

CLINICAL STUDY

Genetic analysis in young patients with sporadic pituitary macroadenomas: besides AIP don't forget MEN1 genetic analysis

Thomas Cuny^{1,20}, Morgane Pertuit², Mona Sahnoun-Fathallah³, Adrian Daly⁴, Gianluca Occhi⁵, Marie Françoise Odou⁶, Antoine Tabarin⁷, Marie Laure Nunes⁷, Brigitte Delemer⁸, Vincent Rohmer⁹, Rachel Desailoud¹⁰, Véronique Kerlan¹¹, Olivier Chabre¹², Jean-Louis Sadoul¹³, Muriel Cogne¹⁴, Philippe Caron¹⁵, Christine Cortet-Rudelli¹⁶, Anne Lienhardt¹⁷, Isabelle Raingeard¹⁸, Anne-Marie Guedj¹⁹, Thierry Brue³, Albert Beckers⁴, Georges Weryha¹, Alain Enjalbert^{2,20} and Anne Barlier^{2,20}

¹Department of Endocrinology, University Hospital of Nancy - Brabois, 54500 Vandoeuvre-les-Nancy, France, ²Laboratory of Molecular Biology, University Hospital of Marseille - APHM - La Conception, 13005 Marseille, France, ³Department of Endocrinology, Centre de Référence des Maladies Rares d'Origine Hypophysaire DEFHY, University Hospital of Marseille - APHM - La Timone, 13385 Marseille cedex 5, France, ⁴Department of Endocrinology, University Hospital of Liège, University of Liège, 4000 Liège, Belgium, ⁵Department of Medicine, University of Padova, Padova, Italy, ⁶Laboratory of Biochemistry and Molecular Biology, University Hospital of Lille, 59037 Lille, France, ⁷Department of Endocrinology, University Hospital of Bordeaux - Haut-Lévêque, 33604 Pessac Cedex, France, ⁸Department of Endocrinology, University Hospital of Reims - Robert Debré, 51092 Reims, France, ⁹Department of Endocrinology, University Hospital of Angers, 49933 Angers Cedex 9, France, ¹⁰Department of Endocrinology, University Hospital of Amiens - Hopital Sud, 80054 Amiens Cedex, France, ¹¹Department of Endocrinology, University Hospital of Brest - Cavale Blanche, 29609 Brest Cedex, France, ¹²Department of Endocrinology, University Hospital of Grenoble - Albert Michallon, 38043 Grenoble, France, ¹³Department of Endocrinology, University Hospital of Nice - Hôpital l'Archet, 06200 Nice, France, ¹⁴Department of Endocrinology, University Hospital of Reunion Island, Saint Pierre, France, ¹⁵Department of Endocrinology, University Hospital of Toulouse - Hopital Larrey, 31059 Toulouse, France, ¹⁶Department of Endocrinology, University Hospital of Lille, 59037 Lille, France, ¹⁷Department of Paediatrics, University Hospital of Limoges, 87000 Limoges, France, ¹⁸Department of Endocrinology, University Hospital of Montpellier - Hôpital Lapeyronie, 34295 Montpellier cedex, France, ¹⁹Department of Endocrinology, University Hospital of Nîmes, 30000 Nîmes, France and ²⁰Laboratoire CRN2M, UMR 7286-CNRS, Faculté de Médecine Nord, Aix-Marseille University, 51 Boulevard Pierre Dramard, 13344 Marseille cedex 15, France

(Correspondence should be addressed to A Barlier at Laboratoire CRN2M, UMR 7286-CNRS, Faculté de Médecine, Nord Aix-Marseille University; Email: anne.barlier@univ-amu.fr)

Abstract

Context: Germline mutations in the aryl hydrocarbon receptor interacting protein gene (*AIP*) have been identified in young patients (age ≤ 30 years old) with sporadic pituitary macroadenomas. Otherwise, there are few data concerning the prevalence of multiple endocrine neoplasia type 1 (*MEN1*) mutations in such a population.

Objective: We assessed the prevalence of both *AIP* and *MEN1* genetic abnormalities (mutations and large gene deletions) in young patients (age ≤ 30 years old) diagnosed with sporadic and isolated macroadenoma, without hypercalcemia and/or *MEN1*-associated lesions.

Design: The entire coding sequences of *AIP* and *MEN1* were screened for mutations. In cases of negative sequencing screening, multiplex ligation-dependent probe amplification was performed for the detection of large genetic deletions.

Patients and settings: One hundred and seventy-four patients from endocrinology departments of 15 French University Hospital Centers were eligible for this study.

Results: Twenty-one out of 174 (12%) patients had *AIP* ($n=15$, 8.6%) or *MEN1* ($n=6$, 3.4%) mutations. In pediatric patients (age ≤ 18 years old), *AIP/MEN1* mutation frequency reached nearly 22% ($n=10/46$). *AIPmut* and *MEN1mut* were identified in 8/79 (10.1%) and 1/79 (1.2%) somatotropinoma patients respectively; they each accounted for 4/74 (5.4%) prolactinoma (PRL) patients with mutations. Half of those patients ($n=3/6$) with gigantism displayed mutations in *AIP*. Interestingly, 4/12 (33%) patients with non-secreting adenomas bore either *AIP* or *MEN1* mutations, whereas none of the eight corticotroph adenomas or the single thyrotropinoma case had mutations. No large gene deletions were observed in sequencing-negative patients.

Conclusion: Mutations in *MEN1* can be of significance in young patients with sporadic isolated pituitary macroadenomas, particularly PRL, and together with *AIP*, we suggest genetic analysis of *MEN1* in such a population.

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Introduction

Familial cases of pituitary adenomas (PA) represent up to 5% of all PA, with 2.7% related to multiple endocrine neoplasia type 1 (MEN1) (1) and nearly 2.5% related to the clinical entity familial isolated pituitary adenomas (FIPA) (2). Together the two syndromes comprise the most common causes of hereditary conditions predisposing to PA (3). In 2006, Vierimaa *et al.* (4) identified mutations in the aryl hydrocarbon receptor interacting protein (AIP) gene in the familial setting of PA. In FIPA kindreds, AIP mutations occur in 15–20% of cases (5), whereas they occur at a very low frequency in sporadic cases, between 0 and 4% (6, 7, 8, 9). Because patients mutated for AIP (AIPmut) have typically early onset disease and larger PA compared with controls (10), Tichomirowa *et al.* (11) performed AIP screening in young patients with isolated sporadic macroadenomas and identified that nearly 12% of patients had germline AIP mutations.

Mutations in the tumor suppressor gene MEN1 predispose to multiple endocrine and non-endocrine diseases including PA (12) that occur in ~40% of MEN1 cases (13). Whereas hyperparathyroidism is frequently reported as the first manifestation of MEN1 syndrome, pituitary disease can be the first lesion diagnosed in about 15% of patients with mutations in MEN1 (MEN1mut) (13, 14). Nevertheless, unlike AIP, there is limited data concerning the prevalence of MEN1 mutations in the specific subset of young patients diagnosed with isolated macroadenomas without any other disease of the MEN1 spectrum.

Therefore, we conducted a prospective study of a large cohort of patients in France that had been diagnosed with sporadic macroadenomas between January 2007 and December 2011 to determine the prevalence of both AIP and MEN1 gene abnormalities (point mutations and large deletions). We enrolled only patients diagnosed before 30 years old.

Materials and methods

Subjects

This genetic screening was performed in 174 patients with sporadic pituitary macroadenoma (maximal diameter ≥ 10 mm on pituitary MRI), diagnosed before 30 years of age and without hypercalcemia (corrected for serum albumin). Patients were enrolled from endocrinology departments of 15 French University Hospital Centers. All subjects provided informed written consent for the genetic screening. A subgroup of 59 patients had previously undergone AIP studies as part of an international collaborative study (11).

There were 79 (45.4%) subjects with somatotropinomas (49 males and 30 females, mean age at diagnosis 24.2 ± 5.9 years), 74 (42.5%) with prolactinomas (PRL; 39 males and 35 females, mean age 20.3 ± 5.2 years),

12 (6.8%) with clinically non-functioning PA (NFPAs, six males and six females, mean age 20.7 ± 6 years), eight (4.6%) with corticotroph adenomas (two males and six females, age 22 ± 5.4 years), and one female, aged 25 years, with a thyrotropinoma.

None of the subjects had a family history of MEN1 or FIPA. Family members of MEN1mut or AIPmut patients were contacted whenever possible and underwent genetic screening, followed by pituitary MRI and hormonal testing in case of positive genetic analysis.

Genomic analysis of AIP and MEN1

Genomic DNA from peripheral blood leukocytes was extracted and the coding exons and exon–intron boundaries of the AIP and MEN1 genes (NM_130799.2, NM_003977.2) were PCR amplified and screened by direct sequencing. Genomic DNA was also analyzed for large deletion in both genes by multiplex ligation-dependent probe (Salsa MLPA probe-mix P244-B1 AIP-MEN1, MRC-Holland, Amsterdam, The Netherlands). A 4.8 Mb region (from 11q13 to 11q13.3) was analyzed using probes localized on the MEN1 gene (exon 11, 10, 5 to 2), SNX15, FAM89B, RELA, SART1, and BRMTS1 genes; AIP gene (exons 1–6); and CCND1 gene. The potential effect of each missense or silent variation on AIP or menin protein was evaluated *in silico* using a battery of tools: Polyphen2 (<http://genetics.bwh.harvard.edu>), UMD-predictor (15), and Alamut 2.2.0 software (including SpliceSiteFinder, MaxEntScan, MNSPLICE, GeneSplicer, Human Splicing finder, RESCUE-ESE).

Haplotype analysis

The AIP p.Gly117Alafs*39 mutation carriers were genotyped using 14 microsatellite markers surrounding both AIP and MEN1 genes, located at 64.3 and 67.0 Mb respectively. Markers were PCR amplified from genomic DNA, separated on an ABI 3730XL DNA sequencer, and analyzed with Peak Scanner v1.0 software (Applied Biosystems). Genetic markers' primers sequences and amplification condition were reported elsewhere (16).

Statistical analysis

The Mann–Whitney *U* test was used for statistical analysis. *P* values below 0.05 were considered to denote statistical significance in this study. The mean age at diagnosis in each group of patients is referred to with s.d. (mean age \pm s.d.).

Results

Genomic DNA AIP and MEN1 mutations among the study cohort

AIP and MEN1 genetic analysis in the study cohort identified 21 patients bearing mutations (21/174,

12%). Fifteen had *AIP* (8.6%) and six had *MEN1* (3.4%) mutations (Table 1). No large genetic deletion was observed in any case using MLPA. In the cohort, the mean age at diagnosis was significantly lower in *AIPmut* patients compared with *MEN1mut* and non-mutated groups (18.7 ± 5 years (*AIP*) vs 22.2 ± 7.6 years (*MEN1*) and 22.7 ± 5.7 years (non-mutated group) respectively, $P < 0.05$).

In the pediatric population (i.e. age ≤ 18 years at diagnostic, $n = 46$), ten patients (21.7%, patients 3-6-7-9-10-13-14-15-16-19) bore mutations (seven in *AIP* and three in *MEN1*). The pediatric population included 30 PRL (65%), 11 somatotropinomas (24%), three NFPAs (6.5%), and two ACTH-secreting adenomas (4.5%). There were 28 females (61%) and 18 males (39%), and the high proportion of females observed is due to the macroprolactinoma subgroup. The age at diagnosis of patients with *AIP* or *MEN1* mutations was similar to their non-mutated pediatric counterparts (mean age 14.7 ± 2.8 years for *AIPmut* group, 15.3 ± 2.1 years for *MEN1mut* group, and 14.6 ± 3.6 years in non-mutated group, NS).

Overall, 11 different *AIP* variants were identified: seven of them led to a premature codon stop (Table 1), suggesting that they are deleterious. The variant p.Gly117Alafs*39 was found in five unrelated patients originating from two geographically close regions (two from Reunion, three from Comoros Islands, all of them are of African origin). To address the issue of a possible founder effect, we genotyped 14 microsatellite markers surrounding the *AIP* gene. Although the lack of information on pedigrees and allele frequencies do not consent to draw final conclusions, our data are strongly suggestive for a common ancestor at least for four out of five subjects sharing a genomic region on chromosome 11 ranging from 4.4 to 7.5 Mb (Table 2).

The four *AIP* remaining variants included two missense variants (p.Lys58Asn and p.Arg304Gln), previously reported as deleterious in the literature (11, 17), and two previously undescribed variants. The variant p.Leu294Pro is localized on exon 6, in the third tetratricopeptide repeat (TPR) domain, known as a key domain for protein-protein interactions and scored as being likely to affect *AIP* protein on *in silico* analyses. The deletion of three bases (c. 735_737 del) in exon 5 induces the loss of glutamine 246 in the second TPR domain (18), therefore supporting a strong pathological role of this mutant.

Genetic screening of family members of affected mutation carriers was possible in three different families (overall 11 subjects tested) and was positive in three subjects, the mother (aged 45) and the maternal grand father (aged 80) of patient 11 (p.Lys58Asn) and the mother (aged 51) of patient 12 (p.Arg304Gln). In all these carriers, pituitary MRI was normal.

Among the six *MEN1* variants identified in our cohort, three of them led to premature stop codons, suggesting a deleterious effect (Table 1). The intronic

mutation (c.655-6C>T) induces a deletion of exon 3 causing a frameshift and a premature stop codon 13 triplets further downstream (19). The two other variants included one missense mutation (p.Pro540Ser), already described but without demonstration of pathogenic effect in the original report (20), and one novel missense variant (p.Asp231His). *In silico* analysis showed a moderate to strong likelihood of a deleterious effect for these two variants (Table 1).

Genetic screening has been conducted in three family members (mother, father, and brother) of patient 16. The genetic analysis was positive in the asymptomatic father and allowed the diagnosis of asymptomatic primary hyperparathyroidism, hitherto unknown, with a normal pituitary MRI. The genetic screening of *MEN1* has also been done in the mother of patient 15 and was negative.

Analysis by phenotype

Nine out of 79 (11.4%) somatotropinoma patients had *AIP* (six males and two females) or *MEN1* mutations (one male, Table 1). Three out of six patients with gigantism were identified with *AIP* mutations (two males and one female) (Table 1). Patient 7, with the novel *AIP* missense variant p.Leu294Pro, was diagnosed at 10 years of age and was resistant to somatostatin analog therapy.

Eight out of 74 (10.8%) PRL patients bore *AIP* (three males and one female) or *MEN1* (two males and two females) mutations (Table 1). The new missense *MEN1* mutant (p.Asp231His) was identified in a 29-year-old male, who was affected by an aggressive PRL that was resistant to dopamine agonist therapy.

Only 12 patients (6.9%) from our whole cohort were affected by NFPAs. Four of them (33%) were identified as having either *AIP* or *MEN1* mutations (Table 1). The deletion of a glutamine (p.Glu246del) in *AIP* protein was found in a young male, aged 20, who had a macroadenoma with partial immunoreactivity for GH (50%) but without any pituitary hormonal hypersecretion *in vivo*.

No mutation was identified in the eight patients diagnosed with corticotroph adenoma and in the female with a thyrotropinoma.

Discussion

Until 2006, mutations in the *MEN1* gene were the main molecular abnormalities seen in cases of familial PA, particularly in association with other endocrine diseases. The implication of germline mutations in the *AIP* gene has since significantly extended the field of genetic analysis in apparent familial predisposition to PA (4).

While the data on *AIP* mutation status in the current study largely supports the emerging profile of the ideal

Table 1 Characteristics of pituitary adenomas in patients with *AIP* and *MEN1* mutations.

Region	Chr position GRCh37 (h19)	Genetic characteristics				Clinical features				Disease features				
		AIP mut/variant (NM_003977.2)	MEN1 mut/variant (NM_130799.2)	Polyphen score ^a	UMD score ^b	Pathogenicity ^c for mutation ^d	Reference for mutation ^d	Gender	Age at diagnosis (years)	Gigantism	GH (mUI)/ IGF1 (ng/ml)	Maximal tumor diameter (mm)	CSI	SSE
<i>In silico</i> analysis														
Acromegaly														
Patient 1	Exon 1	p.Arg22* (c.64 C>T)				Yes	(11)	M	23	N	549/1280	32	Y	Y
Patient 2 ^e	Exon 5	p.Tyr261* (c.783 C>G)				Yes	(11)	M	29	N	257/2449	31	Y	Y
Patient 3 ^e	Exon 3	p.Gly117Ala fs*39 (c.350del)				Yes	(11)	M	18	N	10.4/810	17	N	Y
Patient 4	Exon 3	p.Gly117Ala fs*39 (c.350del)				Yes	(11)	M	20	N	8.3/641	16	N	Y
Patient 5	Exon 3	p.Gly117Ala fs*39 (c.350del)				Yes	(11)	M	20	Y	160/894	20	Y	Y
Patient 6 ^e	Exon 6	p.Arg304* (c.910 C>T)				Yes	(4)	F	18	N	4.7/582	24	N	Y
Patient 7	Exon 6	p.Leu294Pro (c.881 T>C)		1	70	Likely		F	10	Y	185/2013	27	Y	Y
Patient 8	Exon 10		p.Pro540Ser (c.1618 C>T)	0.999	65	Likely	(20)	M	28	N	117.7/1385	14	N	N
Patient 9	Exon 1		p.Glu20* (c.58 G>T)			Yes		M	14	Y	575/NA Prolactin (ng/ml) 2475	18	Y	Y
Prolactinomas														
Patient 10 ^e	Exon 3	p.Gly117Ala fs*39 (c.350del)				Yes	(11)	F	15		24	24	Y	Y
Patient 11 ^e	Exon 2	p.Lys58Asn (c.174 G>C)		0.774	59	Likely	(11)	M	20		72	72	Y	Y
Patient 12	Exon 6	p.Arg304Gln (c.911 G>A)		0.047	47	Yes ^f	(8)	M	27		2180	35	Y	Y
Patient 13	Exon 3	p.Gly117Ala fs*39 (c.350del)				Yes	(11)	M	13		3112	32	Y	Y
Patient 14	Exon 10		p.Arg516Gly fs*43 (c.1546del)			Yes	(36)	M	16		14858	35	Y	Y
Patient 15	Exon 2		p.Leu117Arg fs*11 (c.349_350ins- s26) ^g			Yes		F	13		2973	11	Y	N
Patient 16	Exon 2		p.His139Thr fs*46 (c.415del)			Yes	(37)	F	17		499	14	Y	N
Patient 17	Exon 4		p.Asp231His (c.691 G>C)	0.981	59	Likely		M	29		12500	31	Y	Y
NFPAs														
Patient 18 ^e	Exon 1		p.Asp30Trp fs*14 (c.88_89del)			Yes	(11)	M	19		39	39	Y	Y
Patient 19	Exon 4		p.Asn211Thr fs*4 (c.630del)			Yes		M	15		32	32	Y	Y
Patient 20	Exon 5		p.Glu246del (c.735_737del)			Likely		M	20		35	35	Y	Y
Patient 21	Intron 3		c.655-6 C>T			Likely ^h	(19)	F	30		27	27	Y	Y

Underlined patients belong to pediatric population. CSI, cavernous sinus invasion; SSE, suprasellar extension; NA, not available; Y, yes/N, no.

^aPolyphen score ranges from 0 to 1.

^bUMD score ranges from 0 to 100.

^cPathogenicity is estimated based on *in silico* predictions, clinical data, and data from the literature. Five different categories classified allelic variants: pathogenic (yes), likely pathogenic (likely), of unknown significance, unlikely pathogenic, and not pathogenic (32).

^dThe number refers to the original publication of the first description of the mutation.

^ePatients previously described in the study by Tichomirowa *et al.* (11), with updated clinical data.

^fDespite the low score of *in silico* predictions, this variant was classified as pathogenic considering the numerous publications (8, 11, 17, 25, 29, 33).

^gThe inserted sequence of 26 nucleotides was GAAAGGGGGTCTCCAGCCGTGAGC.

^hThis variant has been found in a patient of African origin. In this population, the allelic frequency may range from 0.7 to 1.4% according to EVS (Exome Variant Server from Exome Sequencing Project) and dbSNP (NCBI's Variant database). On the other side, this variant induces a deletion of exon 3 causing a frameshift and a premature stop codon 13 triplets further downstream according to Roijers *et al.* (19). Moreover, we have found c.655-6C>T in other unrelated index cases with MEN1 lesion (personal not published data). Consequently, we have classified this variant as 'likely pathogenic'.

Table 2 Molecular markers on chromosome 11 and haplotype data of five *AIP* p.Gly117Alafs*39 mutation carriers, three originating from Comoros Islands (C1, C2 and C3) and two from Reunion (R1, R2). Bold represents the more likely at-risk haplotype shared by at least two subjects.

Marker	Genomic position ^a	C1	C2	C3	R1	R2
D11S4076	61.1	154	156	154	154	154
m_11TETRA@61,73	61.7	356	352	356	348	348
CHR11_64_AC_110	64.5	117	121	117	117	117
Chr11-64-TG-110	65.2	142	144	142	142	142
D11S913	65.9	116	120	116	116	116
AFMA190YD5	66.7	269	277	269	269	269
D11S1889	67.1	377	371	377	377	377
ACRO_CHR11_28	67.2	203	203	203	229	203
D11S987	67.6	104	106	104	106	104
D11S4113	68.9	284	286	284	290	284
D11S4095	69.2	115	125	115	125	125
D11S4136	69.6	129	135	129	129	129
D11S4162	70.6	180	178	180	178	178
D11S1314	72.0	346	336	NA	NA	346

NA, not available.

^aBased on the human NCBI36/hg18 genome assembly.

screening candidates, the major new finding relates to *MEN1* screening in young sporadic pituitary macroadenoma patients. There are few data assessing the prevalence of *MEN1* mutations in the specific case of isolated and sporadic macroadenoma. Stratakis *et al.* (21) reported one *MEN1* mutation in a 11-year-old male with macroprolactinoma among six patients with isolated GH- or PRL-secreting adenoma. In our study, *MEN1* mutations were identified in 3.4% of cases and this frequency reaches 6.5% in the pediatric population ($n=3/46$). No large genomic deletion was identified among *MEN1*-sequencing-negative patients; such findings have been reported previously in 1% of *MEN1* families (22). In contrast to *AIPmut* patients, *MEN1mut* patients from our series had the same age at diagnosis as the population without mutation, in agreement with the data from Verges *et al.* (13) on micro- and macroadenomas. In invasive adenoma group from the study by Trouillas *et al.* (23), *MEN1mut* patients tended to be younger than their non-mutated counterparts. In the oncogenetic field, and particularly for *MEN1* and hyperparathyroidism, it is well known that tumors arise earlier in mutated patients than in their non-mutated counterparts. In *MEN1* pituitary tumors, data are missing to claim it. Consistent with the literature (13), PRL are over-represented in our *MEN1mut* patients (4/6, 66% in our cohort). Consequently, our results suggest that *MEN1* mutations should be strongly considered in the young sporadic pituitary macroprolactinoma population, as we found an equal frequency of *AIP* and *MEN1* mutations in our cohort (Fig. 1).

In our cohort, only one *MEN1mut* patient (patient 15) has developed primary hyperparathyroidism to date. Moreover, this hyperparathyroidism was completely asymptomatic and diagnosed about 10 years after the first symptoms of pituitary tumor. In addition, one

family member of *MEN1* proband (patient 16) was subsequently diagnosed with occult hyperparathyroidism thanks to positive genetic screening. Therefore, the genetic screening performed in family members of *MEN1mut* patients could result in a contributive diagnosis and therapeutic intervention. This is in line with the high penetrance of the *MEN1* syndrome estimated near 90% at the age of 50 years (12).

By definition, FIPA families are free of mutations in the *MEN1* gene. While our results show that both *AIP* and *MEN1* contributed to sporadic macroadenomas in young patients, we have not found that *MEN1* mutations can lead to isolated PA in a familial setting. We still have not identified any *MEN1* mutations in the unrelated patients from our FIPA cohort (personal communication). This might be the consequence of the high frequency and penetrance of hyperparathyroidism (24). Hyperparathyroidism should be actively searched in cases of family members with isolated PA to focus genetic analysis either on *MEN1* or on *AIP*. Subsequently, *MEN1* genetic screening may now not be necessary *a priori* before designating kindred with multiple related members with isolated PA as having FIPA (Fig. 1).

The prevalence of *AIP* mutations in patients with sporadic pituitary adenoma without considering the age at diagnosis is low, between 0–4% (6, 9, 25, 26), including in those with sporadic macroadenoma (27). Strikingly, this frequency reaches 12% in young patients (age ≤ 30 years) with sporadic macroadenoma (11), strongly supporting the idea that young patients should be the primary targets of genetic screening. Accordingly, our study identifies an overall mutation prevalence of *AIP* of 8.6% in a similarly selected population. Two studies on patients diagnosed before 40 years have previously reported an *AIP* mutation frequency

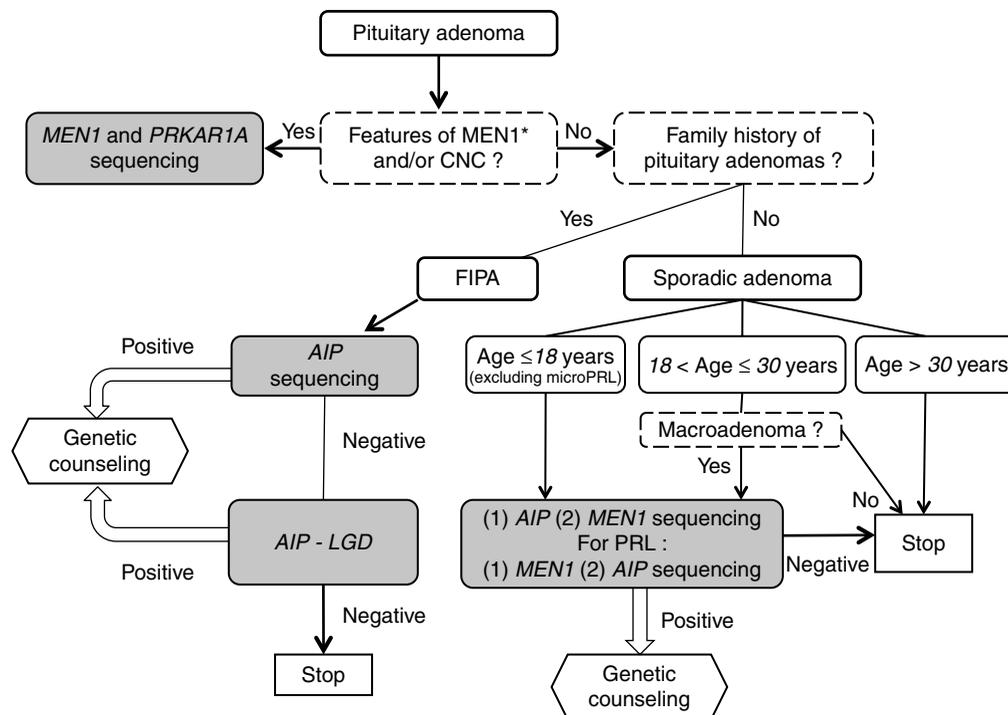


Figure 1 Suggested algorithm for *AIP* and *MEN1* genetic screening in clinically relevant pituitary adenoma (PA). Adapted from references (34) and (35). *Hyperparathyroidism should be actively searched for in all patients with PA. In patients diagnosed before 30 years old with sporadic macroadenoma, we suggest to perform first *AIP* and secondarily *MEN1* genetic screening, except for PRL. In the pediatric arm, we proposed to perform *AIP* and *MEN1* genetic screening in all cases, except for microprolactinomas considering the high frequency of this tumor in young females and even if data are missing to support this proposal. *CDKN1B* is not included in this algorithm because *MEN4* is a very rare syndrome (33) and this algorithm focuses on routine genetic testing. McCune Albright is currently one of the syndromes that could be associated with PA, but it is not a hereditary syndrome, as the mutation of the locus *GNAS* is present as mosaicism. No activating mutation has been reported so far in humans at germinal level, probably because the germinal activating mutation is lethal for the embryo. This algorithm focuses exclusively on PA predisposition syndromes for which subsequent genetic analysis could be performed in the family members. *AIP*, aryl hydrocarbon receptor interacting protein; *CNC*, Carney complex; *LGD*, large gene deletion detection; *MEN1*, multiple endocrine neoplasia type 1; *PRL*, prolactinoma; *PRKAR1A*, regulatory R1A subunit of protein kinase A.

near 7% (7, 8). This prevalence reaches 14.3% in patients with GH-secreting tumors diagnosed before 25 years old (28). All these data lead us to propose to limit the genetic screening to subjects younger than 30 years old (Fig. 1).

In our pediatric population, *AIP* mutation prevalence reaches nearly 15% and as high as 40% ($n=4/10$) in cases of acromegaly. In the literature, the frequency of *AIP* mutations in such populations varied from 2% (1/36) (28) to 20–23% (11, 25). Not only the age at diagnosis but also the size of the tumor is an important criterion that modifies the frequency of *AIP* mutation in isolated sporadic PA. The tumors from *AIPmut* patients in a FIPA cohort were overwhelmingly macroadenomas (10). However, among 74 children with Cushing's disease, one *AIP* mutation was found in a patient diagnosed at the age of 6 years with a 3×4 mm ACTH-secreting adenoma (21). Subsequently, data are missing in the pediatric population to support the exclusion of children with microadenoma from the *AIP* genetic screening that we suggest in Fig. 1.

In our study, the majority of *AIP* mutations were found in somatotropinoma patients (8/15 mutated patients) as previously known (10). No mutation was found in corticotroph adenoma patients and in the single thyrotropinoma patient. However, *AIP* mutations have already been reported in several young patients with isolated sporadic corticotroph adenomas (7, 21), justifying *AIP* screening in such populations (Fig. 1). There is a variable penetrance of FIPA in *AIPmut* families with around 20–30% in the largest cohort studied (5, 29). Indeed, in our study, the positive genetic screening in the three family members was not accompanied by the identification of any PA on MRI. In five *AIPmut* carriers from the study of Cazabat *et al.* (7), no adenoma was identified by MRI, and of the *AIPmut* carriers ($n=2/21$) from the study conducted by Tichomirowa *et al.* (11), two family members (not included in the current study) were diagnosed with a microadenoma and without associated hormonal over-secretion. Whether there are specific *AIP* mutants associated with higher penetrance of the disease

remains unknown. On the one hand, these observations are in agreement with the low penetrance of the FIPA syndrome; on the other hand, it asks the question of substantial benefits of AIP screening for asymptomatic family members. A long prospective study is clearly required to assess the impact of AIP screening in family members of AIPmut patients in order to clarify the natural history of asymptomatic AIPmut carriers.

In mutation-negative patients, we did not identify any large genomic deletion of AIP by MLPA in agreement with three other previous studies (6, 7, 11). This molecular abnormality could account for 9.5% of AIP-negative FIPA kindreds (30), and among 64 unrelated patients from a FIPA cohort of our laboratory, we identified one family member of homogenous FIPA with acromegaly, with large genomic deletion of AIP in exon 1 (A Barlier, personal communication). Considering the cost and the difficulties of this analysis, MLPA analysis should be considered primarily in cases of FIPA that are first shown to be negative on AIP sequencing.

Finally, our study identified an overall mutation prevalence of 12% for AIP and MEN1. Surprisingly, this frequency reaches 33% for patients diagnosed with NFPA ($n=4/12$). However, among the four patients, one of them (AIP mutated) was a silent somatotroph macroadenoma, whereas the remaining three others were non-reactive on immunostaining experiments. Excluding the silent somatotroph case, the mutation frequency of AIP becomes 20% (2/10), still higher than that observed in the study conducted by Tichomirowa *et al.* (6.3%, (11)). NFPA are very rare tumors in the population aged under 30 years old. Therefore, further studies are needed to clarify the mutation prevalence in young NFPA patients.

Although the MEN1 mutation prevalence was only to 3.4% in our series, taking into account the high penetrance of MEN1 syndrome, together with the possibility of up to 10% of *de novo* mutations (31) and the strong impact of MEN1 mutations in terms of genetic counseling and therapeutic management, we suggest to include not only AIP but also MEN1 genetic analysis in young patients with sporadic PA (Fig. 1). Nevertheless, in the current guidelines for MEN1 management, the genetic analysis of MEN1 is not specifically recommended in this kind of population (24). But until now, there were no data on the prevalence of MEN1 mutations in young patients with sporadic and isolated PA, particularly macroprolactinomas. Further investigations are required before including MEN1 genetic screening in clinical practice in such a population. Even if the phenotypes induced by a point mutation or a large deletion of the gene are not different (22, 30), the latter group might be associated with increased penetrance (30). Therefore, according to our results and those of the literature (7, 11), seeking large AIP and/or MEN1 deletions seems unjustified as a routine measure in cases of isolated sporadic PA even in young patients (Fig. 1).

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.

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