Mutations of the Gene for the Aryl Hydrocarbon Receptor-Interacting Protein in Pituitary Adenomas

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Key Words

Aryl hydrocarbon receptor-interacting protein gene · Pituitary adenoma · Familial isolated pituitary adenomas · Isolated familial somatotropinomas · Acromegaly

Abstract

Heterozygous germline mutations in the gene encoding the aryl hydrocarbon receptor-interacting protein (AIP) were first described in two Finnish families with pituitary adenomas. The gene is involved in about 15% of familial isolated pituitary adenomas (FIPA), in about 50% of cases of familial acromegaly and in a small proportion of acromegalic patients with sporadic presentation. This review describes the genetic and clinical features of published patients with AIP, with either familial or sporadic pituitary tumors. A genotype-phenotype correlation is proposed: patients with AIP mutations resulting in a truncated protein are significantly younger than those bearing a mutation which preserves the structure of the C-terminal end of the protein (22.7 ± 9.6 vs. 29.8 ± 10.9 years). Pituitary tumors linked to AIP mutations are almost exclusively somatotrophic (87.5%, $n = 56/64$) or lactotrophic (9.4%, $n = 6$). Patients with AIP mutations are mostly

men (70%, 44 M/19 F), suffer macroadenomas (97%) and are younger at diagnosis (24.4 ± 10.5 years) than unselected patients with pituitary tumors. Thus, AIP is involved in the development of pituitary tumors, especially involving the somatomammotroph lineage. Genetic testing could be discussed for FIPAs and in young acromegalic patients with a sporadic presentation. Functional studies are needed to understand AIP-induced tumorigenesis.

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Introduction

The pathophysiology of pituitary tumors remains largely unknown, despite recent progress in the elucidation of various genetic syndromes associated with pituitary neoplasias. Although almost always benign, these tumors cause significant morbidity in affected patients because of their secretory activities or local development or both. Their prevalence has been largely underestimated in the past with previous estimations being between 2 and 3 per 10,000. However, large autopsy series indicate a prevalence of between 6 and 22% of subclinical pitu-

itary tumors (mean 14%) [1], and pituitary MRI in healthy volunteers reveals a frequency of about 10% among pituitary abnormalities compatible with occult pituitary adenoma [2]. A recent epidemiological study of symptomatic pituitary adenomas reported an overall rate of 1 per 1,064 of the general population, which is three times higher than previously believed [3].

Each anterior pituitary cell type may undergo monoclonal proliferation. The resulting tumors are commonly classified according to their secretion pattern or to immunohistochemical staining pattern. Among clinically symptomatic pituitary adenomas, prolactinomas are the most frequent tumors ($\approx 65\%$), followed by non-secreting tumors ($\approx 15\%$), somatotropinomas ($\approx 13\%$), corticotropinomas ($\approx 6\%$) and thyrotropinomas ($\approx 1\%$) [3, 4].

The large majority of these tumors are sporadic. A small proportion (about 3%) occurs with a familial aggregation, as components of either the multiple endocrine neoplasia type 1 (MEN1) or the Carney complex (CNC), both syndromes associated with a genetic predisposition to multiple endocrine tumors [5, 6]. Isolated familial pituitary adenomas have also been described in the absence of *MEN1* and *PRKARIA* mutations [7], and this situation represents about 1% of all adenomas. It appears that patients in the same families can exhibit either the same pituitary tumor type or different tumor types; it has been suggested that these clinical syndromes should be classified as homogeneous and heterogeneous familial isolated pituitary adenoma (FIPA) kindreds, respectively [8]. Recently, Vierimaa et al. [9] described germline-inactivating mutations in the gene encoding the aryl hydrocarbon receptor interacting protein (AIP) in some of these families. Individuals carrying these mutations presented with combinations of somatotropinomas, mixed GH-prolactin-secreting tumors and prolactinomas. The *AIP* gene maps at 11q13, in the same region as the *MEN1* gene. Analysis of tumor samples from affected individuals showed loss of heterozygosity at the *AIP* locus suggesting that *AIP* acts as a tumor suppressor gene. Further studies found *AIP* mutations in 15% of all FIPAs and about 50% of families presenting with only GH-secreting tumors. *AIP* mutations were also found in a small number of patients with sporadic pituitary adenomas, mostly GH-secreting tumors.

This review addresses the clinical and molecular features of pituitary adenomas occurring in a familial setting or in patients presenting with sporadic tumors but bearing a germinal mutation of the *AIP* gene.

Method

We collected information about all patients with *AIP* mutations reported in the literature since the first description of Vierimaa et al. [9] up to March 2008. In particular the mutation, age at diagnosis, familial or sporadic presentation, tumor type, sex and tumor size (micro- or macroadenoma) for each case are reported when available [9–17]. The Mann-Whitney non-parametric test and χ^2 Pearson test were used for statistical analysis.

Genetic of Pituitary Adenomas

Pituitary adenomas may occur as part of genetic syndromes associated with other endocrine or non-endocrine tumors. The most frequent of these syndromes is MEN1, with an estimated prevalence of 0.02–0.2 per 1,000. MEN1 is an autosomic dominant inherited disease predisposing to primary hyperparathyroidism, endocrine digestive tumors, pituitary adenomas and some other rare lesions. Affected patients bear an inactivating mutation of the *MEN1* gene, located at chromosome 11q13, and the normal allele is lost in the tumors. Pituitary adenomas occur in about 40% of these patients. Pituitary tumors associated with MEN1 are more frequently diagnosed in women than in male (sex ratio 1:5), and this is also the case for non-MEN1 patients. The distribution of immunohistological types is not different from that of sporadic tumors, with two thirds of the patients presenting with prolactinomas [4]. Recently, mutations in the *CDKN1B* gene, located at chromosome 12p13 and encoding the p27kip1 protein, were found in one family with GH-secreting pituitary adenomas and hyperparathyroidism and also in 1 patient with Cushing's disease and hyperparathyroidism. This new entity was called MENX. Other endocrine or non-endocrine tumors have been observed in patients. LOH has been described in a small-cell neuroendocrine cervical carcinoma and suggests that *CDKN1B* may act as a tumor suppressor gene [18, 19]. However, these mutations appear to be very rare among patients with MEN1 phenotype and a normal *MEN1* sequence [18, 20].

Activating mutations of the *GNAS 1* gene, located at chromosome 20q13 and encoding the α subunit of protein Gs (*gsp* oncogene), are responsible for the McCune-Albright syndrome (MAS). These mutations can be found in different tissues (mosaicism) resulting from a post-zygotic event. The same mutation is found in about 40% of sporadic somatotropinomas.

MAS affects various tissues through activation of the cAMP pathway. Café-au-lait spots, fibrous osteodysplasia and endocrine hyperactivity (leading to precocious puberty, GH excess, hyperthyroidism and other manifestations) are the main clinical features [21]. In the same cellular pathway, inactivating mutations in the gene encoding the regulatory subunit R1 α of the protein kinase A (*PRKAR1A*), located at 17q22-24, have been described in CNC. Patients present with myxomas, spotty skin pigmentation (lentigines) and endocrine overactivity including primary pigmented nodular adrenocortical disease (PPNAD) responsible for adrenocorticotrophic hormone-independent Cushing syndrome. *GNAS*-activating mutations increase cAMP levels in tissues [22], and *PRKAR1A*-inactivating mutations result in abnormal PKA activity [23]. In cases of MAS and CNC, the pituitary gland frequently shows somatomammotrope hyperplasia with high growth hormone and IGF-I levels and mild hyperprolactinemia, whereas somatotropinomas or somatomammotrope adenomas are much less common [24, 25]. In contrast to *GNAS* mutations, somatic mutations of *PRKAR1A* and *MEN1* are uncommon in sporadic pituitary tumors [26, 27].

MEN1, *PRKAR1A* and extremely rarely *CDKN1B* may be involved in familial syndromes associating pituitary adenomas and other tumors; in contrast, germline mutations in the *AIP* gene have been described in FIPA [9]. *AIP* mutations have been found in 15–20% of FIPAs (20 families). Families with *AIP* mutations mostly have IFS (isolated familial somatotropinomas) (15/20) or are families with lactotropinomas and somatotropinomas (4/20). So, germline mutations of *AIP* predispose to the development of tumors secreting GH or both GH and prolactin, reminiscent of the pituitary phenotype of pathologies linked to the cAMP pathway (MAS and CNC).

LOH of at the *AIP* gene has been demonstrated in tumors from mutated patients, supporting the hypothesis of a tumor suppressor gene mechanism. Many relatives of affected subjects are asymptomatic carriers suggesting that the disease has a low penetrance.

AIP Protein

AIP, also called aryl hydrocarbon receptor-associated protein 9 (ARA9), has been known for more than 10 years as a protein associated with the aryl hydrocarbon receptor (AHR), a ligand-inducible transcription factor that mediates the cellular response to xenobiotic compounds including various environmental pollutants [28]. In the absence of ligand, the latent receptor is associated with

the 90-kDa heat-shock protein (HSP90, a molecular chaperone) and AIP [28]. Upon ligand binding, AHR accumulates in the nucleus where it forms a transcriptionally active complex. Activation of the AHR-mediated signaling pathway by xenobiotic compounds has numerous toxic effects, including carcinogenesis, teratogenesis, and immunosuppression. AIP, in turn, stabilizes the AHR protein and participates in the regulation of the intracellular localization of the receptor by a cytoplasmic retention mechanism that has not yet been described.

Simultaneously, AIP was found to interact with the hepatitis B virus X-protein, and was therefore also called hepatitis B virus X-associated protein (XAP2) [29]. More recently, Sumanasekera et al. [30] demonstrated that AIP can also bind another xenobiotic receptor, PPAR α (peroxisome proliferator activated receptor α).

AIP is a 330-amino-acid protein belonging to the immunophilin family. It is made up of two domains: the N-terminal FKBP domain and the C-terminal domain containing three tetratricopeptide repeats domains (TPRs). The TPRs region is responsible for protein-protein interactions: this part of AIP binds HSP90 and other regulatory proteins [31]. The five last amino acids of the α -helical C-terminus of AIP, outside the TRPs domain, are absolutely required for binding to AHR; deletion and mutagenesis studies show that all AIP mutations leading to C-terminal truncated proteins or lacking one of these five amino acids are unable to interact with AHR [32]. The AIP N-terminal domain contributes to the stability of the AHR-HSP90-AIP complex and the regulation of its intracellular localization.

The TPRs region is responsible for protein-protein interactions and this part of AIP binds both HSP90 and also phosphodiesterases. Interaction with AIP decreases the activity of the cAMP-specific phosphodiesterase PDE4A5 [33], whereas the binding of PDE2A to AIP inhibits the nuclear translocation of the AHR complex possibly by a local reduction of the cAMP levels [34]. Moreover, the AHR pathway may be activated by cAMP, in a way that appears different from AHR activation by dioxin. Disruption of the physiological cAMP-mediated activation of AHR by xenobiotic compounds has been proposed to be an important mechanism in their toxicity [35]. These possible links between AHR signal transduction and cAMP are obviously of potential significance to endocrine tumorigenesis, especially in mammosomatotrope cells.

AIP is also associated with survivin and regulates its stability [36]; it also interacts with ubiquitin ligase protein C-terminal Hsp70-interacting protein (CHIP) and protects AHR from CHIP-mediated degradation [37].

Table 1. AIP germline mutations and their putative consequences

Mutation	Nucleotide	Putative protein	n	Ref.
<i>C-terminus truncated</i>				
Nonsense	c.40 C>T	p.Gln14X	17	9
	c.64 C>T	p.Arg22X	1	10
	c.424 C>T	p.Q142X	1	12
	c.601 A>T	p.Lys201X	2	11
	c.649C>T	p.Q217X	2	12
	c.715 C>T	p.Q239X	2	12
	c.804 A>C	p.Y268X	2	17
	c.910 C>T	p.Arg304X	6	9, 11, 12
	c.286–287delGT	p.Val96Pro.fsX31	3	14
	c.404delA	p.His135Leu.fs19X	1	11
Frameshift	c.517–521delGAAGA	p.Gln174Gly.fs22X	3	15
	c.542delT	p.I182S.fsX12	1	13
	c.824–825insA	p.His275GlnX13	1	13
	c.854–857delAGGC	p.Gln285fsX18	2	12
<i>C-terminus conserved</i>				
Splicing site	IVS2-1 G>C		1	13
	c.469-2 A>G		1	11
	c.469-1 G>A		1	9
Deletion	c.66–71delAGGAGA	p.del23–24	1	13
	c.138–161del24	p.del47–54	2	12
Deletion and missense	c.878–879 AG>GT and c.880–891delCTGGACCCAGCC	p.Glu293Gly,Leu294– Ala297del	1	13
Missense	c.721 A>G	p.Lys241Gln	2	12
	c.811 C>T	p.R271W	4	12
	c.911 G>A	p.Arg304Gln	3	11, 13

Lin et al. [38] recently suggested that AIP may interact with pathways other than xenobiotic receptor signal transduction, at least in mice. *Ara9*^{–/–} mice (*AIP*^{–/–}) display embryonic lethality and cardiac deformations. The phenotype is different from those of *Ahr*^{–/–} or *Pparα*^{–/–} mice. No pituitary abnormalities have been described in heterozygotes (*AIP*^{+/-}) mice, but long-term follow-up of these animals is required. In view of the lethality of the *AIP*^{–/–} genotype, pituitary-specific AIP knock-down mice may well be more informative.

The role of AIP in tumorigenesis in pituitary and more specifically mammosomatotrope cells is still largely unknown. Functional studies and work with animal models in the future should help resolve this issue.

AIP Mutations in Pituitary Tumors

In May 2006, Vierimaa et al. [9] described the first germline AIP mutations in FIPA in two Finnish families presenting prolactinomas and somatotropinomas, and in

some sporadic patients with acromegaly. They performed whole-genome single-nucleotide polymorphism studies and linkage analysis in these families and found the region 11q12–11q13. This region contains the *MEN1* gene and was already implicated in familial acromegaly, without *MEN1* mutation [39]. The authors looked at expression profiles of genes in this region in lymphocytes of patients with familial pituitary tumor: *AIP* was underexpressed compared with the other genes. They then sequenced the coding region of *AIP* and detected a heterozygous nonsense germline mutation segregating with pituitary adenoma; tumoral DNA analysis showed LOH suggesting a tumor suppressor gene.

Now, about 25 different germline mutations have been reported in patients with pituitary adenomas [9–15, 17] (table 1). No somatic mutations of the *AIP* gene in pituitary adenomas have yet been reported, but only one published study has looked for *AIP* mutations in tumoral DNA [10].

The first described *AIP* mutation, p.Gln14X, has only been observed in the Finnish population and results from

Fig. 1. AIP structure and published mutations. This figure represents functional domains of AIP with different colors: the N-terminal part (NFKBP domain) in blue, and the C-terminal part comprising the three tetratricopeptide repeat (TPR) domains in pink, and the distal C region (DCR) in purple. The C-terminus of the protein binds the aryl hydrocarbon receptor (AHR); the region interacting with the 90-kDa heat-shock protein (HSP90) and phosphodiesterases (PDE) is shown in green. The mutations listed in table 1 are indicated; legends for the symbols are presented beside the figure.

a founder effect; the first two Finnish families described have a common ancestor [40]. In this population, a few patients with the same AIP mutation present with sporadic prolactinomas [9, 16]. A high frequency of AIP mutation is thus observed in Finnish acromegalic patients (16%) [40], and especially in the youngest subjects (40%) [9].

Among the non-synonymous amino acid changes in the AIP protein in patients, three might be rare polymorphisms: the p.Ala299Val [11, 13], p.Val49Met [14], and p.Arg16His [11–13, 41] substitutions [13, 41]. More studies are needed to compare the frequency of these variants between patients and matched controls and to test the functional consequences of the mutant protein. We excluded the patients with these three AIP variants from subsequent analysis.

Most known mutations in the *AIP* gene result in a truncated protein, lacking more or less of the C-terminal sequences important for protein-protein interactions (TPRs); in all cases, the last five amino acids essential for AHR binding are missing. Eight different nonsense mutations and six different frameshift mutations have been described, leading to premature stop codons distributed all along the coding sequence. Other mutations include splice site mutations and deletions not resulting in frameshifts and a few missense mutations. Splice site mutations

involving exons 3 and 4 (two exons with a number of nucleotides divisible by three: 189 and 177, respectively) probably result in exon skipping without frameshift. The c.469-2 A>G mutation leads to an mRNA with deletion of exon 4 [pers. data]. The mutant protein lacks important central regions, as exon 3 encodes a large part of the FKBP-PPI domain and exon 4 encodes the whole first TPR domain, but it retains an intact C-terminal end. Deletions within the coding sequence, not resulting in frameshifts or premature stop codons, would have the same effects. All the known missense mutations map in the C-terminal part of the protein, necessary for protein-protein interactions; the substitution of the arginine at position 271 (a mutation present in two FIPA families) reduces the interaction of AIP with PDE4A5 [33]. However, these mutations result in a protein with a conserved α -helical C-terminus, the structure required for AHR binding (fig. 1).

We compared the phenotypes of the patients bearing the two types of mutations: those resulting in truncated proteins lacking the C-terminal domain and those keeping an intact or generally conserved C-terminus. Patients in the first group are significantly younger (22.7 ± 1.4 vs. 29.8 ± 2.8 years, mean $\pm$ SEM, $p < 0.05$) (fig. 2). They are (not significantly) more frequently diagnosed in

FIPAs than with a sporadic presentation: 37/46 (80%) versus 12/19 (63%). Thus, mutations with roughly conserved C-terminus may be less pathogenic such that age at diagnosis is significantly older; they may also have lower penetrance that could explain the more frequently sporadic presentation. However, this possibility needs confirmation in larger cohorts and by functional analyses that are not yet available.

Concerning tumor secretion, statistically valid genotype/phenotype studies are not possible, because of the small numbers of AIP-mutated patients presenting with non-GH-, non-PRL-secreting adenomas. Note, however, that both the only family with AIP mutation and without somatotropinoma and the only patient with sporadic presentation with neither somatotropinoma nor prolactinoma (Cushing's disease) have missense mutations (p.Lys 241Gln and p.Arg304Gln, respectively).

Clinical Presentation of AIP-Mutated Patients

64 patients with pituitary adenoma and a germline *AIP* mutation have been reported in the literature: 45 with a familial history and 19 with a sporadic presentation (30%). Analysis of the data concerning these patients provides some important clues and raises new questions.

What Is the Frequency of AIP Mutations among Patients with Pituitary Adenoma Occurring in a Familial Setting?

AIP analysis demonstrated an overall frequency of AIP mutations of 15% among FIPAs and of 50% among cohorts with IFS [12]. Thus, AIP mutations are mostly found in families with exclusively GH-secreting adenomas or somatotropinomas and prolactinomas, but can explain only half of such cases. Among families only including prolactinomas or families comprising non-secreting/gonadotroph adenomas or corticotroph adenomas, AIP mutations appear to be very rare. The age of patients in homogeneous families with acromegaly and that of patients in heterogeneous families with GH and PRL adenomas is similar (22.6 ± 7.2 and 19.6 ± 6.6 years, respectively); patients from the only family with non-secreting adenoma and prolactinoma are older (45 and 53 years of age) (fig. 3).

What Type of Secretion Is Associated with AIP Mutation?

In unselected familial pituitary adenomas, prolactinomas are generally more frequent (41% among FIPA cohorts) than somatotropinomas or somatolactotropino-

Fig. 2. Age at diagnosis in the patients according to the type of AIP mutation. The horizontal lines are the medians; the rectangles represent the interquartile range (50% of patients in the center of the distribution), the vertical lines are the adjacent values and atypical values are visualized by dots. The means are represented by white lines and their values are indicated alongside.

mas (30 and 7% of pituitary tumors occurring in a familial context) [42]. Most cases of FIPA with AIP mutation involve somatotropinomas (82% and up to 86% if somatolactotrope adenomas are included (39 of 45 cases)). Prolactinomas are present in only 11% ($n = 5$) of patients in FIPA with AIP mutation. Non-secreting/gonadotrope adenomas and corticotrope adenomas are extremely rare in cases of FIPA with AIP mutation [8]. The only reported familial case of non-secreting adenoma with AIP mutation is associated with a prolactinoma. With the exception of this case, all FIPA families with AIP mutation present with familial GH-secreting tumors in some cases associated with prolactinomas.

Among patients with AIP mutation and sporadic presentation, there is also a large majority of somatotropinomas (84%, $n = 16/19$) and only one case each of somatolactotropinoma, prolactinoma, and corticotroph adenoma.

What Is the Frequency of AIP Mutations in Patients with Apparently Sporadic Pituitary Adenomas?

AIP mutations are rare in unselected patients with sporadic acromegaly, with reported frequencies ranging from 0 [14, 16] through 3–4% in the largest cohorts [11, 13] and up to 16% in Finland [40]. The true frequency is unfortunately not available in some cohorts in which sequence analysis of the *AIP* gene has been limited to the three first

Fig. 3. Distribution of tumor phenotype in AIP-mutated patients with either familial (FIPA) or sporadic presentation. This figure shows the ages of all published FIPA patients with AIP mutation classified into three groups: IFS patients in red (GH), subjects with heterogeneous FIPA with prolactinomas and somatotropinomas in purple (GH PRL), and the 2 patients from the heterogeneous FIPA with a non-secreting tumor (NS) and a prolactinoma (PRL) in orange.

mutations described [43, 44]. In young acromegalic patients, however (<25 or <30 years of age according to the study), AIP mutations have been observed in about 10–15% [11, 13]. In patients with other types of sporadic adenomas, AIP mutations are very rarely reported (1 in 86 cases of Cushing disease, none of 76 non-secreting adenomas), whereas prolactinomas have been described only in the Finnish population [13, 16]. However, few systematic studies are available and consequently additional investigations are required, particularly for young patients.

What Is the Penetrance of Pituitary (or Non-Pituitary) Disease in Patients with an AIP Mutation Diagnosed by Genetic Screening?

So far, data are scarce concerning relatives of patients with sporadic presentation. In our experience in a cohort of apparently sporadic acromegalic patients, no de novo mutation has been observed (in all cases, one of the parents carried the mutation). Thus, the sporadic presentation is likely a result of low penetrance of the disease. The mutated first-degree relatives are clinically asymptomatic (three families) [unpubl. data] and complete clinical,

Fig. 4. Age, sex and AIP status of cases of sporadic acromegaly. This graph represents the sex and age at diagnosis of acromegalic patients with or without AIP mutation [9–11, 13]. All mutated patients are <45 years of age (gray line). All mutated patients <17 years of age are male (gray dotted line). On the right is a French cohort of acromegalic patients with normal AIP sequence [11]. Mean age at diagnosis for each group is indicated with a green line and the values are indicated alongside.

hormonal and morphological analysis would be informative. The penetrance of pituitary disease in patients with AIP mutations has been studied in a few families [8, 15, 17]. In two extensively explored families, 30–50% of clinically asymptomatic mutation carriers have isolated increased levels of IGF1 or prolactin with normal pituitary MRI findings [8, 15, 17]. However, these percentages are based on very preliminary investigations. Extrapituitary disorders related to AIP mutation could be suspected because of the non-endocrine specificity of AIP expression. Available descriptions of patients lack complete clinical data and rare extrapituitary disease cannot be excluded. Note that a 2-year-old child with AIP mutation presents a premature thelarche [15].

Are There Particularities in the Presentation of the Pituitary Disease in Patients Bearing an AIP Mutation?

In unselected FIPA cohorts, women are in the majority (62%), whereas cases in FIPA kindreds with AIP mutation are mostly men (71%, 32 M/13 F), as described for IFS [45]. As expected, patients with sporadic presenta-

tion and AIP mutation are similarly mostly men (67%, 12 M/6 F). Women with AIP mutation tend to be older at diagnosis than men (26.7 ± 9.4 vs. 23.4 ± 11 years, respectively, NS) and gigantism has only been described in men, but clinical information is frequently missing from reports.

Patients with sporadic presentation tend to be older than patients from families (28.5 ± 2.8 n = 19 vs. 22.6 ± 1.4 years n = 41, respectively, $p < 0.05$).

We compared the characteristics of all acromegalic patients with AIP mutation reported in the literature (n = 57) with the characteristics of a French cohort of apparently sporadic acromegalic patients (n = 155) in which AIP mutation had been excluded. We are aware that this comparison introduces a bias because it incorporates two variables simultaneously: the presence or absence of the mutation, and also two different populations (patients of various origins and a French cohort). As expected, age at diagnosis was significantly younger for mutated patients (23.2 ± 8.1 vs. 42.9 ± 14.1 years, $p < 0.0001$) and there was a significant male preponderance among mutated patients (38 M/15 F vs. 70 M/85 F, $p < 0.001$); all acromegalic patients with AIP mutations and <17 years of age are male (fig. 4).

In patients with AIP mutation, tumors are almost exclusively macroadenoma (97%, 37/38 with information available), whereas they represent 80.1% in the French control cohort of acromegalic patients ($p = 0.01$). However, patients with AIP mutations are younger and the frequency of macroadenoma is not significantly different from that in age-matched controls.

More detailed clinical descriptions of patients with AIP mutation are needed to assess tumor invasiveness, and in particular comparisons between these patients and other acromegalic subjects; few publications provide such information.

Conclusion

The study of pituitary adenomas as a part of hereditary syndromes brings additional elements to our understanding of pituitary tumorigenesis. The identification of *menin* and *PRKAR1A* are examples of such advances. More recently, the description of AIP mutations in FIPAs has opened up new fields of investigation. Although the relevance of the cAMP pathway remains obvious in the physiopathology of GH-secreting tumors, the unexpected involvement of a protein interacting with the AHR provided insight into the interrelation between various

intracellular signaling pathways. Future work will reveal whether loss of AIP function alters the cAMP pathway or if other molecular mechanisms lead to proliferation of pituitary cells. The possible role of environmental pollutants in pituitary tumorigenesis also deserves further investigation.

AIP mutations may predispose to non-pituitary tumors. Possible missense mutations were found in patients with colic cancer, and premature pubarche has been observed in one girl with an AIP-inactivating mutation. Careful analysis of families may reveal further different diseases. Determining the complete phenotype of animal models could help, although the AHR system in rodents is somewhat different from that in man.

It would also be informative to conduct functional analysis and other investigations of AIP variants not clearly associated with AIP dysfunction (missense mutations, neutral polymorphisms and sequence variants possibly associated with low penetrance predisposition).

Appropriate clinical management remains the ultimate goal. Molecular studies should be oriented by careful analysis of patient phenotype, hormonal results, pituitary MRI and family history.

Screening for AIP mutations in patients with any type of *pituitary adenoma occurring in a familial setting* is certainly warranted because of the high frequency of AIP mutations in this situation; possibly, IFS and GH-PRL families should be targeted first. Investigations of MEN1 should remain the first line of screening in the evaluation of families without mammosomatotrope tumor.

Indications for genetic screening for AIP mutations in patients with *apparently sporadic pituitary tumors* require careful consideration, and some questions have yet to be resolved.

AIP mutations in patients with unselected, apparently sporadic, acromegaly are rare, from 0 to 3–4% depending on the study, and except in the Finish population where the frequency is 16%; thus, systematic screening is probably not justified. However, the frequency of germline AIP mutations is higher in young patients with GH-secreting macroadenomas (about 10–15% in patients <30 years). Therefore, and until further studies are available in large cohorts of well-characterized patients, genetic testing of apparently sporadic acromegalic patients <40 years of age seems reasonable.

Should clinically asymptomatic relatives of AIP-mutated patients be screened? Until now, consequences of bearing the AIP mutation for asymptomatic subjects are not well known. So, although genetic screening of first-degree relatives would be useful to improve our under-

standing, and potentially beneficial for these subjects, they must be given all available information and provide informed consent prior to testing. Hormonal evaluation in asymptomatic subjects bearing the mutation may include plasma prolactin and IGF1 measurement, GH/HGPO and pituitary MRI.

More clinical and functional studies are needed to determine the pathogenic potential of rare AIP variants and elucidate the mechanism of cellular proliferation associated with AIP disruption. Such work is worthwhile be-

cause the understanding of this new molecular pathway may allow the development of new therapeutic tools for these patients with aggressive pituitary tumors, some poorly responsive to standard treatments.

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