting	F	33	Amenorrhea	Macro	c.974C>T	p.(T325I)
6	Prolactin-secreting	F	29	Primary amenorrhea	Micro	c.1294C>T	p.(P432S)
7	ACTH-secreting	F	26	Cushing's syndrome	Micro	c.365T>Y	p.(I122T)
8	ACTH-secreting	F	21	Cushing's syndrome	Micro	c.924 G>C	p.(E308D)
9	ACTH-secreting	M	38	Visual defects	Giant	c.924 G>C	p.(E308D)
10	Nonfunctioning	M	71	Panhypopituitarism	Macro	c.924 G>C	p.(E308D)
11	Nonfunctioning	M	35	Pituitary apoplexy	Macro	c.1074C>T	p.(=)
12	TSH and GH-secreting	M	32	Visual defects	Macro	c.354C>T	p.(=)

HPT, hyperparathyroidism. Genetic analysis in patient number 1 did not identify any germline mutations or deletions in *MEN1* and *CDKN1B* genes.

^aPreviously reported cases (2, 5).

with and without *GPR101* mutation (36 years (21–71) vs 39 years (11–87), not significant). *In silico* prediction of the pathogenicity of the rare *GPR101* variants is shown in Table 3. The MAFs of the p.(E308D) mutant and of the rare *GPR101* variants are shown in Table 4. The overall MAF of the *GPR101* p.(E308D) mutation and variants was higher in patients with pituitary adenomas than in unaffected individuals included in the ExAC database.

Six patients had the germline p.(E308D) mutation. Three of them (patients number 1, 2 and 3 in Table 2) had adult-onset sporadic acromegaly due to a pure GH-secreting adenoma and have been described elsewhere (2, 5). Regarding the two patients with ACTH-secreting adenomas and p.(E308D) mutation, the female patient had typical early-onset Cushing's disease and a microadenoma, whereas the male patient had a giant pituitary adenoma responsible for visual impairment but only subclinical hypercortisolism. One p.(E308D)-carrying male patient had a nonfunctioning macroadenoma revealed by panhypopituitarism.

Six patients had rare germline *GPR101* variants. One patient with sporadic acromegaly (patient number 4 in Table 2) presented with the p.(D366E) variant and has been reported in (5). This likely pathogenic variant is not reported in the ExAC database. In addition, we found four rare *GPR101* variants c.354C>T, p.(=), p.(I122T), p.(T325I) and c.1074C>T, p.(=), also not reported in the ExAC database (Table 3). The silent variant c.1074C>T, p.(=) is known in NCBI as #rs537038614, but data on MAF are not available. Finally, the p.(P432S) variant is known as #rs114879480 in NCBI and is reported in the ExAC database, but its MAF was higher in patients with prolactinomas than in unaffected individuals (Table 4). Of note, this likely pathogenic variant was identified in a patient with early-onset prolactinoma and primary amenorrhea (Table 2). Further, the likely pathogenic variant p.(I122T) was identified in a young patient with Cushing's syndrome and the silent variant c.354C>T, p.(=) was identified in a patient with a mixed TSH- and GH-secreting adenoma. The missense variants p.(I122T), p.(T325I),

Table 3 *In silico* prediction of the pathogenicity of the new rare *GPR101* variants.

Variant	Protein-level consequence	Location in protein	Pathogenicity	Conservation
c.354C>T	p.(=)	ICL2	VUS	Yes
c.365T>Y	p.(I122T)	TM3	Likely pathogenic	Yes
c.974C>Y	p.(T325I)	ICL3	VUS	Yes
c.1074C>T	p.(=)	ICL3	VUS	Yes
c.1098C>A	p.(D366E)	ICL3	Likely pathogenic	Yes
c.1294C>T	p.(P432S)	ECL3	Likely pathogenic	Yes

ICL2, intracellular loop 2; TM3, 3rd transmembrane segment; ICL3, intracellular loop 3; ECL3, extracellular loop 3; VUS, variants of uncertain significance.

Clinical Study	A-L Lecoq, J Bouligand and others	<i>GPR101</i> mutations and pituitary adenoma	174:4	527
-----------------------	-----------------------------------	---	--------------	------------

Table 4 Minor allele frequencies of the p.(E308D) *GPR101* mutation and original rare *GPR101* variants identified in this cohort of patients with sporadic pituitary adenomas.

Mutation or variant	Protein consequence	Adenoma type	Number of variant carriers	Allele frequency (%)	P value vs general population
c.354C>T	p.(=)	TSH and GH-secreting	1	5.56	NR, <0.0001
c.365T>Y	p.(I122T)	ACTH-secreting	1	0.83	NR, <0.0001
c.924G>C	p.(E308D)	GH-secreting	3	0.94	0.09
		ACTH-secreting	2	1.67	0.0199
		Nonfunctioning	1	0.41	0.90
		Total	6	0.51	0.42
c.974C>Y	p.(T325I)	Prolactin-secreting	1	0.24	NR, <0.0001
c.1074C>T	p.(=)	Nonfunctioning	1	0.41	NR, <0.0001
c.1098C>A	p.(D366E)	GH-secreting	1	0.31	NR, <0.0001
c.1294C>T	p.(P432S)	Prolactin-secreting	1	0.24	0.0488
All variants			12	1.02	0.0013

NR, not reported in the ExAC database.

p.(D366E) and p.(P432S) affect highly conserved amino acids (data not shown).

Patients with *AIP* mutations or rare *AIP* variants

Germline *AIP* mutations or rare variants were identified in 32 (4.2%) of the 766 patients with pituitary adenomas. Sixteen *AIP* mutation carriers have previously been reported in our study of 443 patients with sporadic pituitary adenomas (14). Among the 16 new patients with *AIP* mutations, three had pathogenic and four likely pathogenic *AIP* mutations, seven had VUS, and two had

likely benign *AIP* variants (Table 5). Altogether, we identified 25 different *AIP* mutations or rare variants in our cohort, five of which are new (Table 5).

Patients with *AIP* mutations were younger at diagnosis than both patients without *AIP* mutations and patients with *GPR101* mutations (26 years (11–70) vs 40 years (13–87), $P<0.0001$ and vs 36 years (21–71), $P=0.0117$) (Fig. 1). Among patients with acromegaly, those with *AIP* mutations were younger at diagnosis than those without *AIP* mutations (19 years (14–42) vs 40 years (14–77); $P=0.0009$). Six of the 10 *AIP* mutation carriers with GH-secreting or GH- and prolactin-secreting adenomas

Table 5 Clinical, morphological and genetic characteristics of previously unreported patients with *AIP* mutations or rare *AIP* variants.

Patient No.	Adenoma type	Sex	Age at diagnosis (years)	Adenoma size	Mutation or variant	Protein	Pathogenicity
1	GH- and prolactin-cosecreting	F	42	Macro	c.2T>C	p.?	Pathogenic
2	GH-secreting	M	17	Giant	c.*14C>A	p.?	Likely benign
3	ACTH-secreting	F	40	Macro	c.26G>A	p.(R9Q)	VUS
4	ACTH-secreting	F	11	Micro	c.47G>A	p.(R16H)	Likely benign
5	Prolactin-secreting	M	46	Macro	c.100-6C>A	p.?	VUS ^a
6	Prolactin-secreting	F	29	Macro	c.100-18C>T	p.?	Likely pathogenic
7	Prolactin-secreting	M	23	Macro	c.382C>T	p.(R128C)	VUS ^a
8	Prolactin-secreting	M	37	Macro	c.382C>T	p.(R128C)	VUS ^a
9	Nonfunctioning	F	26	Giant	c.382C>T	p.(R128C)	VUS ^a
10	Prolactin-secreting	F	26	Micro	c.591G>A	p.(E197=)	Likely pathogenic
11	GH- and prolactin-cosecreting	M	16	Macro	c.646G>T	p.(E216*)	Pathogenic
12	Prolactin-secreting	F	32	Macro	c.753G>A	p.(L251=)	VUS ^a
13	Prolactin-secreting	M	17	Macro	c.772C>T	p.(L258F)	Likely pathogenic ^a
14	Nonfunctioning	M	48	Giant	c.896C>T	p.(A299V)	VUS
15	Prolactin-secreting	M	36	Micro	c.911G>A	p.(R304Q)	Pathogenic
16	Nonfunctioning	M	70	Macro	c.973C>A	p.(R325=)	VUS ^a

VUS, variants of uncertain significance.

^aMutations described here for the first time.

Figure 1

Age at diagnosis among 766 patients with sporadic pituitary adenomas, according to *GPR101* and *AIP* mutation status.

Median age at diagnosis is indicated by lines. * $P<0.05$;

*** $P<0.001$.

were giants, compared with 16 giants among 253 *AIP* mutation-negative acromegalic patients ($P<0.0001$). Nine of the ten *AIP* mutation-positive acromegalic patients were males, compared with 138 males among 253 *AIP* mutation-negative acromegalic patients ($P=0.0268$). None of the 766 patients harbored germline mutations in both the *GPR101* and *AIP* genes.

Discussion

We describe the results of genetic screening for germline *GPR101* mutations in the largest reported cohort of unselected patients with sporadic pituitary adenomas managed in a tertiary care center for pituitary diseases. For the first time, we report that the *GPR101* p.(E308D) mutation and rare *GPR101* variants are not restricted to patients with sporadic acromegaly but also occur in a small fraction of patients with sporadic pituitary adenomas that are not GH-secreting. We identified the p.(E308D)

mutation in two *simplex* patients with ACTH-secreting pituitary adenomas and one patient with a nonfunctioning pituitary adenoma. In addition, we found original rare *GPR101* variants in one patient with acromegaly, two patients with prolactin-secreting adenomas, one patient with Cushing's disease, one patient with a TSH- and GH-cosecreting adenoma and one patient with a nonfunctioning pituitary adenoma. Genetic screening for germline *AIP* mutations, conducted in parallel, identified new *AIP* mutation-positive patients and allowed us to analyze phenotypic differences between *GPR101* and *AIP* mutation carriers.

The overall minor allelic frequency of the *GPR101* p.(E308D) mutation and the rare *GPR101* variants was higher in our cohort than in the ExAC database, which contains sequence data for about 61 500 unrelated and apparently unaffected individuals. However, germline *GPR101* genetic variation was very rare in this large cohort of patients with sporadic pituitary adenomas and therefore does not seem to have major pathophysiological significance for pituitary tumorigenesis.

The *GPR101* p.(E308D) substitution is considered to be a pathogenic gain-of-function mutation, as overexpression of the p.(E308D) mutant in rat GH3 cells affects cell proliferation and GH secretion (2). However, overexpression of the p.(E308D) mutant did not result in a stronger cyclic AMP response elements activation than overexpression of the non-mutant *GPR101* (2). In addition, it should be noted that p.(E308D) is also present in unaffected persons, with an allelic frequency of 0.36% according to the ExAC database (16). In our cohort, the p.(E308D) allele was over-represented in patients with ACTH-secreting adenomas. A similar trend towards a higher MAF was also observed in patients with GH-secreting adenomas but it did not reach statistical significance. A study of thousands rather than hundreds of patients would be necessary to determine the true importance of p.(E308D) in the pathophysiology of sporadic pituitary adenomas. Indeed, the reported lack of the p.(E308D) mutation in FIPA families (2) could be due to an inadequate sample size.

The phenotype of our *GPR101* mutation-positive patients was variable and markedly different from that of the *AIP* mutation carriers. Half the carriers of the *GPR101* mutant or variants were women. Likewise, half the *GPR101* mutation-positive acromegalic patients were female, contrasting with the reported male predominance among *AIP* mutation-positive patients with sporadic acromegaly (1). However, in contrast to previous reports, in a recent study from a large international cohort there

was no predominance of male patients among the *AIP* mutation positive familial cases (10). Age at diagnosis did not differ between *GPR101* mutation-positive and -negative patients, whereas *AIP* mutation carriers were diagnosed at a younger age than non carriers (Fig. 1). In particular, GH excess was diagnosed after the age of 40 years in all four of our *GPR101* mutation-positive acromegalic patients, whereas most *AIP*-mutated acromegalic patients are diagnosed before the age of 30 years (1). None of these four patients had GH- and prolactin-secreting adenomas, whereas this type of adenoma is frequently observed in *AIP* mutation-positive acromegalic patients (1). Unlike patients with *AIP* mutations, none of our 22 patients with sporadic non-syndromic gigantism harbored germline *GPR101* mutations. Childhood onset of GH excess seems to require *GPR101* gene overexpression rather than just its activation by a point mutation (2). Given the variable clinical features of *GPR101* mutation-positive patients with sporadic pituitary adenomas, clinical findings cannot help to identify potential mutation carriers.

The low penetrance of sporadic pituitary adenomas and their variable clinical features among *AIP* mutation carriers suggests the involvement of other disease-modifying genes. None of the patients screened here harbored germline mutations in both the *GPR101* and *AIP* genes, but the low frequency of *GPR101* and *AIP* mutations in this cohort means that digenism cannot be ruled out. Trivellin *et al.* (2) reported that at least one *GPR101* mutation-positive patient had a somatic *GPR101* mutation in the adenoma but no germline mutation. Somatic *GPR101* mutations might thus have a disease-modifying role in *AIP*-mutated pituitary adenomas (1, 10).

This study has several limitations. As already mentioned, recruitment of a larger population through international collaboration would enable the identification of more mutation carriers and provide a more comprehensive analysis of their phenotype. As our cohort was composed only of patients who had a hospital work-up, many patients only seen in an outpatient clinic were excluded, explaining why microprolactinomas were underrepresented. Finally, functional *in vitro* studies would be necessary to confirm the pathogenicity of the rare *GPR101* and *AIP* variants predicted by *in silico* analysis.

In conclusion, genetic screening for *GPR101* mutations in the largest reported single-center cohort of patients with sporadic pituitary adenomas of various histological types showed a very low prevalence of *GPR101* germline mutation and rare variants. The phenotype of *GPR101* mutation-positive patients

was variable and differed from that of *AIP* mutation-positive patients. None of the patients harbored germline mutations in both the *GPR101* and *AIP* genes. Whether and how germline genetic variation in *GPR101* contributes to pituitary tumorigenesis remains to be further investigated.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

A-L Lecoq was the recipient of a Poste d'Accueil INSERM and a grant from Association de Recherche contre le Cancer (ARC). P Kamenický was the recipient of a Contrat d'Interface INSERM.

Acknowledgements

We are indebted to Elodie Dupuis and Zaina Ait Arkoub for excellent technical assistance and to the nursing staff of Service d'Endocrinologie of Hôpital Bicêtre.

References

- 1 Lecoq A-L, Kamenický P, Guiochon-Mantel A & Chanson P. Genetic mutations in sporadic pituitary adenomas – what to screen for? *Nature Reviews Endocrinology* 2015 **11** 43–54. (doi:10.1038/nrendo.2014.181)
- 2 Trivellin G, Daly AF, Fauchz FR, Yuan B, Rostomyan L, Larco DO, Scherthaner-Reiter MH, Szarek E, Leal LF, Caberg J-H *et al.* Gigantism and acromegaly due to Xq26 microduplications and *GPR101* mutation. *New England Journal of Medicine* 2014 **371** 2363–2374. (doi:10.1056/NEJMoa1408028)
- 3 Beckers A, Lodish MB, Trivellin G, Rostomyan L, Lee M, Fauchz FR, Yuan B, Choong CS, Caberg J-H, Verrua E *et al.* X-linked acro-gigantism syndrome: clinical profile and therapeutic responses. *Endocrine-Related Cancer* 2015 **22** 353–367. (doi:10.1530/ERC-15-0038)
- 4 Cho-Clark M, Larco DO, Semsarzadeh NN, Vasta F, Mani SK & Wu TJ. GnRH-(1-5) transactivates EGFR in Ishikawa human endometrial cells via an orphan G protein-coupled receptor. *Molecular Endocrinology* 2014 **28** 80–98. (doi:10.1210/me.2013-1203)
- 5 Kamenický P, Bouligand J & Chanson P. Gigantism, acromegaly, and *GPR101* mutations. *New England Journal of Medicine* 2015 **372** 1264. (doi:10.1056/NEJMc1500340#SA1)
- 6 Vierimaa O, Georgitsi M, Lehtonen R, Vahteristo P, Kokko A, Raitila A, Tuppurainen K, Ebeling TML, Salmela PI, Paschke R *et al.* Pituitary adenoma predisposition caused by germline mutations in the *AIP* gene. *Science* 2006 **312** 1228–1230. (doi:10.1126/science.1126100)
- 7 Daly AF, Vanbellighen J-F, Khoo SK, Jaffrain-Rea M-L, Naves LA, Guitelman MA, Murat A, Emy P, Gimenez-Roqueplo A-P, Tamburrano G *et al.* Aryl hydrocarbon receptor-interacting protein gene mutations in familial isolated pituitary adenomas: analysis in 73 families. *Journal of Clinical Endocrinology and Metabolism* 2007 **92** 1891–1896. (doi:10.1210/jc.2006-2513)
- 8 Leontiou CA, Gueorguiev M, van der Spuy J, Quinton R, Lolli F, Hassan S, Chahal HS, Igreja SC, Jordan S, Rowe J *et al.* The role of the aryl hydrocarbon receptor-interacting protein gene in familial and sporadic

Clinical Study	A-L Lecoq, J Bouligand and others	GPR101 mutations and pituitary adenoma	174:4	530
----------------	-----------------------------------	--	-------	-----

- pituitary adenomas. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 2390–2401. (doi:10.1210/jc.2007-2611)
- 9 Daly AF, Tichomirowa MA, Petrossians P, Heliövaara E, Jaffrain-Rea M-L, Barlier A, Naves LA, Ebeling T, Karhu A, Raappana A *et al.* Clinical characteristics and therapeutic responses in patients with germ-line *AIP* mutations and pituitary adenomas: an international collaborative study. *Journal of Clinical Endocrinology and Metabolism* 2010 **95** E373–E383. (doi:10.1210/jc.2009-2556)
 - 10 Hernández-Ramírez LC, Gabrovská P, Dénes J, Stals K, Trivellin G, Tilley D, Ferraù F, Evanson J, Ellard S, Grossman AB *et al.* Landscape of familial isolated and young-onset pituitary adenomas: prospective diagnosis in *AIP* mutation carriers. *Journal of Clinical Endocrinology and Metabolism* 2015 **100** E1242–E1254. (doi:10.1210/jc.2015-1869)
 - 11 Barlier A, Vanbellinghen J-F, Daly AF, Silvy M, Jaffrain-Rea M-L, Trouillas J, Tamagno G, Cazabat L, Bours V, Brue T *et al.* Mutations in the aryl hydrocarbon receptor interacting protein gene are not highly prevalent among subjects with sporadic pituitary adenomas. *Journal of Clinical Endocrinology and Metabolism* 2007 **92** 1952–1955. (doi:10.1210/jc.2006-2702)
 - 12 Cazabat L, Libè R, Perlemoine K, René-Corail F, Burnichon N, Gimenez-Roqueplo A-P, Dupasquier-Fediaevsky L, Bertagna X, Clauser E, Chanson P *et al.* Germline inactivating mutations of the aryl hydrocarbon receptor-interacting protein gene in a large cohort of sporadic acromegaly: mutations are found in a subset of young patients with macroadenomas. *European Journal of Endocrinology* 2007 **157** 1–8. (doi:10.1530/EJE-07-0181)
 - 13 Tichomirowa MA, Barlier A, Daly AF, Jaffrain-Rea M-L, Ronchi C, Yaneva M, Urban JD, Petrossians P, Elenkova A, Tabarin A *et al.* High prevalence of *AIP* gene mutations following focused screening in young patients with sporadic pituitary macroadenomas. *European Journal of Endocrinology* 2011 **165** 509–515. (doi:10.1530/EJE-11-0304)
 - 14 Cazabat L, Bouligand J, Salenave S, Bernier M, Gaillard S, Parker F, Young J, Guiochon-Mantel A & Chanson P. Germline *AIP* mutations in apparently sporadic pituitary adenomas: prevalence in a prospective single-center cohort of 443 patients. *Journal of Clinical Endocrinology and Metabolism* 2012 **97** E663–E670. (doi:10.1210/jc.2011-2291)
 - 15 Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E *et al.* Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics in Medicine* 2015 **17** 405–424. (doi:10.1038/gim.2015.30)
 - 16 Roohi J. Gigantism, acromegaly, and *GPR101* mutations. *New England Journal of Medicine* 2015 **372** 1264–1265. (doi:10.1056/NEJMc1500340)

Received 22 October 2015

Revised version received 2 January 2016

Accepted 20 January 2016