

## Familial Isolated Pituitary Adenomas (FIPA) and the Pituitary Adenoma Predisposition due to Mutations in the Aryl Hydrocarbon Receptor Interacting Protein (*AIP*) Gene

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Pituitary adenomas are one of the most frequent intracranial tumors and occur with a prevalence of approximately 1:1000 in the developed world. Pituitary adenomas have a serious disease burden, and their management involves neurosurgery, biological therapies, and radiotherapy. Early diagnosis of pituitary tumors while they are smaller may help increase cure rates. Few genetic predictors of pituitary adenoma development exist. Recent years have seen two separate, complimentary advances in inherited pituitary tumor research. The clinical condition of familial isolated pituitary adenomas (FIPA) has been described, which encompasses the familial occurrence of isolated pituitary adenomas outside of the setting of syndromic conditions like multiple endocrine neoplasia type 1 and Carney complex. FIPA families comprise approximately 2% of pituitary adenomas and represent a clinical entity with homogeneous or heterogeneous pituitary adenoma types occurring within the same kindred. The aryl hydrocarbon receptor interacting protein (*AIP*) gene has been identified as causing a pituitary adenoma predisposition of variable penetrance that accounts for 20% of FIPA families. Germline *AIP* mutations have been shown to associate with the occurrence of large pituitary adenomas that occur at a young age, predominantly in children/adolescents and young adults. *AIP* mutations are usually associated with somatotropinomas, but prolactinomas, nonfunctioning pituitary adenomas, Cushing disease, and other infrequent clinical adenoma types can also occur. Gigantism is a particular feature of *AIP* mutations and occurs in more than one third of affected somatotropinoma patients. Study of pituitary adenoma patients with *AIP* mutations has demonstrated that these cases raise clinical challenges to successful treatment. Extensive research on the biology of *AIP* and new advances in mouse *Aip* knockout models demonstrate multiple pathways by which *AIP* may contribute to tumorigenesis. This review assesses the current clinical and therapeutic characteristics of more than 200 FIPA families and addresses research findings among *AIP* mutation-bearing patients in different populations with pituitary adenomas. (*Endocrine Reviews* 34: 0000–0000, 2013)

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Abbreviations: AHR, Aryl hydrocarbon receptor; AIP, aryl hydrocarbon receptor interacting protein; ARNT, Ahr nuclear translator; CNC, Carney complex; EGF, epidermal growth factor; EGFR, EGF receptor; ER $\alpha$ , estrogen receptor  $\alpha$ ; FIPA, familial isolated pituitary adenoma; GNAS,  $\alpha$ -subunit of the stimulatory guanine nucleotide-binding protein; GR, glucocorticoid receptor; HIF1 $\alpha$ , hypoxia-inducible factor 1 $\alpha$ ; HSP90, 90-kDa heat-shock protein; LOH, loss of heterozygosity; miR-107, micro-RNA-107; MLPA, multiplex ligation-dependent probe amplification; MRI, magnetic resonance imaging; NFPA, nonfunctioning pituitary adenoma; PAP, pituitary adenoma predisposition; PDE, phosphodiesterase; PPAR $\alpha$ , peroxisome proliferation-activated receptor  $\alpha$ ; RB1, retinoblastoma; RET, rearranged during transfection; TR $\beta$ 1, thyroid hormone receptor  $\beta$ 1; XRE, xenobiotic response element.

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## I. Introduction

**P**ituitary adenomas are one of the most frequent intracranial tumors, particularly in young patients. Clinically relevant pituitary adenomas were shown to occur in 1:1064 of the population in Liège, Belgium, which has been confirmed in similar cross-sectional studies in the United Kingdom and Switzerland, giving an overall prevalence of 78–94 cases per 100,000 population (1–3). Their classically benign histology belies their medical impact. Due to their position close to vital local structures and the potent biological effects of pituitary hormone hypersecretion or deficiency, pituitary adenomas can cause some of the most severe classical conditions in endocrine practice, including acromegaly and Cushing disease. Diagnosis and management of pituitary adenomas often involves a multidisciplinary approach that combines endocrine, neurosurgical, and radiological specialists. Pituitary adenomas can also present late with already locally expansive and invasive disease. Although multiple effective modalities are available, the challenges of aggressive disease behavior remain and provide an impetus for research.

The study of pituitary adenoma pathophysiology can take a number of directions. Some groups have highlighted the molecular and signaling abnormalities within human and animal pituitary adenomas at a somatic level. These somatic molecular genetic abnormalities are numerous due to the complexity of the pituitary gland itself (4–6). Some of these discoveries have led to the experimental use of novel therapies, such as tyrosine kinase inhibitors in this setting (7–10). Another direction of research is into the realm of inherited endocrine neoplasia syndromes, such as multiple endocrine neoplasia type 1 (MEN1) and Carney complex (CNC) (11–13). These challenging, protean clinical syndromes are caused by germline genetic mutations that impact many tissues and cause multiple endocrine tumors, including pituitary adenomas as a characteristic feature (14, 15). Study of these disrupted genes and the impact of absent or abnormal protein on cellular signaling and regulation can also provide information about normal pituitary physiology and the pathophysiology of pituitary adenomas occurring outside these specific genetic settings. These diseases provide an important clinical opportunity,

namely, to allow the early diagnosis of at-risk carriers by germline genetic sequencing.

While MEN1 and CNC have been widely studied, numerous patients and families with apparently inherited endocrine neoplasia have been identified that have no genetic abnormalities in causative genes such as *MEN1* and *PRKAR1A*. Interest in these cases has led to the identification of new conditions, such as MEN4, that are only beginning to be studied. In particular, familial isolated pituitary adenoma (FIPA), consisting of kindreds with two or more related members having pituitary adenomas in the absence of known genetic causes, was identified and characterized over the last decade, and research interest in FIPA has led to hundreds of new kindreds being identified worldwide. In parallel, a new gene, aryl hydrocarbon receptor interacting protein (*AIP*), was found to cause an inheritable propensity for pituitary adenomas, which have since been proven to represent an important cause of pituitary adenomas in young patients. Together, these two complimentary clinical and genetic projects have highlighted novel findings that illustrate a group of more aggressive pituitary adenomas and a molecular pathway through AIP that opens a new understanding of pituitary adenoma pathophysiology.

## II. Familial Isolated Pituitary Adenomas (FIPA)

### A. Historical background

The medical literature on pituitary adenomas before the naming of acromegaly by Pierre Marie was relatively sparse (16). The promulgation of Marie's report and de Souza Leite's subsequent follow-up series led to a well-spring of clinical cases of acromegaly in the literature (17). Focus on the signs and symptoms of acromegaly, allied with interest in the surgical and pathological findings, quickly led to the accumulation of a large body of fundamental literature on pituitary adenomas in general, including work from leading lights such as Harvey Cushing (18). Within these historical works are found the first scientific descriptions of pituitary tumors in a familial setting. The genetic etiology of these early reports is uncertain, with some, like the report by Erdheim in 1903 of pituitary and parathyroid adenomas, being strongly suggestive of MEN1 (19). Indeed, the clinical reports of syndromic pituitary adenomas (*i.e.*, those occurring in association with other clinical abnormalities) led Wermer (20) to characterize MEN1 clinically (as Wermer syndrome), and he suggested correctly the inheritance mode. Like Wermer, Carney also undertook a meticulous clinicopathological characterization of his eponymous syndrome.

CNC was defined as a syndrome of myxomas, spotty pigmentation, and endocrine dysregulation, with acromegaly seen in about 10% of the original case series (21, 22).

Although syndromic pituitary adenomas in the historical literature can likely be ascribed to MEN1 or other diseases, some reports noted the occurrence of isolated pituitary adenomas occurring in a familial setting. Rare cases of familial gigantism from the press and advertising materials of the 17th to 19th centuries have been well documented by de Herder (23, 24). An early but controversial scientific description that was later attributed to familial acromegaly is Friedreich's report (25) of the Hagner brothers. Ostensibly, this report was one of bone and joint deformities, and a plate illustrating one of the brothers does not appear classically affected by acromegaly. A monograph on acromegaly by the Austrian physician Sternberg (26) (aided by Atkinson's 1899 translation) served as a detailed examination of the etiological and clinical aspects of the disease. Here, initial cases of familial acromegaly were discussed, such as cases reported by Schwoner (27). Fraenkel *et al.* (28) later described the case of a 50-yr-old male ("Herr Gleiche") who presented in 1898 and had first-degree relatives with acromegalic features (father and two siblings), and they also discussed a three-member familial acromegaly kindred. The two Hugo brothers were a very well-recognized familial pituitary tumor kindred with acrogigantism whose media fame crossed over into the scientific realm. While traveling to New York, one of the brothers died of a fulminant infection. On autopsy, the familial pituitary etiology was strongly supported by the finding of a pituitary macroadenoma larger than 40 mm in diameter (29).

In 1925, Bailey and Davidoff (30) reported the clinical features and tumor pathology of a series of patients treated by Cushing at the Peter Bent Brigham Hospital in Boston, Massachusetts. Case III was a 25-yr-old man who had onset of acromegalic features as an adolescent and, as the authors note, "came of a family of tall people. . . His paternal great uncle was 7 feet 1 1/2 inches tall (217 cm) . . . the "Kentucky giant." One could speculate that this was the same Kentucky giant (Jim Porter) whom Charles Dickens encountered at Portland, Kentucky, in 1842 and described in the book *American Notes for General Circulation*. Dickens' description of Mr. Porter is both sympathetic and medically suggestive: "He had a weakness in the region of the knees, and a trustfulness in his long face . . . He was only 25 years old, he said, and had grown recently . . . (he) went bobbing down the cabin, among men of six feet high and upwards, like a lighthouse walking among lamp-posts" (31). In 1937, Gray (32) outlined the clinical history of a middle-aged male patient with acrogigantism who had been a silent movie actor and per-

former and noted also a reputed history of familial gigantism in his grandfather. These descriptions represent individual patients or relatively small case series. In contrast, Atkinson (the translator of Sternberg's monograph in 1899) revisited the subject of acromegaly in a monograph of his own in 1932 (33). This publication is an exhaustive review of the literature up to the end of 1930 in which Atkinson reviewed and codified 1319 cases of acromegaly, most being derived from individual case reports. The review is, even today, one of the most extensive on the presentation and clinical symptomatology of acromegaly. Among the listings of cases, Atkinson notes eight in which acromegaly was reported to be familial in first-degree relatives (seven in parents and one in a grandparent).

Kindreds with familial acromegaly continued to accrue over the mid-to-late 20th century. The advent of immunoassays permitted the definitive linking of acromegaly to excess GH secretion, and probably the first case of familial acromegaly with defined hypersecretion of GH was described by Levin *et al.* (34). Thereafter, a variety of other individual case reports of familial acromegaly and acrogigantism were reported in the literature (35–42). The genetic causes of both MEN1 and CNC were discovered in the 1990s. The *MEN1* gene was initially localized to a specific region chromosome of 11q13 (43). The *MEN1* gene was ultimately cloned by researchers at the National Institutes of Health (NIH) in 1997 (44). Mutations in the protein kinase A  $\alpha$  regulatory subunit gene (*PRKAR1A*) on chromosome 17q were discovered to be related to CNC in 1998 and subsequently have been implicated in over 70% of cases (45, 46).

Therefore, by the end of the 1990s, the concept of familial acromegaly that was unrelated to existing conditions could be both clinically and genetically delineated. Before the identification of the *MEN1* gene, familial acromegaly kindreds without genetic linkage to the *MEN1* locus had been described initially by Benlian *et al.* (47) (three members— two living, one historical) and later by Yamada *et al.* (48) (three living members). These studies noted linkage to chromosome 11q13 and loss of heterozygosity (LOH) on chromosome 11q13 in tumor samples. Subsequent to the identification of the *MEN1* gene, a series of studies demonstrated multiple kindreds with familial acromegaly and no *MEN1* mutation (49–53). Such kindreds included families with more than three affected members, indicating a very strong likelihood of a common genetic cause. Most families had only somatotropinomas, but kindreds with prolactinomas alone or in conjunction with somatotropinomas were reported in Japan and Belgium (54–56). An early, very extensive Australian kindred of five affected persons was reported by Pestell *et al.* (38) in 1989. Although acromegaly predominated, one subject

had a prolactinoma, whereas another had likely co-secretion of GH and prolactin from the pituitary tumor. A subsequent study of three new kindreds from Liège, Belgium, reviewed the clinical findings of these and 17 other reported kindreds (45 cases) (57). An autosomal dominant inheritance pattern with reduced, age-dependent penetrance was the most parsimonious model to explain the recurrent pattern (57). Kindreds were usually small (two affecteds), and in 50% of cases only siblings were affected. An early onset of disease was noted (puberty through 30 yr of age), and tumors—usually somatotropinomas, but also somatomammotrope and plurihormonal cases—were frequently large and invasive. These findings are mirrored by those of Gadelha *et al.* (58), also in 1999, who used the term “isolated familial somatotropinoma” to describe these seemingly acromegaly-only kindreds. In addition to the characteristics of large tumor size and early age at diagnosis, they also noted a male preponderance and frequent gigantism among their studied population.

#### **B. The emergence and characterization of FIPA**

In contrast to the clear evidence for familial occurrence of acromegaly-gigantism, the older historic scientific literature lacks firm reports of other familial pituitary tumor types. It was not until the 1980s that viable studies of kindreds with other pituitary tumor types came to light. Three kindreds with familial Cushing disease were published; two of the cases involved second-degree relatives (aunt/nephew, aunt/niece), and one case was of Cushing disease in two sisters (59–61). Berezin and Karasik (61) reported three prolactinoma-only kindreds from Israel. From Japan, there was a description of nonfunctioning pituitary adenoma (NFPA) occurring in a familial setting (62). Many of these kindreds were reported before the advent of *MEN1* gene mutation screening, but in those cases, the clinical descriptions suggest a low likelihood of *MEN1*.

When taken together with data from other families noted in the previous section (38, 54–57), it became apparent that pituitary adenomas other than somatotropinomas could occur in a familial setting. To investigate this, a specific research program was undertaken at the University of Liège to identify kindreds with two or more pituitary adenomas (not limited *a priori* to somatotropinomas) in which *MEN1* or *CNC* did not play a role. We defined this condition as FIPA to describe the clinical findings accurately and to use terminology in concordance with analogous conditions in hereditary endocrine neoplasia (*e.g.*, familial isolated hyperparathyroidism, familial medullary thyroid carcinoma). That study identified 27 FIPA patients with somatotropinomas, prolactinomas, and nonsecreting pituitary adenomas among a group of

1500 patients (63). Patients within the same family could have the same pituitary tumor type in all affected members (homogeneous FIPA), or different pituitary tumor types could exist within the same family (heterogeneous FIPA). To confirm that the phenotype of FIPA was seen more generally, we performed an international case-finding study from 2000–2004 that identified new FIPA kindreds at 22 European and North American centers (64). Negative *MEN1* genetic screening was available in at least one affected member of each FIPA kindred, and *MEN1* was also ruled out clinically. For *CNC*, the situation was more focused, given the more limited repertoire of pituitary tumor types associated with this disease (normal *PRKAR1A* gene sequencing was available in at least one affected member of each homogeneous somatotropinoma kindred); all FIPA kindreds were clinically screened to exclude other features suggestive of *CNC*.

The clinical characteristics of FIPA patients ( $n = 138$ ) and how they compared statistically with those of matched sporadic nonfamilial pituitary adenoma cases ( $n = 288$ ) were first described by Daly *et al.* (65) in 2006. In about 75% of that cohort, the relationship between affected members was sibling/filial in nature (first degree). In that group, a mean of 15.4 individuals per family were assessed, and families with two, three, and four affected members were seen, whereas subsequent families of as many as seven or eight persons have since been classified as FIPA kindreds (66, 67). However, FIPA remains predominantly a condition of two to three, usually closely related persons per kindred, albeit kindreds are usually relatively small due to limited familial anamnesis in many reported families. In certain FIPA kindreds where extensive familial study has been possible, second- and third-degree relationships can be noted, particularly when photographs and other historical documents (old medical records) of deceased individuals are available for assessment. In general, FIPA is present in a minority of cases of pituitary adenomas overall, with Daly *et al.* (65) finding FIPA in approximately 2% of cases from two reference centers in one study. Multicenter epidemiological studies on the true prevalence of FIPA in large groups of pituitary adenoma patients are needed.

#### **C. Clinical characteristics of FIPA**

FIPA patients are diagnosed on average 4 yr before patients with sporadic pituitary adenomas (65). In families with vertical relationships between affecteds, patients in the later generations are diagnosed with pituitary adenomas at a statistically significantly younger age as compared with their parents or grandparents (on average 20 yr before). This earlier disease diagnosis in multigenerational families is particularly pronounced for homogeneous

FIPA kindreds irrespective of the tumor phenotype (65). This is probably due to improvements over time in the availability of diagnostic modalities [*e.g.*, magnetic resonance imaging (MRI)] and improved awareness of pituitary disease in the general medical community. Also, the sensitizing effect of having one family member with a pituitary adenoma already on improved recognition of pituitary related symptoms in another member should not be underestimated.

The rank order of frequency of pituitary adenoma subtypes in individual patients from our FIPA cohort is: prolactinoma (37.5%), somatotropinoma (35.0%), NFPA (14.5%), somatolactotropinomas (6.4%), Cushing disease (2.9%), gonadotropinomas (2.0%), plurihormonal tumors (1.2%), and thyrotropinomas (0.5%) (65, 68, 69), and unpublished observation of A. Beckers and A. F. Daly. Therefore, as compared with the frequencies of pituitary adenoma subtypes in the general epidemiological population, the proportion of FIPA patients with prolactinoma is conspicuously lower (66 *vs.* 37.5%), although they remain the most frequent overall. The proportion of patients with somatotropinomas is consequently much higher in the setting of FIPA than in the general epidemiological data (35.0 *vs.* 13%) (1, 65, 68, 69). Prolactinomas in FIPA are most frequently microadenomas that occur in premenopausal females; this does not differ from general characteristics of sporadic prolactinomas. Also reflecting the case with sporadic prolactinomas, males with prolactinomas in the FIPA setting comprise a minority of cases, but frequently present with macroadenomas. However, when prolactinomas occur in the setting of heterogeneous FIPA kindreds, they appear to have more aggressive characteristics than sporadic adenomas, being significantly more frequently invasive and extending toward the optic chiasm (65). This term “aggressive” is used in a relative sense of tumors that are more difficult to control therapeutically or larger tumor size. There has only been one pituitary carcinoma (a malignant prolactinoma) that was subsequently found to occur in a heterogeneous two-member FIPA kindred (a sibling had a large nonmalignant NFPA) (70). In our experience, somatotropinomas in FIPA are almost equally divided between homogeneous and heterogeneous FIPA kindreds (65, 69, 71). Patients from FIPA kindreds with homogeneous acromegaly correspond to the previous terminology of isolated familial somatotropinoma and share their features, having a larger adenoma diameter and tumors that have an earlier age of onset (57, 58, 72, 73). NFPA in FIPA mainly occur in heterogeneous families and have a significantly younger age at onset than their sporadic counterparts (mean, 8 yr) (65). FIPA-associated NFPA are significantly more frequently invasive than sporadic NFPA (84.6 *vs.* 59.6%, respectively). NFPA in FIPA

may be true null-cell adenomas or can be silent gonadotropin-positive, silent GH positive, silent corticotrope (type II), or plurihormonal tumors (65, 69, 74, 75). FIPA families with Cushing disease, TSH adenomas, and secreting gonadotropinomas are too rare to compare reliably against the characteristics of sporadic groups.

### III. Pituitary Adenoma Predisposition due to Aryl Hydrocarbon Receptor Interacting Protein (AIP) Gene Mutations

#### A. Genome-wide studies and the discovery of the role of AIP mutations

In sporadic somatotropinomas, LOH at 11q13 has been detected in a variable proportion of pituitary tumors (57, 76–78). As noted above, studies of familial acromegaly kindreds during the 1990s pointed to the involvement of a region of chromosome 11q13 that was separate from the *MEN1* locus (47, 48, 50, 55). These studies gave rise to important genetic linkage work that further refined the specific region involved to an area between microsatellite markers D11S956 and D11S527 (79, 80). By 2005, Soares *et al.* (81) had narrowed the region in question down to a 2.21-Mb stretch of DNA on 11q13.3, but targeted sequencing of potential candidate genes within that region proved negative. Among these acromegalic patients, macroadenomas were relatively common, gigantism was reported, and patients were relatively young at disease onset (57, 73, 82).

The hypothesis of a possible pituitary adenoma predisposition (PAP) gene in Finland was developed independently when three clusters of *MEN1*-negative families segregating acromegaly/gigantism and prolactinomas were detected in northern Finland. This familial occurrence of isolated pituitary adenomas not limited to acromegaly and without features of known syndromes prompted consideration of a previously uncharacterized form of low-penetrance PAP. Pedigree genealogy reaching back to the 1700s was available by combining information from the Finnish population register and parish registers. The genealogy analysis established a link between two families, whereas the third cluster appeared to be separate. The two linked clusters were found to have a common ancestor couple born in 1763 and 1770. Names in the pedigree genealogy were then combined with data from a previously characterized, population-based cohort of 54 acromegaly patients, diagnosed with GH-secreting adenomas between 1980 and 1999 in Oulu University Hospital, northern Finland (83). This enabled the construction of a complete pedigree with affected status (Fig. 1). Altogether, 11 affected individuals were identified. This genealogy

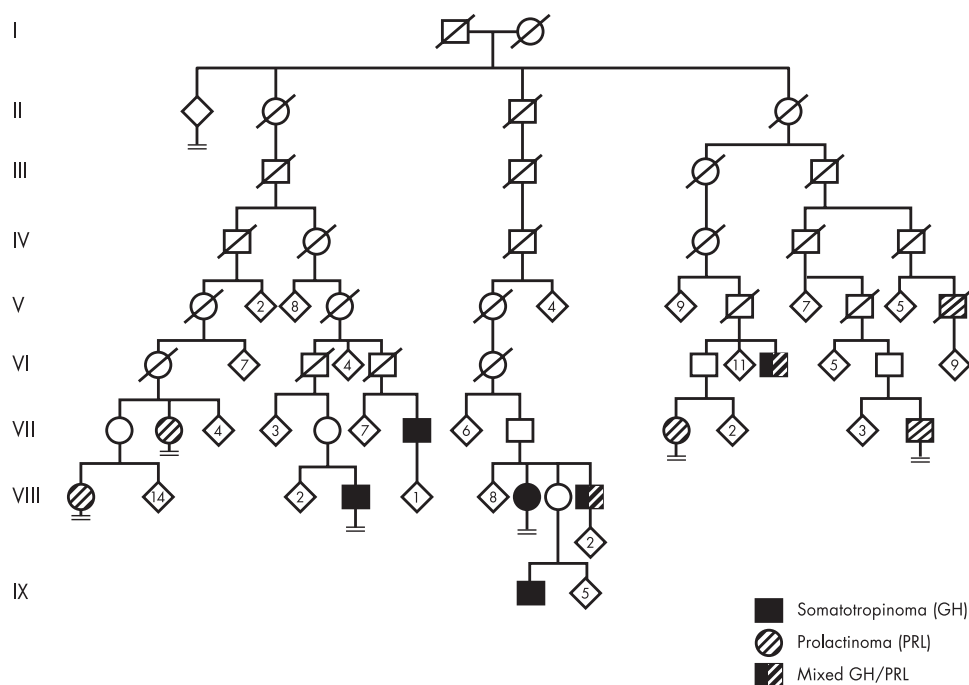
**Figure 1.**

Figure 1. Pedigree of original Finnish family with pituitary adenoma due to a Q14X founder mutation in *AIP*. Generations are indicated with *Roman numerals*. Generation I is from the 18th century. *Numbers within diamonds* indicate number of children. *Circles*, Females; *squares*, males; *diagonal line*, deceased. Pedigree has been modified for confidentiality.

was not limited to acromegaly only because there were five members with prolactinoma, four with somatotropinoma, and two with mixed GH/prolactin-secreting pituitary adenoma. The disease predisposition locus was identified in these families using whole genome single-nucleotide polymorphism genotyping. This, together with fine mapping of the most prominent candidate region on chromosome 11q12–13 (LOD score, 7.1), provided unambiguous evidence for susceptibility locus identification (84). The locus and pathology were termed “pituitary adenoma predisposition” (PAP) (OMIM no. 102200). The linked, more than 7-Mb long, founder haplotype segregated perfectly with the acromegaly phenotype in both pedigrees. Because the disease-associated haplotype was extremely gene rich, containing 295 genes, a novel approach was needed to select candidate genes. For this purpose, gene expression profiles were generated from peripheral blood samples of patients/obligatory carriers and healthy controls. In the gene expression experiment, we sought genes that were underexpressed, based on the earlier literature supporting the inactivation of a tumor suppressor gene in this chromosomal area locus (50, 51, 53). In the linked region, 172 probe sets fulfilled the criteria, and of these, 27 reached a  $P$  value  $\leq 0.05$ . Two probe sets representing the *AIP* gene occupied the first two positions, with  $P$  values of 0.00026 and 0.00114. Therefore, *AIP*

was chosen as the primary candidate gene. Direct genomic DNA sequencing in both families revealed an early stop codon mutation, c.40C>T/p.Q14X, in the first exon of *AIP*. The mutation was not detected in 209 population-matched healthy controls. The change segregated perfectly in patients with somatotropinoma and somatolactotrope type of adenoma and was also present in three prolactinoma patients. Interestingly, two other prolactinoma patients with microadenomas did not share the founder haplotype harboring the *AIP* mutation, thus representing phenocopies. The existence of such phenocopies is unsurprising given that prolactin-secreting microadenomas are the most frequently seen pituitary adenoma in the general population (1). Mutation screening of a northern Finland population-based group of 45 apparently sporadic acromegaly patients revealed the Finnish founder mutation p.Q14X in six patients and a splice-acceptor site (IVS3-1G>A) mutation in one patient. Overall, these two mutations accounted for 16% of all patients diagnosed with GH-secreting adenomas and for 40% of patients younger than 35 yr of age at diagnosis, indicating that the young age at onset is a useful indicator for the PAP caused by *AIP* mutations. The identification of *AIP* as a novel PAP gene was further confirmed when a late stop codon mutation in exon 6 (c.910C>T/p.R304X) was identified in Italian siblings affected with somatotropinoma at the

age of 18 yr (previously reported in Ref. 53). In all available tumor samples from the mutation carriers in Finland and Italy (including somatotropinomas, mixed GH/prolactin-secreting tumors, and prolactinomas), the wild-type allele was lost. This biallelic inactivation of *AIP* in the tumors strengthened the assumption that *AIP* is likely to act as a tumor suppressor (84).

## B. *AIP* mutations in specific populations

Studies have examined the prevalence of *AIP* mutations in FIPA kindreds, in unselected populations of sporadic pituitary adenomas, and in focused populations of pituitary adenoma patients, such as young patients, and in other nonpituitary tumors. Together, these studies have confirmed and extended the initial findings and helped to delineate the characteristics and epidemiology of pituitary adenomas associated with *AIP* mutations. For clarity, sporadic populations mentioned below refer to populations that had no known family history of pituitary adenomas. After genetic testing, pituitary adenoma patients with *AIP* mutations and no known family history of pituitary tumors (irrespective of whether they have unaffected mutation carriers as family members) are referred to as “simplex” cases by some authors (85)

### 1. *AIP* mutations in FIPA

After the report of Vierimaa *et al.* (84) describing *AIP* as a gene associated with a predisposition to pituitary adenomas in a familial setting, the role of *AIP* mutations in the pathogenesis of FIPA was investigated. Daly *et al.* studied an international cohort of 73 FIPA families ( $n = 156$  patients) from Europe and the Americas (71). Ten different germline *AIP* mutations were noted in that study; all but one were novel (the p.R304X mutation was noted in another Italian family). One of the changes, R16H, although highly conserved, has since been reclassified as probably being a variant of no pathogenic effect that does not map with the FIPA phenotype in some families (86–88). This study illustrated some of the characteristics of *AIP* mutation-positive FIPA patients. *AIP* mutations with pathogenic effects explained only a minority of FIPA families (<20%). A missense mutation, p.R271W, involving an important, conserved residue was found to cause different tumor patterns in two unrelated families, with acromegaly/gigantism in one kindred (described initially in Ref. 47) and somatotropinoma/prolactinoma in the second; this indicated that the same mutation in *AIP* could give rise to varying pituitary adenoma phenotypes in different kindreds. The p.K241E missense mutation, also in an important conserved residue, was associated with a two-sibling FIPA family (gonadotrope and  $\alpha$ -subunit-positive NFPA patient and a prolactinoma patient), dem-

onstrating that *AIP* mutations are not invariably related to somatotropinoma and prolactinoma-expressing kindreds only, and also included NFPA. In terms of demographic characteristics, the mean age at diagnosis was significantly lower in FIPA subjects with *AIP* mutations than those FIPA patients without mutations, and the mean maximal diameter of pituitary adenomas in the *AIP* mutation-bearing patients was significantly larger as compared with those without *AIP* mutations. Family screening also identified asymptomatic *AIP* mutation carriers. Because the PAP associated with *AIP* mutations is a condition with incomplete penetrance, asymptomatic mutation carriers are relatively common (67, 71, 84, 86, 89, 90).

The role of *AIP* mutations in FIPA families has also been studied by other groups, both as individual kindreds (89, 91–94) and as collaborative studies of multiple kindreds (67, 86, 90, 95–97). Two studies led by the Korbons group in London have accrued and assessed the characteristics of 64 FIPA kindreds (160 patients) worldwide and studied the role of *AIP* mutations in the population (67, 86). These FIPA studies are of particular interest also because they identified *AIP* mutations as the cause of many previously identified familial acromegaly families reported by Frohman's and Gadelha's research groups, Pestell *et al.*, and others (38, 39, 51, 79, 81). In their series, the mean age at diagnosis in the *AIP* mutation-positive FIPA cases was also significantly lower than in the *AIP* mutation-negative FIPA cases (approximately 16 yr). The Korbons group found that families with *AIP* mutations had a significantly higher number of affected ( $3.2 \pm 1.8$  cases) than *AIP* mutation-negative FIPA families ( $2.2 \pm 0.4$  cases) (86).

A combined analysis of the published results on FIPA cohorts, including 45 novel, genetically characterized, previously unreported FIPA kindreds, shows that a total of 211 FIPA families have been described in a manner that permits data analysis. These subdivide into 127 homogeneous families (60.2%) and 84 heterogeneous families. As shown in Fig. 2A, among homogeneous FIPA kindreds, somatotropinoma families (including those with somatotrope tumors) and prolactinoma families make up 90% of the total, with homogeneous NFPA (7.2%) being the only other frequent familial type. The heterogeneous FIPA cohort (Fig. 2B) contains 11 different combinations of pituitary adenomas, although subtypes such as somatotropinoma-prolactinoma and combinations of somatotropinomas and/or prolactinomas with NFPA account for nearly 90% of cases. However, FIPA families with heterogeneous presentation of Cushing disease, thyrotropinomas, and gonadotropinomas also have been identified. Among the 211 FIPA families reported comprehensively in the literature or studied by the authors, 43 *AIP*

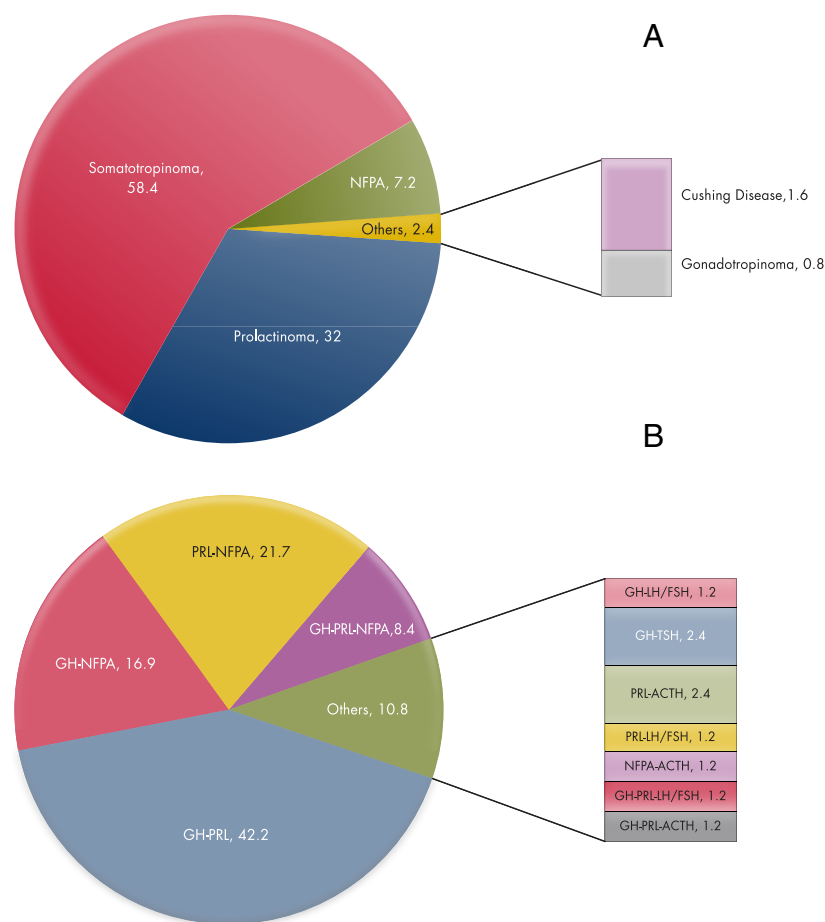
**Figure 2.**

Figure 2. Proportions of 211 FIPA kindreds with homogeneous (A) or heterogeneous (B) presentation of pituitary adenomas within the same family. GH, Somatotropinoma (includes also somatolactotrope tumors); PRL, prolactinoma; ACTH, Cushing disease; LH/FSH, gonadotropinomas; TSH, thyrotropinomas.

mutation-bearing FIPA kindreds have been identified. Thus, *AIP* mutations appear to explain only a minority of FIPA kindreds (20.4%): 29 homogeneous families (28 somatotropinoma, one NFPA), and 14 heterogeneous families (10 somatotropinoma-prolactinoma, one somatotropinoma-NFPA, two prolactinoma-NFPA, and one prolactinoma-Cushing disease family). There was a higher proportion of *AIP* mutation positivity among homogeneous FIPA families (22.8%) as compared with heterogeneous FIPA kindreds (16.7%), which is largely due to *AIP*-positive homogeneous acromegaly kindreds. The proportion of homogeneous acromegaly kindreds explained by *AIP* mutations was 36.1%. It is important to note that to date no homogeneous prolactinoma ( $n = 40$ ), Cushing disease ( $n = 2$ ) or gonadotropinoma ( $n = 1$ ) FIPA kindreds have been shown to be *AIP* mutation positive in our analysis or in those from the other large international collaborative group (67, 86, 98).

## 2. *AIP* mutations in unselected sporadic populations

A germline *AIP* mutation was initially identified in 16% of seemingly sporadic acromegaly patients without a known family history of pituitary adenoma from the same geographical region of Finland (84). Studies of unselected sporadic pituitary adenoma patients have shown a low rate of *AIP* mutations. A general population-based study of 460 pituitary adenoma patients representing genetically heterogeneous populations from Europe and North America had an overall prevalence of *AIP* mutations of less than 2% (99). Using sequencing and multiplex ligation-dependent probe amplification (MLPA) for *AIP* mutations in 148 patients (germline and somatic DNA), Barlier *et al.* (100) noted an even lower prevalence (0.7%). More recently, Cazabat *et al.* (101) performed a large, single-center screening approach in 443 patients with sporadic pituitary adenomas. This study noted that *AIP* mutations account for no more than 3.6% of unselected pituitary adenomas overall (4.5% of prolactinoma patients, 4.1% of acromegalic patients, 6.8% of Cushing disease patients, and <1% of NFPA patients). Taking these and other studies in general sporadic pituitary adenoma populations together, *AIP* mutations occur at a relatively low frequency of less than 4%, meaning that unselected screening efforts are probably not a highly efficient method

for identifying *AIP* mutation-positive cases in the general, nonfamilial pituitary tumor patient population (67, 91, 92, 95, 100–104).

## 3. *AIP* mutations in young adult patients

In the original study on *AIP* mutations and pituitary tumors, the Finnish founder mutation p.Q14X and the splice site mutation IVS3–1G>A accounted for a significant fraction of population-based acromegaly patients, especially the ones diagnosed at an early age (six of 15 aged <35 yr at diagnosis) (84). Since then it has been established that young age at onset/diagnosis is a characteristic feature of *AIP*-related pituitary adenomas. Among large heterogeneous international populations of pituitary adenoma patients, *AIP* mutations tend to occur rarely (0–3.6%) when unselected populations are studied (67, 92, 95, 100, 101, 105, 106). However, those cases that are



identified are almost invariably found in younger patients. For example, Georgitsi *et al.* (99) reported that *AIP*-related pituitary adenomas occurred in 5.5 and 7.4% of those aged less than 45 and less than 40 yr of age at diagnosis, respectively. Although in unselected cases the prevalence of *AIP* mutations fell to 0–1.8%, all but three cases had disease onset before the age of 30, and one patient was an 8 yr old child. Similarly, Barlier *et al.* (100) found that only one of 148 (0.7%) unselected cases from Marseille/Liège bore an *AIP* mutation: a somatotropinoma in a male aged 24 yr at diagnosis. In the large Parisian cohort of unselected pituitary adenoma patients noted above, *AIP* mutations were detected in 3.6% (16 of 443) of cases, of which seven were diagnosed before the age of 18 yr (107). Occhi *et al.* (95) reported a similar overall rate of deleterious *AIP* changes in Italian patients (3.1%), although the age at diagnosis was higher with all four patients age 30 yr or older at diagnosis. Studies in FIPA kindreds from various groups have shown that *AIP* mutation-bearing patients are generally young at diagnosis (67, 71, 86) and significantly younger than FIPA cases without *AIP* mutations (67, 71, 86). We performed a specific screening study that was limited to sporadic patients with two of the most characteristic features of *AIP* mutation-related pituitary adenomas: young age (<30 yr of age) and macroadenoma at diagnosis (108). Germline *AIP* mutations were found to occur in 19 of 163 patients (11.7%), rising to 20.5% of pediatric/adolescent patients (aged <18 yr at diagnosis). Overall, 13% of somatotropinomas, 11.5% of prolactinomas, and one of 16 (6.3%) NFPA had germline *AIP* mutations. Although these patients had no known history of FIPA, six of seven families of affected patients that permitted testing revealed *AIP* mutation carriers (two asymptomatic microadenomas were diagnosed in carriers).

#### 4. *AIP* mutations in pediatric and adolescent patients

The occurrence of pituitary tumors among children and adolescent patients is rare, and approximately 2–6% of all surgically treated pituitary adenomas occur in young patients—prolactin- and ACTH-secreting adenomas being the most common types. Pediatric somatotropinomas are usually more aggressive than those in adults (106, 109, 110). Studies have examined *AIP* mutation prevalence among populations that specifically included children and adolescents (*i.e.*, those aged <18 yr at diagnosis or disease onset). A screening study was performed by Georgitsi *et al.* (104) in a specific, sporadic, non-FIPA pediatric population ( $n = 36$ ) aged less than 18 yr at diagnosis or at the time of first signs/symptoms of a pituitary adenoma. Two patients were found to have pathological *AIP* mutations; one was a male with gigantism due to a large somatotropi-

noma, and the other had a NFPA at the age of 15 yr, giving an overall prevalence in this pediatric series of 5.6%. Subsequently, Stratakis *et al.* (109) reported a series of patients from the NIH Clinical Center in the United States. This was a diverse population of patients with and without familial or associated syndromic disease features. One of 74 pediatric patients (1.4%) with isolated sporadic Cushing disease had an *AIP* mutation; this patient was diagnosed at age 6 yr with a microadenoma that recurred postsurgically and required radiotherapy to achieve control, resulting in panhypopituitarism. Two pediatric patients with non-FIPA sporadic somatotropinomas ( $n = 1$ ) or prolactinomas ( $n = 1$ ) had *AIP* mutations; both were macroadenomas, and one was a *de novo* mutation, which remains the only case reported to date. Finally, one 11-yr-old patient from a heterogeneous FIPA family with a somatolactotrope macroadenoma had an *AIP* mutation, which was poorly responsive to somatostatin analogs and required three operations and radiotherapy. Interestingly, that patient's tumor had zones of hyperplasia on pathological analysis, a novel feature that was later reported in fraternal twin sisters with silent somatotrope adenomas and a separate *AIP* mutation (75). In the latter study, it was noted that while the adenoma tissue had LOH for *AIP*, as would be expected by the Knudson two-hit hypothesis, both the hyperplastic and normal pituitary tissues did not have LOH for *AIP*. This suggests that if the hyperplastic and adenomatous tissue were pathologically part of the same process, then loss of the wild-type allele may be a relatively late event in tumorigenesis in *AIP*-mutated FIPA kindreds. Overall in the NIH series, eight of 88 (9.1%) patients had mutations in either *AIP* or *MEN1* genes, of which *AIP* comprised four of 88 cases (4.5%), although this population was predominantly Cushing disease patients, in which the rate of *AIP* mutations is low. A higher rate of *AIP* mutations appears to be present in pediatric and adolescent populations with macroadenomas, because Tichomirowa *et al.* (108) reported 20.5% of patients aged less than 18 yr at diagnosis had a germline *AIP* mutation. Similar evidence has been noted in a French series of 443 sporadic pituitary adenoma cases (101); of these cases, 30 were aged less than 18 yr at diagnosis, and 23.3% were noted to have germline *AIP* mutations. Pediatric/adolescent cases of *AIP* mutation-related pituitary adenomas, like their adult counterparts, are generally somatotropinomas, prolactinomas, mixed GH/prolactin-secreting adenomas, although Cushing disease cases are also seen.

#### C. Founder *AIP* mutations

The p.Q14X mutation has been described in the Finnish population and from haplotype and genealogical data

is considered a founder mutation. A patient from Estonia with the same mutation has been noted and is possibly due to migration of this patient's ancestors from Finland (111). Founder mutations for a number of diseases (often recessive) have been described in Finland due to specific patterns of geographically delimited genetic drift in original founding populations after the last Ice Age, to which gene flow from Scandinavian populations to the west and south has created some striking differences between southern/western and northern/eastern Finland. We cannot speculate on when the founding *AIP* mutation occurred, in fact, although Finnish genealogical and parish information provide an excellent database. Historical evidence does point to many relevant cases coming from the same region of Finland. Daniel Cajanus, a northern Finnish man from Paltamo (modern province of Kainuu) was affected by gigantism in the early 18th century, and his sister and cousin also reputedly suffered from tall stature and enlarged extremities, respectively. Another man with gigantism from Tornio, northern Finland, was also noted some 70 yr previously (112), whereas other well-known patients with acrogigantism either came from or had family from Paltamo (Väinö Myllyrinne) or the nearby Puolanka [Lauri (Louis) Moilanen]. All of these cases came from or had their origin in a limited geographical area separated by no more than 200 km and also very close to Oulu, where many of the p.Q14X cases are currently cared for.

The p.R304X mutation has also been shown to be a founder mutation. The first published evidence of this came from Occhi *et al.* (96) who compared the microsatellite marker pattern around the loci for the *AIP* and *MEN1* genes in three Italian families, two of which were previously described (71, 84). Haplotyping showed that two of the families shared alleles that suggest a common ancestor, probably in the Lazio region from which they originated. Subsequently, Chahal *et al.* (90) connected a historic case of acrogigantism to four FIPA families from Northern Ireland, via a common p.R304X mutation of *AIP* with a common ancestor between approximately 375 and 3750 yr ago. Among patients with gigantism, the case of Charles Byrne has probably been the best documented in the popular and scientific literature (113, 114). He was born in County Derry in the Irish Province of Ulster, and following his death in 1783, his skeleton has been on public display, currently at the Hunterian Museum in London. Extracting DNA from the teeth, Chahal *et al.* (90) demonstrated that Charles Byrne's gigantism (and enlarged pituitary fossa as shown by Cushing a century before) was also due to a p.R304X *AIP* mutation and had a shared haplotype with the four modern-day FIPA families. The p.R304 residue of *AIP* is a hot spot for truncating mutations (c.910 C>T) and also for a missense p.R304Q mu-

tation (c.911 G>A), due to its being a CpG site; multiple FIPA families and simplex cases with these mutations have been described across the globe (Table 1). Similarly, other pathological mutations, such as p.R271W, p.K241E/p.K241X, and p.R81X also have been shown to act as hot spots in multiple kindreds (71, 109, 115–117).

#### D. *AIP* mutation screening: current status

Based on a review of the published literature, online resources such as GenBank and the authors' own unpublished screening results, a total of 215 patients with *AIP* mutations and pituitary adenomas have been reported. As shown in Table 1, to date, 70 different *AIP* mutations have been identified. Mutations in *AIP* occur relatively evenly throughout the coding region of the gene. All of these mutations have been germline mutations, and no somatic *AIP* mutations have been found in pituitary tumors. Different mutation types include nonsense, missense, splice site, insertion, deletion, frameshift, and promoter region mutations, as well as heterozygous deletion of most of or the entire *AIP* gene. Nonsense mutations and frameshifts leading to truncations account for half of reported *AIP* mutations. The missense mutations shown in Table 1 involve residues that are highly conserved and/or have been shown to correlate with altered *in vitro* activity in the published literature. Similarly, a variety of groups have used various *in silico* and *in vitro* methods to verify the pathogenicity of many intronic/splicing mutations. However, in cases where clear deleterious effects or correlates of genetic variants are not clearly present, caution should be used before labeling them as mutations, and a more conservative terminology such as "a variant of unknown significance" may be employed.

The most common mutations of the *AIP* gene are p.R304X (n = 35 patients), p.Q14X (n = 19 patients), p.R271W (n = 10 patients), and p.R304Q (n = 10 patients). As screening procedures have expanded globally, other mutations such as p.R81X appear to be growing in frequency. As seen in Fig. 3A and Table 1, the pituitary adenomas definitively diagnosed in association with *AIP* mutations consist of 132 somatotropinomas, 19 somatolactotropinomas, 29 prolactinomas, 13 NFPAs, four Cushing disease, one thyrotropinoma, one gonadotropinoma, and one plurihormonal tumor (tumor type not available; n = 15). The population remains predominantly male (61.2%), and the majority of patients present with macroadenomas (88.3%). Most cases present as FIPA (68.2%). Other cases may be pure simplex cases in which only one known *AIP* mutation-positive pituitary adenoma patient exists among a family of *AIP* mutation carriers (7.6%). So-called sporadic cases (24.2%) are likely to be a mix of classifications. Many will be cases in

**TABLE 1.** *AIP* mutations identified in patients with pituitary adenomas and the main associated clinical/demographic features

Mutation/variant	Protein prediction	Affected patients (n)	FIPA, sporadic, familial/simplex cases	Pituitary tumor type	Gender	Age at diagnosis	Macroadenoma/microadenoma (n)	Gigantism	Country (no. of patients)
Truncating mutations									
c.40C>T	Q14X	19	FIPA (13), sporadic (4), familial (1), N/A (1)	Somatotropinoma (11), somatolactotropinoma (2), NFPA (3), prolactinoma (2), N/A (1)	M (12), F (6), N/A (1)	11, 14, 17, 18, 19, 21, 22, 23 (2), 24, 28, 29, 31, 34, 35, 41, 42, 48, N/A (1)	Macroadenoma (17), Microadenoma (1), N/A (1)	5	Finland (18), Estonia (1)
c.64C>T	R22X	2	Familial (2 separate)	Somatotropinoma (2)	M (2)	11, 22	Macroadenoma (2)	1	France (1), Spain (1)
c.70G>T	E24X	7	FIPA (7)	Somatotropinoma (3), somatolactotropinoma (1), N/A (3)	M (3), F (4)	13, 15, 16, 17 (2), 18, 24	Macroadenoma (3), microadenoma (1), N/A (3)	5	Brazil (7)
c.241C>T	R81X	4	FIPA (4)	Somatotropinoma (4)	M (2), F (2)	14, 25, 34, 36	Macroadenoma (4)	1	United States (2), Brazil (2)
c.424C>T	Q142X	3	FIPA (3)	Somatotropinoma (3)	M (2), F (1)	17 (2), 29	Macroadenoma (3)	1	Italy (3)
c.490C>T	Q164X	2	FIPA (2)	Somatotropinoma (2)	M (1), F (1)	20, 23	Macroadenoma (2)	2	Germany (2)
c.550C>T	Q184X	1	Familial	Somatotropinoma (1)	M (1)	21	Macroadenoma (1)	1	Italy
c.601A>T	K201X	2	Sporadic (2)	Somatotropinoma (2)	M (1), F (1)	24, 27	Macroadenoma (2)		France (2)
c.646G>T	E216X	2	FIPA (2)	NFPA (silent somatotrope; 2)	F (2)	12, 17	Macroadenoma (2)		France (2)
c.649C>T	Q217X	2	FIPA (2)	Somatotropinoma (1), somatolactotropinoma (1)	M (1), F (1)	23, 28	Macroadenoma (1), microadenoma (1)		Belgium
c.662dupC	E222X	2	FIPA (2)	Somatotropinoma (2)	M (2)	24, 28	Macroadenoma (2)		United Kingdom
c.715C>T	Q239X	2	FIPA (2)	Somatotropinoma (2)	M (2)	14, 15	Macroadenoma (2)	2	France (2)
c.721A>T	K241X	1	Sporadic ( <i>de novo</i> )	Prolactinoma	M (1)	18	Macroadenoma (1)		United States (1)
c.783C>G	Y261X	2	Sporadic (2)	Somatotropinoma (1), somatolactotropinoma (1)	M (2)	17, 28	Macroadenoma (2)	2	France (2)
c.804A>C	Y268X	3	FIPA (2), familial (1)	Somatotropinoma (2), prolactinoma (1)	M (1), F (2)	23, 24, 29	Macroadenoma (3)		Brazil (3)
c.910C>T	Q285X R304X	1 35	Familial FIPA (28), sporadic (2), familial (2), N/A (3)	Somatotropinoma (1), Somatotropinoma (27), somatolactotropinoma (2), prolactinoma (3), N/A (3)	M (1), M (16), F (16), N/A (3)	60 6, 8, 13, 16 (2), 17 (4), 18 (2), 19 (3), 20 (3), 21, 24 (2), 25 (2), 26, 27, 32 (2), 42, 62, N/A (7)	Macroadenoma (1) Macroadenoma (29), Microadenoma (1), N/A (5)		The Netherlands United Kingdom (19), Italy (7), New Zealand (2), Romania (2), Belgium (1), France (1), India (1), United States (1)
Initiation codon mutation									
c.2T>C	M1?	1	Familial	Somatotropinoma (1)	M (1)	8	Macroadenoma (1)	1	France
Mutations leading to frameshift and truncation									
c.3_4insC	R2fsX43	4	FIPA (4)	Somatotropinoma (2), NFPA (silent somatotrope; 2)	M (4)	8, 12, 41, N/A (1)	Macroadenoma (1), microadenoma (1), empty sella (1), N/A (1)	1	Greece
c.88_89del GA	D30TfsX14	1	Sporadic (1)	NFPA	M (1)	19	Macroadenoma		France
c.74_81delins7	L25PfsX130	5	FIPA (5)	Somatotropinoma (3), somatolactotropinoma (1), prolactinoma (1)	M (3), F (2)	13, 25, 27, 32, 38	Macroadenoma (4), microadenoma (1)	1	Australia
c.244_248delGAAGG	E82fsX7	1	Sporadic (1)	Somatotropinoma (1)	M (1)	15	Macroadenoma (1)		Bulgaria
c.249G>T	G83AfsX15	3	FIPA (3)	Somatotropinoma (1), prolactinoma (2)	M (3)	14, 60, N/A	Macroadenoma (2), N/A (1)	1	United Kingdom
c.286_287delGT	V96PfsX32	3	FIPA (3)	Somatotropinoma (3)	M (3)	22, 26, 52	Macroadenoma (2), empty sella (1)	2	Japan
c.350delG	E117AfsX39	3	Sporadic (3)	Somatotropinoma (1), prolactinoma (2)	M (2), F (1)	16, 18, 30	Macroadenoma (3)		France
c.338insACCC	P114fsX	1	Sporadic (1)	Somatotropinoma (1)	F (1)	12	Macroadenoma (1)	1	United States
c.404delA	H135LfsX21	1	Sporadic (1)	Somatotropinoma (1)	M (1)	14	Macroadenoma (1)	1	France
c.500delC	P167HfsX3	6	FIPA (6)	N/A (6)	M (2), F (4)	N/A	N/A (6)		Malaysia
c.517_521delGAAGA	E174fsX47	3	FIPA (3)	Somatotropinoma (2), somatolactotropinoma (1)	M (1), F (2)	17, 25, 35	Macroadenoma (2), microadenoma (1)	1	Brazil
c.543delT	L181fsX13	6	FIPA (6)	Somatotropinoma (2), prolactinoma (1), NFPA (1), N/A (2)	M (4), F (2)	16, 17, 32, 48, 65, N/A (1)	Macroadenoma (4), microadenoma (1), empty sella (1)	1	Spain
c.752delT	L251RfsX52	1	Sporadic (1)	Cushing disease (1)	F (1)	25	Macroadenoma (1)		France
c.824_825insA	H275QfsX12	1	Sporadic (1)	Somatotropinoma (1)	M (1)	8	N/A (1)		United States

(Continued)

TABLE 1. Continued

Mutation/variant	Protein prediction	Affected patients (n)	FIPA, sporadic, familial/simplex cases	Pituitary tumor type	Gender	Age at diagnosis	Macroadenoma/microadenoma (n)	Gigantism	Country (no. of patients)
c.854_857delAGGC	Q285fsX16	2	FIPA (2)	Somatotropinoma (2)	M (2)	20, 33	Macroadenoma (2)	1	Italy
c.919insC	Q307fsX104	2	FIPA (2)	Somatotropinoma (1), prolactinoma (1)	M (1), F (1)	11, N/A (1)	Macroadenoma (2)		United States
Inframe duplication c.805_825 dup	F269_H275dup	4	FIPA (3), sporadic (1)	Somatotropinoma (3), Somatolactotropinoma (1)	M (4)	14, 15 (2), 29	Macroadenoma (3), N/A (1)	3	United Kingdom, (3), France (1)
bp Substitutions c. (-270_-269CG>AA) and c.(220G>A)	Reduced promoter activity	2	FIPA (2)	Somatotropinoma (2)	F (2)	10, 14	Macroadenoma (2)	2	Japan
Inframe deletions c.66_71delAGGAGA	delG23_E24	1	Sporadic (1)	Somatotropinoma (1)	M (1)	20	N/A (1)		Germany
c.138_161del24	delG47_R54	2	FIPA (2)	Somatotropinoma (2)	M (2)	25, 28	Macroadenoma (2)		Argentina
c.742_744delTAC	delY248	1	Familial	Somatotropinoma (1)	M (1)	19	Macroadenoma (1)	1	Italy
Extensive deletions c.878_879AG>GT and c.880_891 delCTGGACCCAGCC	E293G; and del L294_A297	2	FIPA (2)	Somatotropinoma (2)	M (1), N/A (1)	20, N/A (1)	N/A (2)		Germany
c.1104_-109_279 + 578	Exon 1 and 2 deletion	2	FIPA (2)	Somatotropinoma (2)	M (1), F (1)	24, 46	Macroadenoma (1), N/A (1)		Germany
c.100-1025_279 + 357del	delA34_K93 (exon 2 deletion)	4	FIPA (4)	NFPA (2), somatolactotropinoma (1), plurihormonal tumor (GH, prolactin, gonadotrope; 1)	M (2), F (2)	17, 18 (2), 29	Macroadenoma (2), microadenoma (2)	1	United Kingdom
c.1-?_993+?del	Full gene (exon 1-6) deletion	3	FIPA (3)	Somatotropinoma (3)	M (3)	19, 23, 35	Macroadenoma (3)		United Kingdom
Full gene deletion	Exon 1-6 deletion	2	FIPA (2)	Somatotropinoma (2)	M (1), F (1)	10, 20	Macroadenoma (2)	2	Serbia
Missense mutations c.26G>A	R9Q	2	Sporadic (2)	Cushing disease (1), prolactinoma (1)	F (2)	14, 39	Macroadenoma (1), Microadenoma (1)		France
c.166C>A	R56C	1	Sporadic (1)	Prolactinoma	M (1)	26	Macroadenoma (1)		Bulgaria
c.174G>C	K58N	2	Sporadic (2)	Prolactinoma (1), gonadotropinoma (1)	M (1), F (1)	20, 32	Macroadenoma (2)		France
	L70M	2	FIPA (2)	Somatolactotropinoma (1), prolactinoma (1)	M (1), F (1)	22, 60	Macroadenoma (2)		Italy
c.250G>A	E84K	1	Sporadic (1)	Somatolactotropinoma	F (1)	22	Macroadenoma (1)		Ukraine
c.308A>G	K103R	1	Sporadic (1)	Cushing disease	M (1)	6	Microadenoma (1)		United States
c.509T>C	M170T	1	Sporadic (1)	Somatotropinoma	M (1)	32	Macroadenoma (1)	1	France
c.563G>A	R188Q	1	Sporadic (1)	Prolactinoma	F (1)	24	Microadenoma (1)		France
c.584T>C	V195A	1	Familial	Prolactinoma	M (1)	12	Macroadenoma (1)		Brazil
c.713G>A	C238Y	3	FIPA (3)	Somatotropinoma (3)	M (3)	19, 21, 23	Macroadenoma (3)		Mexico
c.718T>C	C240R	3	FIPA (3)	Somatotropinoma (3)	M (3)	13, 15, 25	Macroadenoma (3)	1	France
c.721A>G	K241E	2	FIPA (2)	Prolactinoma (1), NFPA (1)	M (1), F (1)	39, 53	Macroadenoma (2)		Belgium
c.769A>G	I257V	1	Familial	Thyrotropinoma	M (1)	39	Macroadenoma (1)		Spain
c.803A>G	Y268C	1	Familial	Prolactinoma	M (1)	28	Macroadenoma (1)		Belgium
c.811C>T	R271W	10	FIPA (9), sporadic (1)	Somatotropinoma (8), Somatolactotropinoma (1), prolactinoma (1)	M (8), F (2)	10, 12, 16, 18, 21, 22, 29, 42, N/A (2)	Macroadenoma (8), microadenoma (2)		France (4), New Zealand (3), India 3 (2), Italy (1)
c.829G>C	A277P	1	Familial	Somatolactotropinoma (1)	M (1)	12	Macroadenoma (1)	1	Italy
c.871G>A	V291M	1	Sporadic (1)	Somatolactotropinoma (1)	F (1)	30	N/A (1)		Italy
c.872T>A	V291E	1	Sporadic (1)	Somatolactotropinoma (1)	M (1)	21	Macroadenoma (1)	1	France
c.911G>A	R304Q	10	FIPA (2), familial (2), sporadic (6)	Somatotropinoma (6), prolactinoma (3), Cushing disease (1)	M (2), F (7), N/A (1)	15, 17, 25, 26, 30, 37, 38, 67, N/A (2)	Macroadenoma (7), microadenoma (2), N/A (1)		Italy (3), Romania (2), The Netherlands (1), Poland (1), Belgium (1), France (2)
c.974G>A	R325Q	1	Sporadic (1)	Prolactinoma	F (1)	18	Macroadenoma (1)		France

(Continued)

TABLE 1. Continued

Mutation/variant	Protein prediction	Affected patients (n)	FIPA, sporadic, familial/simplex cases	Pituitary tumor type	Gender	Age at diagnosis	Macroadenoma/microadenoma (n)	Gigantism	Country (no. of patients)
Silent/synonymous splice site mutations									
c.591G>A	E197E	2	Sporadic (2)	Somatotropinoma (2)	F (2)	23, 66	Macroadenoma (2)		Italy (1), Lebanon (1)
c.807C>T	F269F	5	FIPA (2), sporadic (3)	Somatotropinoma (3), prolactinoma (1), NFPA (1)	M (3), F (2)	10, 47, 58, N/A (2)	Macroadenoma (4), N/A (1)		United Kingdom (2), Belgium (1), Bulgaria (1), Italy (1)
Intronic mutations									
IVS3–2A>G		2	Sporadic (2)	Somatolactotropinoma (1), prolactinoma (1)	M (2)	16, 40	Macroadenoma (2)		France (2)
IVS3 + 1G>A		1	Sporadic (1)	Somatotropinoma	F (1)	62	Microadenoma (1)		Italy
IVS3–1G>A		1	Sporadic (1)	Somatolactotropinoma	F (1)	26	Microadenoma (1)		Finland
IVS2–1G>C		1	Sporadic (1)	Somatotropinoma	M (1)	17	Macroadenoma (1)		United States

The predicted effect on protein, where known, is included. Missense mutations are included based on having demonstrable effects *in vitro*, *in silico*, or occurring in highly conserved domains within the AIP molecule. M, Male; F, female; N/A, not available.

which familial screening for pituitary disease and/or *AIP* mutations was not offered, was declined, or is not reported, and as such, many could represent either unknown FIPA kindreds or simplex cases. Only one *de novo* mutation in *AIP* has been reported in a sporadic patient (109). Figure 3B shows the distribution of age at diagnosis among the known cases of *AIP*-associated pituitary adenomas. This confirms the consistent impression since the original studies on *AIP* that young age at diagnosis is a clear feature of this disease (71, 84, 99). A total of 78.0% of patients were diagnosed at or before the age of 30, and only a further 11.5% were diagnosed between 30 and 40 yr of age. Three patients had empty sella and a distant history of headache and acute illness that suggested apoplexy. As noted by Igraja *et al.* (86) in their series, apoplexy was a presenting feature in 8% of their 63 cases and has been noted in acute presentation by others in the FIPA setting (75, 86, 90, 118). Specific studies on series of patients presenting with pituitary tumor apoplexy may confirm this to be a suggestive feature of *AIP* mutation carriage. Gigantism occurred in more than one third of somatotropinoma cases (36.0%) and accounts for one fourth of all cases of *AIP* mutations reported to date. This confirms the finding from a specific comparison of *AIP* mutation-related somatotropinomas *vs.* sporadic *AIP*-intact cases that gigantism is significantly more common in the setting of *AIP* mutations and represents a characteristic phenotypic feature of the disease (115).

Most of the *AIP* mutations noted in Table 1 were identified by sequencing approach. Some patients harbor large germline *AIP* deletions, which are undetectable by traditional sequencing methods (86, 105). To identify such germline mutations, the use of the MLPA technique is needed and has proven useful in the demonstration of *AIP* genetic abnormalities, although in a relatively limited number of cases (86, 100, 105), and in numerous large studies no mutations were revealed by this method (95, 101,

108). MLPA studies in populations that are negative for *AIP* mutations on standard sequencing are, however, useful to identify those rarer instances of gene deletions (85).

#### E. Treatment outcomes in *AIP*-mutated pituitary adenomas

The characteristics of *AIP*-mutated pituitary adenomas as being large and expansive tumors occurring at a young age suggest a negative impact on treatment outcomes. Also, early evidence pointed to a possibility for *AIP*-mutated somatotropinomas to be relatively resistant to the effects of somatostatin analogs (67, 89). These and other characteristics were studied specifically in a series of 96 patients with *AIP* mutations and pituitary adenomas to determine whether larger tumor size translated into difficulties in disease control (115). Among the group, somatotropinomas were the most frequent type ( $n = 75$ ), and these patients were compared with a control group of 232 acromegaly patients without *AIP* mutations (Table 2). The *AIP* mutation group had significantly more males than control acromegaly patients. As expected from previous studies, *AIP* mutation-related tumors were significantly larger and more frequently had extrasellar extension at diagnosis. Fifty-two percent of acromegaly cases in the *AIP* mutation group had first symptoms before the age of 18 as compared with less than 5% of controls. Gigantism was significantly more common among the *AIP* mutation group *vs.* controls, and all cases of gigantism in that group occurred in males. The increased tumor size was associated with higher GH secretion at diagnosis in the *AIP*-mutated group *vs.* controls, whereas prolactin co-secretion was present in 56 and 29% of the *AIP*-mutated and control populations, respectively. These comparisons were all statistically significant (115).

After a follow-up of at least 1 yr, acromegaly patients with *AIP* mutations had a similar overall rate of disease (70.4%) as the controls (80.5%). Specifically, patients

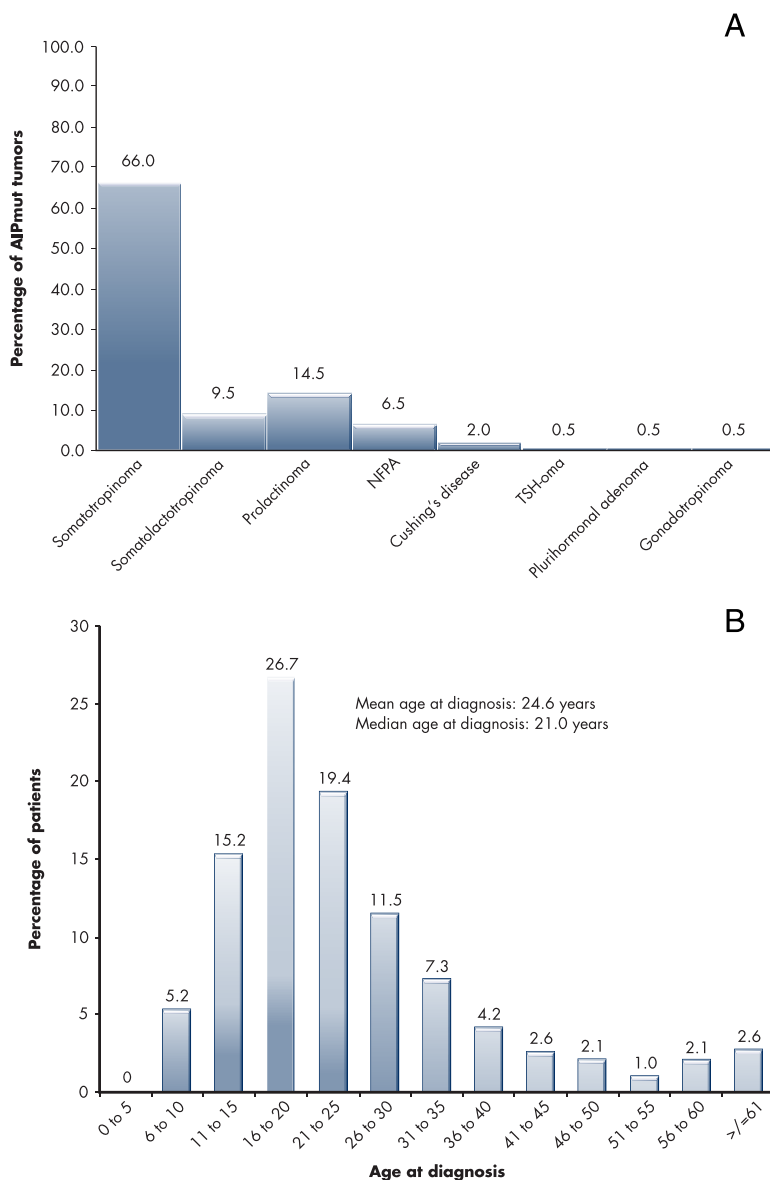
**Figure 3.**

Figure 3. A, Percentage of patients with different pituitary adenoma types seen in patients (n = 215) with germline AIP mutations. B, Distribution of AIP mutation-positive pituitary adenoma population by age at diagnosis (divided into 5 yr cohorts).

with somatotropinomas in the setting of AIP mutations required a second or third neurosurgical intervention significantly more frequently than controls (22 vs. 6%, respectively). Although radiotherapy was employed more frequently in the AIP mutation group (41%) as compared with controls (25%), this did not reach statistical significance. In patients with AIP mutations treated with somatostatin analogs, the median percentage decreases in GH and IGF-I secretion were significantly lower than in the acromegalic controls (Fig. 4). These differences were unaffected when preoperative, primary, and postopera-

tive somatostatin analog use was assessed separately. In parallel, a smaller degree of tumor shrinkage was noted with somatostatin analogs in the AIP mutation group vs. control patients. Four AIP mutation-bearing patients had complete resistance to somatostatin analogs and tumor growth while on treatment.

Similar to the case with somatotropinomas, patients with AIP mutation-related prolactinomas (n = 13) also displayed features that suggested relative resistance to treatment. More than three fourths of patients were males who were at a young age at the time of first symptoms (50% were younger than 18 yr). As with somatotropinomas, tumors were also large at diagnosis (only one was a microadenoma), and invasion of local structures had already occurred at diagnosis in nine of 13 cases. All but one case received dopamine agonist treatment, and overall efficacy was relatively poor (five of 12 cases controlled initially), leading to a need for surgery in seven patients, of whom one had four neurosurgical interventions and three underwent two transphenoidal operations each. Importantly, relatively poor responses to dopamine agonists were seen, including cases of primary and secondary resistance. The reason for this characteristic remains unclear because dopamine D2 receptor studies were not performed on the tumor tissues from these patients. Information on NFPA and other tumor types was based on small patient numbers, and conclusions are difficult to draw from the limited data.

Overall, patients with AIP mutation-related pituitary adenomas usually have somatotropinomas/somatolactotropinomas or prolactinomas; in the course of clinical management, these tumors appear to have relatively poor medical therapy responses and require more frequent reoperation. The basis for the reduced

response to somatostatin analogs in terms of hormonal reduction and tumor size changes is unknown. Further study of important determinants, such as tumor expression of somatostatin receptors 2 and 5 and particularly the truncated form of somatostatin receptor subtype 5 or other factors that determine octreotide responses is needed (119–125). Recent work from Chahal *et al.* (126) points to a potential mechanism via the zinc finger transcription factor ZAC1. ZAC1 is highly expressed in normal pituitary but is down-regulated in pituitary adenomas (123, 125); importantly, AIP expression is increased in

**TABLE 2.** Clinical characteristics of 75 *AIPmut*-associated and 232 control non-*AIPmut* somatotropinoma (n = 232) patients

	Somatotropinoma groups		P value
	<i>AIPmut</i>	Control	
n	75	232	
Percentage males	61.3%	46.5%	0.027
Age at diagnosis (yr)	22.0 (8.0–60.0)	43.0 (16.0–72.0)	<0.000001
Age at first symptoms (yr)	17.5 (4.0–50.0)	38.0 (14.0–70.0)	<0.000001
Maximum tumor diameter (mm)	22.5 (7.0–60.0)	16.0 (3.0–48.0)	0.00026
Macroadenoma	93.1	80.8	0.026
Extrasellar extension (%)	65.1	49.8	0.018
Invasion (%)	51.7	38.8	0.11
GH level at diagnosis (ng/ml)	28.5 (3.3–183.0)	17.4 (1.7–180.0)	0.00068
IGF-I level at diagnosis (%ULN)	217.0 (116.0–1090.0)	210.5 (20.0–550.0)	0.48
Prolactin co-secretion (%)	56.1	28.9	0.00023
Gigantism (%)	32.0	6.5	<0.000001

Extrasellar extension represents superior or lateral extension of the tumor beyond the sella on MRI/computed tomography or at surgery. Invasion represents evidence of the presence of pituitary tumor tissue invading or penetrating the normal border of the pituitary gland. Age at diagnosis, age at first symptoms, maximum tumor diameter, and GH and IGF-I levels at diagnosis are presented as median (range). ULN, Upper level of normal. [Adapted from A. F. Daly *et al.*: Clinical characteristics and therapeutic responses in patients with germ-line *AIP* mutations and pituitary adenomas: an international collaborative study. *J Clin Endocrinol Metab* 95:E373–E383, 2010 (115), with permission. © The Endocrine Society.].

tumors from patients previously treated with somatostatin analogs before surgery (126).

#### F. Role of *AIP* mutations in other tumor types

To date, the only tumors that have been found to unambiguously associate with *AIP* mutations are pituitary adenomas, which contrasts with *MEN1*, *MEN4*, and *CNC*, where several other tumor types are found among the typical manifestations. Mutation screening in a total of 499 colorectal, breast, and prostate cancers was performed (127). These tumors, apart from being the most prevalent among men and women worldwide, have been associated with acromegaly, particularly colorectal neoplasia. In this material, no somatic *AIP* mutations were identified. The occurrence of somatic *AIP* mutations has been studied also in nonpituitary endocrine tumors and familial nonmedullary thyroid cancer (128, 129). Altogether, 79 sporadic tumors of endocrine system, including thyroid, adrenal, and parathyroid lesions, carcinoids and adenocarcinoids, paragangliomas, and pancreatic tumors were screened with negative results; no germline *AIP* mutations were detected in familial nonmedullary thyroid cancers either.

Interestingly, loss of wild-type allele was recently reported in an adrenocortical carcinoma of an acromegaly patient with a germline *AIP* mutation (p.R81X) (130). However, the 11q13 LOH, in the region where the *MEN1* gene is also located, could also be accompanied by a germline defect in another, as yet unidentified, tumor suppressor gene at 11q13. The existence of such a gene related to adrenocortical tumorigenesis has been suggested previously (12, 131). Although other groups have also noted nonpituitary tumors in patients with *AIP* mutations, no

consistent pattern has emerged (67), and recently an association between meningioma and *AIP* mutation was discounted in a patient with a pituitary adenoma (117). Identification of rare tumor associations with *AIP*, which is already a quite low penetrance gene, may require further long-term follow-up of large numbers of affected individuals and mutation carriers. Hibernomas are benign neoplasms displaying cytogenetic rearrangements involving chromosome band 11q13. A recent study of Nord *et al.* (132) suggested *MEN1* and *AIP* deletion to be involved in the pathogenesis of these brown fat tumors, but these tumors have, as yet, not been identified in carriers of germline *AIP* mutations.

#### IV. Mouse Models and *in Vitro* Studies of *AIP* in Pituitary Tumor Biology

Mouse models have been widely used to study pituitary development, function, and disease to gain important insight into the role of particular genes in different pathways and the nature of tumor development in the pituitary gland. Moreover, if the mouse model recapitulates the phenotype of human disease, it can offer a platform in which to test new drugs or targeted therapies for patients.

##### A. Phenotypes of *Aip* knockout mouse models

The first *Aip* mouse model was published by Lin *et al.* (133). Homozygous *Aip* loss was associated with embryonic lethality due to the congenital cardiovascular abnormalities such as a double-outlet right ventricle, ventricular septal defects, and pericardial edema at an

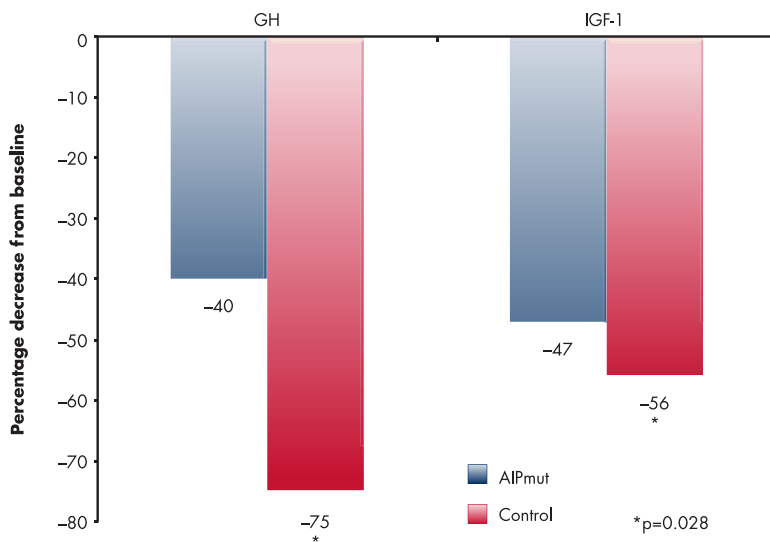
**Figure 4.**

Figure 4. Relative resistance to somatostatin analogs in *AIP* mutation-related somatotropinomas vs. controls. Patients treated with somatostatin analogs for acromegaly who had germline *AIP* mutations ( $n = 75$ ) had a statistically significantly lower percentage decrease from baseline in serum GH and serum IGF-1 concentration as compared with 232 wild-type *AIP* control patients that were matched for age, sex, and decade of diagnosis. [Derived from A. F. Daly et al.: Clinical characteristics and therapeutic responses in patients with germ-line *AIP* mutations and pituitary adenomas: an international collaborative study. *J Clin Endocrinol Metab* 95:E373–E383, 2010 (115), with permission. © The Endocrine Society.]

embryonic age of 10.5–14.5 d. In this same study, heterozygous (*Aip*<sup>+/-</sup>) mice were phenotypically normal and fertile. Moreover, Lin et al. (134) created a hypomorphic *Aip* mouse model, which displayed a reduced *Aip* expression. Hypomorphic *Aip* mice showed a patent ductus venosus resulting in reduced liver size (134). Interestingly, failure of ductus venosus closure has also been detected in aryl hydrocarbon receptor (*Ahr*) and *Ahr* nuclear translocator (*Arnt*) mouse models (135, 136), suggesting that AIP plays an important role in the AHR-mediated developmental pathway.

A conditional *Aip* mouse model where the *Aip* gene was deleted in hepatocytes showed that AIP deficiency leads to reduction of functional cytosolic AHR in the liver and eliminates/reduces dioxin-induced hepatotoxicity (137). AIP seemed to be required for the expression of AHR response genes, albeit that some of the dioxin-response genes were not dependent upon AIP coexpression. This differential dependence on AIP demonstrates that mammalian genome appears to contain more than one class of AHR-response genes and AIP may have a significant role in the up-regulation of a subset of these (137).

Possible tumor predisposition was not a focus of the above-mentioned *Aip* mouse model studies. To model

the PAP caused by germline *Aip* mutations and to clarify the tumor spectrum, Raitila et al. (138) created a conventional *Aip* mouse model. The mouse model was generated by inserting a gene trap vector construct into an intronic region of *Aip*, which produced a truncated AIP protein. Homozygous knock-out (*Aip*<sup>-/-</sup>) mice died during embryogenesis, this result being consistent with the earlier study of Lin et al. (133). Heterozygous *Aip* mice were highly prone to pituitary adenomas. *Aip*<sup>+/-</sup> mice developed pituitary tumors localized in the pars distalis, which corresponds to the human anterior pituitary, at the age of 6 months. No tumors were detected at 3 months, which could be explained by the true rarity of pituitary adenomas in this age group or possibly by the lesions being too small to be detected with routine immunostaining. AIP immunohistochemistry, as well as LOH screening, showed biallelic inactivation of *AIP*. Complete penetrance of pituitary adenomas was reached at the age of 15 months (Fig. 5), emphasizing the fundamental importance of *Aip* for pituitary tumorigenesis. The majority of mice developed somatotropinomas (88%), although mixed GH/prolactin, prolactinomas, and ACTH-positive adenomas were also detected; some adenomas grew to a very large size (Fig. 5, B and C) (138). *Aip*<sup>+/-</sup> mice with somatotropinomas had significantly elevated IGF-1 (*Igf-1*) expression levels and evidence of increased internal organ weight. The incidence of pituitary lesions in heterozygous *Aip* mice is extremely high as compared with known prevalence of these tumors in mice. However, no clear excess of any other tumor types was detected, although a slight excess of hyperplasia of adrenal glands was seen.

In humans, *AIP*-associated tumors can appear already in childhood or early adulthood. In the *Aip* mouse model, the first lesions were detected at the age of 6 months, thus in adulthood. The other main difference between the human and mouse *AIP* disease phenotype was the complete penetrance of pituitary adenomas in the *Aip* mouse model. Despite genomic conservation between the species (139), many biological functions differ, and any given response in humans may not occur in precisely the same way in mice (140). It is possible that such differences can explain these phenotypic discrepancies observed between human and mouse pituitary tumor onset and penetrance. Nevertheless, this conventional *Aip* mouse model greatly resembles human disease, displaying a pituitary tumor phenotype



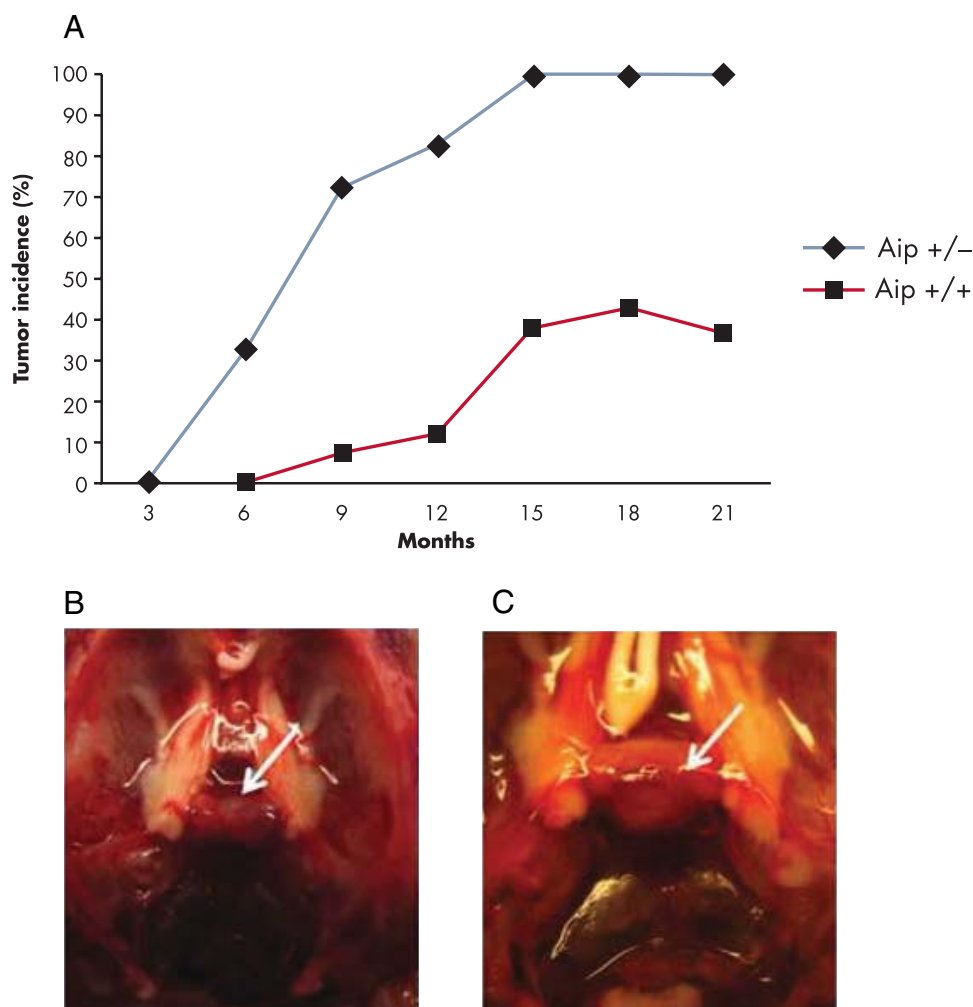
**Figure 5.**

Figure 5. *Aip* mouse model phenotype. A, Pituitary adenoma prevalence in heterozygous (*Aip*<sup>+/-</sup>) and wild-type (*Aip*<sup>+/+</sup>) mice. B, Normal pituitary gland of wild-type mouse. C, Macroadenoma of *Aip*<sup>+/-</sup> mouse. Pituitary glands are depicted by white arrows.

with a predominance of somatotropinomas. This suggests that the factors underlying *AIP* tumorigenesis are similar in mice and humans. Therefore, this mouse model provides an extremely useful tool to further study the *AIP*-associated pituitary tumorigenesis, and it is potentially a valuable platform for testing new therapeutic strategies for management of patients with treatment-resistant pituitary adenomas.

#### **B. Molecular and pathological characteristics of *AIP/Aip*-associated tumors**

The inactivating nature of the germline mutations, the loss of the normal allele in tumors, as well as recent functional evidence imply that the *AIP* gene acts as a tumor suppressor gene (67, 84, 138, 141). Wild-type *AIP* expression was shown to reduce cell proliferation in a rat somatomammotroph pituitary adenoma (GH3) cell line,

and human fibroblast (HEK293 and TIG 3) cells and the mutant form of *AIP* protein lost the ability to block cell proliferation (67). This finding was echoed in the subsequent work of Heliövaara *et al.* (141), which found that *Aip* small interfering RNA silencing leads to a clear increase of cell proliferation rates in the GH3 cell line.

Human *AIP*-associated pituitary tumors have more aggressive features as compared with *AIP* mutation-negative tumors (115), and elevated staining for Ki-67, a marker of cell proliferation, has been demonstrated in some human *AIP* mutation-related pituitary adenomas (93, 142). To assess the aggressiveness of *Aip*-related tumors, the proliferation rates were evaluated in a set of mouse *Aip*-deficient pituitary tumors using Ki-67 immunohistochemistry. The *Aip*-associated tumors had significantly higher proliferation rates compared with *Aip*-proficient tumors.

In *Aip* mutation-positive somatotropinomas and prolactinomas, the proliferation rates were 6.1 and 10.1%, respectively. In wild-type prolactinomas, the fraction of Ki-67-positive tumor cells was 3.6%. Hence, this result supports the view of a more aggressive disease profile of *AIP* mutation-positive tumors (138).

In a study of the histopathological characteristics of normal pituitary and adenoma tissues, Leontiou *et al.* (67) showed that in normal pituitary, *AIP* was present in GH- and prolactin-positive cells but not in other cell types. In sporadic pituitary adenomas without *AIP* mutations, *AIP* immunostaining was present in all adenomas irrespective of hormonal subtype; however, subcellular colocalization of *AIP* and hormone was only seen with GH in somatotropinomas. In *AIP* mutation-positive tumors from FIPA families (F269\_H275dup and R304X mutations), double immunofluorescence staining showed that *AIP* colocalized with GH. On electron microscopy of normal pituitary, Leontiou *et al.* (67) showed that immunogold staining for *AIP* occurred only in GH- and prolactin-positive cells, and this staining was localized to the secretory granules. Whereas in sporadic somatotropinomas immunogold staining for *AIP* was also localized to the GH secretory vesicles, in sporadic prolactinomas this association between *AIP* and prolactin in secretory vesicles did not exist (*AIP* immunogold staining was cytoplasmic, as in corticotropinomas and nonfunctioning adenomas). Sparse and dense granulation patterns on electron microscopy have long been noted to associate with relatively poor and good responses to somatostatin analogs, respectively (143–145). Furthermore, cytokeratin staining of somatotropinomas in dot and perinuclear patterns mirror the electron microscopic classifications of sparse and dense granulation, respectively. Interestingly, dot pattern cytokeratin staining somatotropinomas tend to be larger sized, to occur in younger individuals, and to have poorer responses to test doses/treatment with somatostatin analogs (146). This combination of features echoes the findings of *AIP*-mutated somatotropinomas, suggesting an overlapping pathological relationship, which is further strengthened by a high frequency of sparsely granulated adenomas in somatotropinoma patients with *AIP* mutation in FIPA kindreds (67).

Recently, it has been suggested that *AIP* may be a factor in tumorigenesis and treatment responses of somatotropinomas without *AIP* mutations. Jaffrain-Rea *et al.* (142) demonstrated in a large series of *AIP* mutation-positive and -negative tumors that *AIP* mRNA levels and immunostaining intensities correlated with tumor phenotype and aggressiveness. Somatotropinomas, independently of their germline mutation status, showed lower *AIP* protein levels in invasive tumors as compared with noninvasive tumors. Supporting this notion, Gadelha's group (147) in

Brazil recently noted that *AIP* immunostaining may be a more useful marker of invasiveness than Ki-67 labeling. Generally, patients with *AIP*-associated somatotropinoma have poor response to the somatostatin analog therapy (115). Kasuki *et al.* (148) studied whether the *AIP* protein expression could act as a predictor of treatment response to the somatostatin analog, octreotide, in *AIP* mutation-negative somatotropinomas. They reported that 22% of patients with low *AIP* levels in tumor were controlled with octreotide, whereas 65% of patients with high *AIP* protein expression were controlled. When both high *AIP* and somatostatin receptor 2 expression were used as predictors, control was achieved in 79% of patients. They concluded that the *AIP* protein expression alone is a good predictor of treatment response octreotide.

Because the regulatory actions of somatostatin analogs are mediated via five different somatostatin receptors, Chahal *et al.* (126) studied whether the reduced expression of the somatostatin receptor 1–3 and 5 proteins could explain the poor response to somatostatin analogs in *AIP* mutation-positive tumors. They did not detect reduction of somatostatin receptor subtypes, although the subtype 5 receptor had a somewhat higher expression in *AIP*-mutated tumors as compared with sporadic somatotropinomas (126). Importantly, *AIP* expression in tumors was increased in patients that were pretreated with somatostatin analogs before surgery. In that study, the effects of a somatostatin analog on *AIP* expression were examined in the GH3 rat somatomammotrope pituitary cell line. Octreotide treatment increased expression of *AIP* and *ZAC1*. Furthermore, overexpression of wild-type *AIP* was found to increase *ZAC1* expression, whereas silencing of *AIP* reduced *ZAC1* mRNA levels. Previously it was shown that *Zac1* acts downstream of somatostatin receptor subtype 2, and knockdown of *Zac1* (via RNA interference) prevented the effects of somatostatin analogs. Also, immunoreactivity for *ZAC1* in somatotropinomas has previously been shown to significantly correlate with IGF-I normalization and tumor shrinkage with somatostatin analogs (123–125). These observations suggest that in somatotropinomas *AIP* may be involved in the regulation of the action of somatostatin analogs via the *ZAC1* pathway. Furthermore, this interplay between the *AIP* and *ZAC1* genes might also explain the statistically significantly reduced response to somatostatin analogs in somatotropinoma patients with *AIP* mutations (115).

### C. Analysis of the biological functions of *AIP*

The *AIP* gene [also called the hepatitis B virus X-associated protein 2 (XAP2) (149), aryl hydrocarbon receptor-

Figure 6.

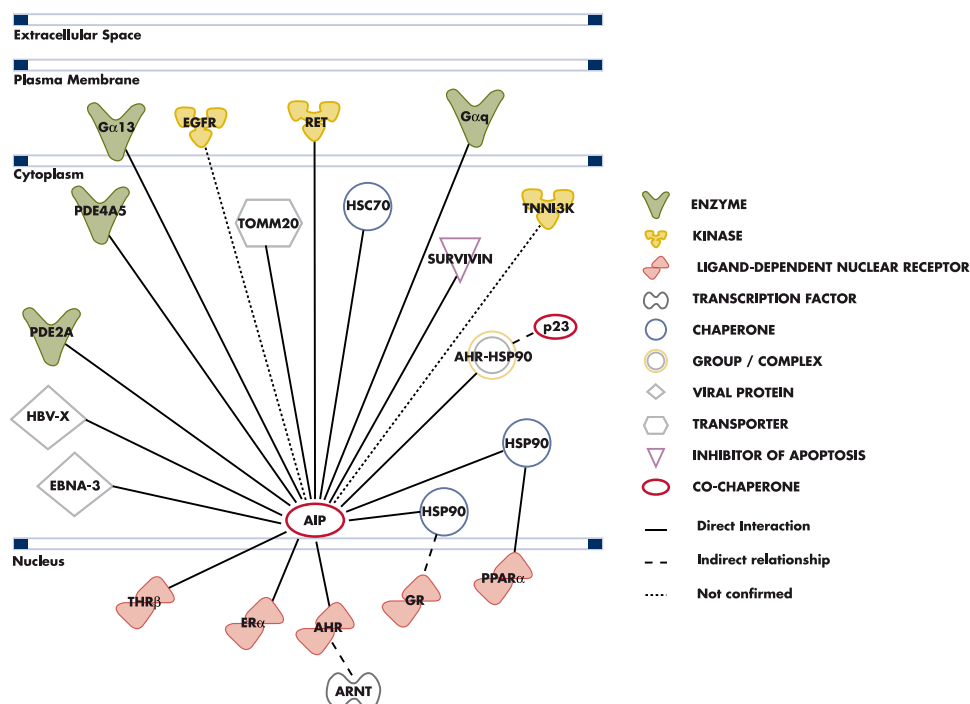


Figure 6. AIP interaction partners. Nodes represent proteins, with their shape indicating the functional class of the protein. The protein-protein interaction network was generated with Ingenuity Pathway Analysis (IPA) software ([www.ingenuity.com](http://www.ingenuity.com)).

activated protein 9 (ARA9) (150), or FK506-binding protein 37 (FKBP37) (151)] contains six exons, and it encodes a cytoplasmic protein of 330 amino acids (37 kDa). The AIP protein was originally identified by its interaction and inhibition of the hepatitis B virus X protein 2 (152, 153). AIP displays structural similarity to AIP-like 1 and the immunophilin co-chaperones, FK506-binding protein (FKBP) 51 and FKBP52 (149, 150, 154). Despite homology with immunophilins, AIP is not considered a true immunophilin because it does not bind and mediate effects of immunosuppressive drugs (155). AIP contains three tetratricopeptide repeats and an  $\alpha$ -helix in the C-terminal region, and this consensus tetratricopeptide repeat motif mediates various intra- and intermolecular protein interactions (156, 157). AIP can also self-associate and form a multimeric complex (158), and these AIP complexes might act as a reservoir for monomeric AIP that can be further used in the formation of AHR complexes.

AIP is considered as being ubiquitously expressed, but its expression levels vary considerably among different tissues (149, 153, 159). In normal pituitary, the AIP protein is expressed in somatotrophs and lactotrophs, where it associates with cytoplasmic secretory vesicles (67). Scattered AIP-expressed cells are detected also in the pars intermedia (142). In sporadic pituitary adenomas, AIP is expressed in all tumor types. In spo-

radic somatotropinomas, AIP colocalizes with GH in the secretory vesicles, similar to normal somatotrophs. In sporadic prolactinomas, corticotropinomas, and nonfunctioning adenomas, AIP resides in the cytoplasm (67). At present, the function of the AIP protein in normal pituitary or in pituitary adenoma cells is not known. Although it is generally acknowledged that AIP germline mutations predispose to pituitary adenomas, little is known about the molecular mechanisms leading to pituitary tumorigenesis. AIP has multiple cellular interaction partners (Fig. 6), and thus, AIP inactivation has the potential to interfere with a wide spectrum of cellular and environmental signals.

### 1. Xenobiotic signaling

The best-characterized AIP binding partner is AHR, also known as the dioxin receptor. AHR is a ligand-activated transcription factor belonging to the basic-helix-loop-helix (bHLH)/PER-ARNT-SIM homology region (PAS) family. In the cytoplasm, AIP forms a complex with AHR, two 90-kDa heat-shock proteins (HSP90), and the co-chaperone p23 (159–163), although Hollingshead *et al.* (158) suggested in their work that increased AIP levels can displace the HSP90-associated p23 from the AHR complex. AIP is involved in the cytoplasmic retention of AHR and decreases its proteosomal degradation by pro-

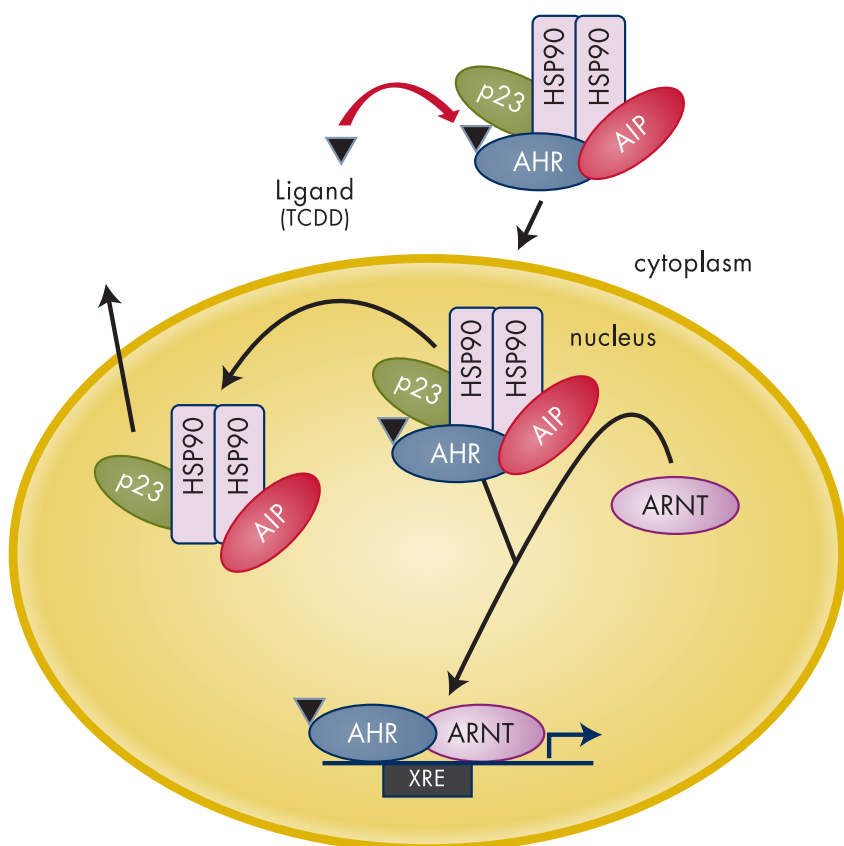
**Figure 7.**

Figure 7. A schematic figure of the xenobiotic signaling. AHR exists in a dormant state in cytoplasm in association with a complex of HSP90, AIP, and the co-chaperone p23. Upon ligand binding, AHR is activated through conformational change and translocates to the nucleus. It forms a heterodimer with ARNT. The heterodimer binds to the XRE and alters expression of genes involved in the metabolism of xenobiotic agents.

tecting it against ubiquitination (152, 160, 164, 165). Upon ligand binding, the HSP90 dimer is released from the complex, and AHR translocates into the nucleus, where it undergoes a conformational change and interacts with ARNT, also known as HIF1 $\beta$ . AHR-ARNT complex regulates the transcription of detoxification enzymes by binding to the xenobiotic response elements (XREs) (Fig. 7) (166, 167). This association is essential for transcriptional activation of these genes in the presence of environmental contaminants such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and polycyclic aromatic hydrocarbons. There are conflicting data about the role of AIP in regulating the activity of AHR. Some studies indicate that AIP appears to facilitate the transcriptional activity of AHR (149, 150, 168), whereas others suggest that AIP interaction inhibits AHR activation (169–171). The reason for the controversy regarding the effect of AIP interaction on AHR function may arise from species differences, tissue-specific effects, and the presence or absence of a ligand.

Exogenous AHR ligands, especially dioxin, are known to inhibit cell proliferation and induce cell cycle arrest (172), and therefore, the role of AHR/ARNT signaling was studied in *AIP*-mediated tumorigenesis (141). Immunostaining revealed that ARNT protein expression was significantly reduced in human *AIP*-associated tumors. In line with this finding, the *Aip* mouse model revealed the total lack of either ARNT or ARNT2 protein, but not both, in *Aip*-related pituitary lesions. Both proteins were always present in *Aip*-proficient pituitary tumors (138). Also, a trend toward increased nuclear expression of AHR was detected. Supporting the nuclear accumulation of AHR in the absence of functional AIP, Nakata *et al.* (168) demonstrated that small interfering RNA silencing of *Aip* caused a weak nuclear accumulation of AHR in ARNT-deficient mouse hepatoma cell line. In contrast, in the work of Jaffrain-Rea *et al.* (142), no nuclear expression of AHR was detected in AIP-associated pituitary tumors. Such a discrepancy might arise from antibody epitope differences and the type of mutations studied.

Down-regulation of ARNT proteins in *AIP* mutation-positive adenomas may disturb the AHR/ARNT complex formation and lead to aberrant expression of

xenobiotic response target genes (Fig. 8A). To study this aspect, expression of p27(Kip1), was studied in human *AIP*-deficient pituitary tumors (141). The expression of p27(Kip1) protein was, however, found to be equal in *AIP*-deficient and -proficient pituitary tumors (141). Although germline mutations in *CDKN1B* gene encoding p27(Kip1) cause MEN4 syndrome, such mutations are not associated with pituitary tumorigenesis in FIPA (173–175). Thus, it is not likely that p27(Kip1), acting via xenobiotic signaling would contribute to *AIP*-associated tumorigenesis. Nevertheless, dioxin-related compounds interfere with the body's endocrine system and may produce adverse developmental, reproductive, neurological, and immune effects (176). In the study of Pesatori *et al.* (177), where the authors analyzed the occurrence of pituitary adenomas in the Seveso population in Italy, after a severe 2,3,7,8-tetrachlorodibenzo-*p*-dioxin exposure accident in 1976, no statistically significant increase in the prevalence of pituitary tumors was found. However, an

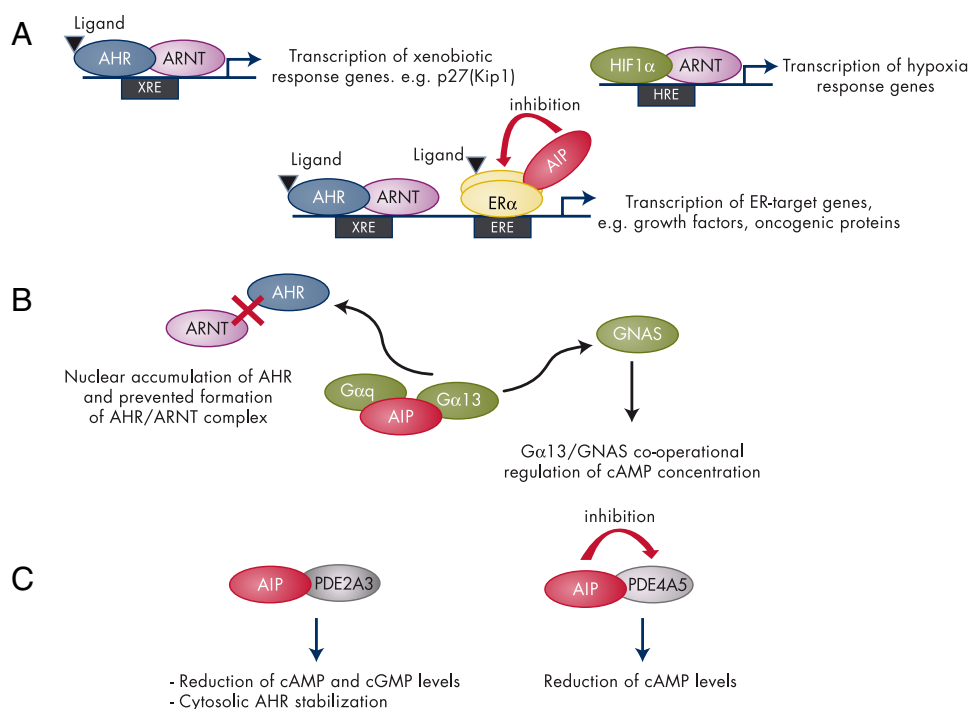
**Figure 8.**

Figure 8. A, Role of AHR-ARNT heterodimer in transcriptional regulation of xenobiotic, hypoxia, and estrogen signaling. B, Role of AIP in regulation of cAMP signaling via G $\alpha$ -proteins and C, via PDEs. HRE, Hypoxia response element; ERE, estrogen response element.

increased prevalence of acromegaly has been observed in highly polluted industrial areas (178), leading to the suggestion that such pollutants may act in some way to induce or promote pituitary tumorigenesis.

## 2. Other AHR-regulated signaling cascades

Although the best-described AHR signaling pathway is the xenobiotic response, recent findings suggest that AHR is a multifunctional protein involved in the regulation of other signaling cascades and undertakes cross talk with several other pathways. Perhaps the best-characterized cross talk with the AHR pathway concerns steroid hormone receptors. AHR is involved in the regulation of estrogen response signaling, mediated by estrogen receptors  $\alpha$  and  $\beta$  (ER $\alpha$  and ER $\beta$ ) (179, 180). Also, the heterodimerization partners of AHR, ARNT and ARNT2, coactivate both ER $\alpha$  and ER $\beta$  (181). The cross talk between the AHR/ARNT and ER signaling is interesting because the estrogen receptor signaling pathway is known to act in the biosynthesis and secretion of hormones of the anterior pituitary and to stimulate the proliferation of lactotropes and gonadotropes (182). Moreover, estrogen-induced transcriptional targets include growth factors [e.g., IGF, epidermal growth factor (EGF), TGF, and vascular endothelial-derived growth factor], and several oncogenic proteins (c-myc, c-fos, erb, c-myb, pttg) (183).

More recently, it has been demonstrated that AIP acts as a negative regulator of estrogen signaling through the interaction with ER $\alpha$  (Fig. 8A) (184). Thus, AIP may have a role in preventing cell proliferation in ER $\alpha$ -dependent tumors.

Apart from their AHR interaction, ARNT and ARNT2 are also binding partners of hypoxia-inducible factor 1 $\alpha$  (HIF1 $\alpha$ ). The HIF1 $\alpha$ -ARNT complex binds to hypoxia-responsive elements and activates the transcription of hypoxia response target genes (Fig. 8A) (185). This complex is involved in the adaptive response to oxygen deprivation in tumor cells, and it controls the up-regulation of a number of factors that are important for solid tumor expansion (185–189). To study the impact of the AIP tumorigenesis-associated ARNT protein imbalance on estrogen and hypoxia responses, expression of ER $\alpha$  and HIF1 $\alpha$  was studied in human and mouse *AIP*-deficient pituitary adenomas (138, 141). Immunostaining showed that the ER $\alpha$  and HIF1 $\alpha$  protein intensities were uniform in *AIP* mutation-positive and -negative tumors. These findings suggest that estrogen and hypoxia responses seem to be functional and that these signaling cascades are not necessarily involved in *AIP*-mediated tumorigenesis (138, 141).

Both ARNT proteins interact also with the single-minded 1 and 2 homolog (SIM1, SIM2) proteins (190,

191). SIM 1 and 2 belong to the basic helix-loop-helix/Per-Arnt-Sim homology (bHLH/PAS) protein family and require heterodimerization with either ARNT or ARNT2 to function. In the absence of *Sim1* function, the paraventricular nucleus and supraoptic nucleus of the hypothalamus fail to develop. Both the paraventricular and supraoptic nuclei play important roles in modulating hormone secretion of the pituitary to maintain homeostasis (192). The *Sim2* gene is required to produce a full complement of anterior hypothalamic cells expressing TRH and somatostatin (193, 194). Thus, it is possible that aberrant ARNT/ARNT2 function might have an impact on SIM1/2-controlled development and regulation of the pituitary/hypothalamic axis in AIP-associated pituitary tumorigenesis.

Of late, it has become evident that AHR also has a role in controlling the cell cycle. AHR appears to act as both a pro-proliferative and an anti-proliferative gene, depending on the cell type. Some studies indicate that AHR can promote cell cycle progression in the absence of exogenous ligands (195, 196), whereas more recent studies are suggesting that AHR may function as a tumor suppressor gene that becomes silenced during the tumor formation, *e.g.*, in prostate and liver tumorigenesis (197, 198). This controversy regarding the role of AHR in tumorigenesis (pro-proliferative *vs.* anti-proliferative) may arise from tissue specificity and the absence or presence of ligand (199). For the most part, the exact role of AHR in signaling cascade responsible for modulating the cell cycle is unknown. One mechanism by which AHR has anti-proliferative potential is through the interaction with the retinoblastoma (RB1) protein. In the presence of mitogenic signals, AHR interacts with RB1 and cooperates in repressing cell cycle progression, particularly in the G<sub>1</sub> to S phase transition (200). It is noteworthy that loss of the RB1 chromosomal region or promoter hypermethylation of the gene appears to be related to aggressive pituitary adenomas and carcinomas (201, 202).

Although it has been shown that low levels of AIP correlate with low levels of AHR in pituitary tumors (132), relatively little is known about the function of AHR in pituitary tissue. However, the AHR-ARNT imbalance associated with AIP-related tumorigenesis, as well as the role of AHR in the regulation of cell cycle progression, makes AHR an interesting candidate to be involved in the AIP-mediated tumorigenesis. Moreover, AHR has been found to be sensitive to cAMP signaling (169, 203). The ability of cAMP to modulate the biological function of AHR is very intriguing because cAMP is known to play a central role in the tumorigenesis of somatotropinomas.

### 3. Role of AIP in the regulation of cAMP signaling

cAMP signaling is a large network that generates interactions between different pathways and integrates signals from distinct receptors (204). In certain tissues, such as in thyroid, adrenal cortex, and pituitary somatotroph cells, cAMP stimulates cell proliferation, and aberrant cAMP signaling is directly implicated in several diseases including the genesis of somatotropinoma (205).

Guanine nucleotide-binding proteins (G proteins) form a large family of signal-transducing molecules. They are heterotrimeric proteins formed by G $\alpha$ -, G $\beta$ -, and G $\gamma$ -subunits. Ligand-receptor binding switches G protein to an active state and permits G $\alpha$  activation of second messenger signaling cascades. G proteins communicate signals from many hormones, neurotransmitters, and other signaling factors and have an essential role in the regulation of cAMP levels. Alteration of the cAMP-protein kinase A pathway is known to be involved in somatotrope tumorigenesis via oncogenic somatic mutations of the  $\alpha$ -subunit of the stimulatory guanine nucleotide-binding protein (GNAS, also known as G $\alpha_s$ ) (MIM: 102200). It has been estimated that up to 40% of sporadic somatotropinomas harbor a somatic mutation in GNAS (206–210). High cAMP levels have also been linked to the pathogenesis of other syndromic conditions with pituitary adenomas such as McCune-Albright syndrome (MIM: 174800) and CNC (MIM: 160980) (211, 212). Although G proteins are known to be involved in the cAMP response regulation and share highly conserved primary structure, at present GNAS is the only G protein gene that has been identified as a target for mutations that are unequivocally associated with pituitary tumorigenesis (205).

AIP interacts with two G $\alpha$  proteins, G $\alpha_{13}$  and G $\alpha_q$ . These interactions were shown to inhibit the interaction between AHR and AIP (168). The G $\alpha_{13}$  signal activation was also found to cause ligand-independent nuclear translocation of AHR (Fig. 8B), and similar to cAMP-mediated nuclear AHR, the G $\alpha_{13}$ -induced nuclear AHR adopts a structure that prevented a formation of active transcriptional complex with ARNT (168, 169). Additionally, it has been demonstrated that G $\alpha_{13}$  is able to regulate cAMP concentration by cooperating with GNAS (213, 214), thereby providing a possible mechanism for pituitary tumorigenesis related to AIP germline mutations.

Phosphodiesterases (PDEs) are a large family of enzymes that degrade and deactivate cAMP. Thus, the finding that AIP interacts with PDE2A and PDE4A5 provides an additional mechanism by which AIP might contribute to the genesis of somatotropinomas (169, 170, 215). PDE2A hydrolyzes both cAMP and cGMP (216). The interaction of PDE2A with AIP is intriguing because the AIP-PDE2A interaction has been shown to inhibit cAMP-

induced nuclear translocation of AHR (Fig. 8C) and to reduce the expression of AHR-regulated target genes (169, 215). Thus, it is possible that the lack of AIP can lead to an aberrant expression of AHR target genes through elevated cAMP levels, a hallmark of somatotropinomas. MacKenzie *et al.* (217) found that GH-mediated differentiation of 3T3-F442A cells was accompanied by increased cAMP PDE activity by a specific PDE4A isoform that they called PDE4A5. AIP binding to the cAMP-specific PDE4A5 inhibited its enzymatic activity (Fig. 8C) and attenuated the ability of cAMP-dependent protein kinase to phosphorylate PDE4A5 (170). As a method to assess the functional validity of the effects of specific germline mutation of *AIP* seen in FIPA families and simplex patients, many mutations completely abolished or significantly attenuated the interaction of AIP with PDE4A5 (67, 86). However, because elevated cAMP levels are associated with pituitary tumorigenesis, it is unclear how AIP could exert its tumor suppressor action through PDE4A5.

#### 4. Other AIP-associated proteins and implications in AIP-mediated tumorigenesis

The rearranged during transfection (RET) proto-oncogene is a transmembrane tyrosine kinase. In the absence of ligand, RET induces apoptosis (218). Gain-of-function mutations of RET are associated with MEN2A and MEN2B (MIM: 171400 and 162300), and familial medullary thyroid carcinoma (MIM: 155240). Loss-of-function mutations of RET predispose to Hirschsprung's disease (MIM: 142623) (219–221). In the pituitary, RET is expressed in somatotropes where it is associated with apoptosis and differentiation and stimulates expression of pituitary transcription factor-1 and *p53* (218). Among pituitary tumors, RET is expressed in somatotropinomas and a subset of corticotropinomas (222). AIP was recently found to interact with the proapoptotic domain of RET (223). However, pathogenic *AIP* and *RET* mutations that were introduced into cells did not impair the AIP-RET interaction. In addition, no RET mutations have been found in somatotropinomas or FIPA families (223, 224). Survivin belongs to the family of inhibitors of apoptosis. The survivin-AIP-HSP90 complex stabilizes survivin, but in the presence of RET, AIP is unable to bind and protect survivin from degradation, leading to enhanced apoptosis (223, 225). Although there is cross talk between RET and survivin and both of the proteins are involved in the regulation of apoptosis, the relevance of these proteins in pituitary tumorigenesis remains uncertain.

Trivellin *et al.* (226) recently highlighted yet another manner in which AIP may be involved in pituitary tumor pathology, namely via regulation by the micro-RNA-107 (miR-107). They noted that *AIP* was a target of miR-107

via a 6-mer site 65–70 bp downstream of the *AIP* stop codon on the 3' untranslated region of *AIP*. This microRNA was capable of inhibiting AIP expression, and it was suggested that the overexpression of miR-107 in pituitary adenomas could explain the decreased AIP expression seen particularly in aggressive somatotropinomas. This effect may differ in other pituitary tumor subtypes, such as nonfunctioning tumors, where AIP expression levels are possibly less important in determining pathological behavior.

The EGF receptor (EGFR) is a transmembrane glycoprotein, a member of the protein kinase superfamily. AIP was reported to interact with EGFR in a large-scale interaction screen (227). However, the AIP-EGFR interaction status is uncertain because this interaction has not been further validated. EGFR is activated by binding of its specific ligands, including EGF and TGF $\alpha$ . Both of these ligands act as mitogens in endocrine pituitary cells. Recent work from Melmed's group has built a body of significant evidence that indicates that targeting EGFR is a valuable potential therapy for pituitary adenomas (8–10, 228). It is also noteworthy that dioxin-activated AHR can trigger sequential activation of EGFR and ERK, leading to the increased expression of TGF $\alpha$  (229). Thus, the confirmation of AIP-EGFR interaction would be important, given the aggressive phenotype and relative therapeutic resistance associated with *AIP* mutation-related pituitary adenomas (115).

AIP is involved in various nuclear receptor signaling pathways. In addition to AHR and ER $\alpha$ , other nuclear receptors capable of binding AIP include peroxisome proliferation-activated receptor  $\alpha$  (PPAR $\alpha$ ), thyroid hormone receptor  $\beta$ 1 (TR $\beta$ 1), and glucocorticoid receptor (GR). PPAR $\alpha$  regulates the expression of genes involved in fatty acid  $\beta$ -oxidation and is a major regulator of energy homeostasis. The cytosolic PPAR $\alpha$ -HSP90-AIP complex has a repressor effect on PPAR $\alpha$  (230, 231). TR $\beta$ 1 is a nuclear hormone receptor and mediates the biological activity of thyroid hormone. *AIP* silencing is known to abolish the TR $\beta$ 1-mediated thyroid hormone expression (232). GR interacts with AIP through HSP90. The effect of AIP on the GR signaling is inhibitory because AIP delays the nuclear accumulation of GR. In the nucleus, GR regulates genes controlling development, metabolism, and immune response. LOH has been observed at the GR gene in about one third of ACTH tumors, suggesting a possible role of GR at least in corticotrope tumorigenesis (233, 234).

AIP was first identified as a partner of the X antigen of the hepatitis B virus, a human DNA virus causing acute and chronic hepatitis. AIP seems to act as a negative regulator of the X protein, and the interaction may have a role in the hepatitis B virus pathology (149). Another viral

protein that interacts with AIP is Epstein-Barr virus encoded nuclear antigen-3 (235). It has been suggested that the AHR pathway can be involved in virus-induced cell transformation (236). AIP has been reported to interact with the translocase of the outer membrane of mitochondria 20, a subunit of a translocator complex that imports mitochondrial pre-proteins into mitochondria (237). In addition to HSP90 binding, AIP can also interact with another heat-shock protein, the heat-shock cognate protein 70 (165). AIP interaction with the cardiac troponin I-interacting kinase (238) is intriguing because a recent study by Lin *et al.* (133, 134) showed that *Aip* knockout mice died during embryogenesis due to cardiac malformations.

As described above, AIP has multiple interaction partners (Fig. 6). Through these proteins, alterations in normal AIP function has the potential to affect a large number of different pathways and signaling cascades. It appears that a number of these interacting partners are potential candidates to promote *AIP* mutation-related pituitary tumorigenesis, particularly the role of AIP in the regulation of cAMP levels and the cross talk between AHR and cAMP pathways. Work based on the *Aip* knockout mouse model and human pituitary tumors is under way to determine the proteins and molecular mechanisms underlying genesis of *AIP*-associated pituitary tumorigenesis. Characterization of these proteins and signaling cascades might reveal novel therapeutic opportunities for the patients with these relatively treatment-resistant pituitary tumors.

## V. Genetic Testing for *AIP* in Selected Pituitary Adenoma Populations

### A. Defining the ideal testing populations

The main aim of genetic testing for *AIP* germline mutations is to identify those at risk of potentially aggressive pituitary adenomas and permit early diagnosis of such adenomas at the microadenoma and noninvasive stage, where treatment is more likely to be effective or curative (239). While *AIP* mutation-related pituitary adenomas appear to be associated with a decreased rate of control with medical therapies (somatostatin analogs and dopamine agonists), not enough is known about the molecular pathways involved in *AIP* mutation-related pituitary tumorigenesis to permit specific choices of medications to be recommended. Therefore, *AIP* mutation-positive pituitary adenomas should be managed according to current guidelines for acromegaly, prolactinoma, *etc.* (240–242).

Published results to date provide firm evidence that *AIP* mutation screening in unselected general pituitary adenoma populations is not strictly justifiable to identify af-

ected patients. Two large studies in more than 400 subjects each have shown overall rates of *AIP* germline mutations of about 4% of unselected sporadic cases (99, 101). However, taking together these results and those from subgroup studies focused on acromegaly, pediatric studies in sporadic and syndromic cases and the clinical characteristics of large cohorts of *AIP* mutation-positive FIPA cases, some approaches to effective *AIP* mutation screening become apparent (67, 71, 75, 84, 86, 89, 90, 93, 95–101, 104–109, 115, 116, 118, 127, 142, 243, 244). The highest likelihood of identifying *AIP* mutation-positive cases is among patients with gigantism, who comprise 25% of those with *AIP* mutations overall and a third of *AIP*-mutated somatotropinomas. In particular, those patients with gigantism in a familial setting of gigantism have the highest probability of having an *AIP* mutation (85). The PAP associated with *AIP* mutations leads to the early development of large pituitary adenomas, usually somatotropinomas, so consistent excessive gain in height in children and adolescents when a pituitary adenoma is suspected should lead the clinician to consider *AIP* testing. More practically, for *AIP* mutation-positive children from families with a known *AIP* mutation, monitoring abnormal gain in height should be an integral part of clinical surveillance.

Up to 20.5% of pediatric patients (those aged less than 18 yr at diagnosis) with a macroadenoma have been reported to be *AIP* mutation positive (108). Macroadenomas occurring in young adults (<30 yr old) also appear to be a valuable indicator of a possible *AIP* germline mutation (11%) and may in the future represent a condition in which routine screening may be effective. The next readily identifiable group in which focused *AIP* genetic screening could be beneficial is among FIPA cohorts. Combining the results of major national and international collaborations, about 20% of FIPA kindreds are *AIP* mutation positive, with the overall rate being only slightly higher in homogeneous *vs.* heterogeneous kindreds (22.8 *vs.* 16.7%). Many different mixtures of pituitary tumor types are now associated with *AIP* mutations in the FIPA setting, although the majority of patients with *AIP* mutations come from families with somatotropinomas, somatolactotropinomas, prolactinomas, and NFPA.

### B. Disease penetrance

As originally described, germline *AIP* mutations were associated with a low penetrance of pituitary adenomas in affected families in Finland (84). Since then, a large number of kindreds with *AIP* mutations have been reported (67, 71, 86), and some particularly large families have been studied genetically and clinically (84, 89, 90). In general, the larger and more completely studied kindreds have



a penetrance that is in the low range (20% penetrance approximately). From the studies of FIPA cohorts together, the penetrance rate is more variable. Based on initial studies on selected families, a penetrance rate of up to 50% or more was suggested (67, 69). Based on a calculation that took into account affected patients, obligate carriers, and half of the subjects with a 50% risk of inheriting a mutation, Igreja *et al.* (86) reported a mean penetrance rate of pituitary tumors in *AIP* mutation-positive FIPA families of  $42 \pm 21\%$ . These figures varying from 20–66% can be explained by a number of potential factors. First, many FIPA kindreds were initially described as clusters of closely related individuals, and *AIP* sequencing in these families also tended to discover clusters of affecteds and carriers that led to relatively high penetrance rates. Expansion of kindred studies to second- and third-degree relatives can serve to lower penetrance rates. In contrast, certain FIPA kindreds with large numbers of affected patients (five or more) have been described (67, 86). Such kindreds show that there appears to be an inherent variability in the penetrance, with some families having many affected subjects and a low number of carriers, whereas other families with similar truncating *AIP* mutations have a low number of affecteds among a wealth of unaffected adult carriers. In addition, numerous cases of familial mutations with only a single affected patient have been discovered (“simplex” cases), whereas other unrelated families with the same mutation (R304X, R271W) have multiple members with aggressive, early-onset mac-

roadenomas. These findings can be explained at least in part by ascertainment bias; the families with striking occurrence of a disease enter genetic studies much more often than more subtle clusters. At this time, the penetrance of *AIP* mutations in the FIPA setting can be considered as low to perhaps moderate penetrance. The variability raises the possibility that, in addition to simple chance, some unknown endogenous genetic factors (*i.e.*, other gene variants/mutations or modulators/repressors) or extraneous environmental effects (*i.e.*, toxins, dioxin, *etc.*) related to or separate from *AIP* and its molecular pathway play a role in determining which mutation carriers develop pituitary adenomas.

One important feature of *AIP* mutation-positive FIPA kindreds is that of phenocopy in which a member of a FIPA kindred has a pituitary adenoma but has a normal *AIP* sequence, unlike the other affected members of the family that have an *AIP* mutation (73, 87). Phenocopies are of particular importance in the setting of FIPA because in the general population pituitary adenomas occur in about 1:1000 people, whereas incidental tumors (without clinical correlates) occur in more than 15% of individuals (1, 245). Hence, the genetic investigation of *AIP* mutation-positive FIPA kindreds can be confounded by the presence of such phenocopies and can lead to unnecessary study and follow-up of nonmutation carriers. The challenges are illustrated in Fig. 9, in which a kindred presented as a three-member heterogeneous FIPA family, with somatotropinomas in father and son (cases III-6 and IV-7, re-

**Figure 9.**

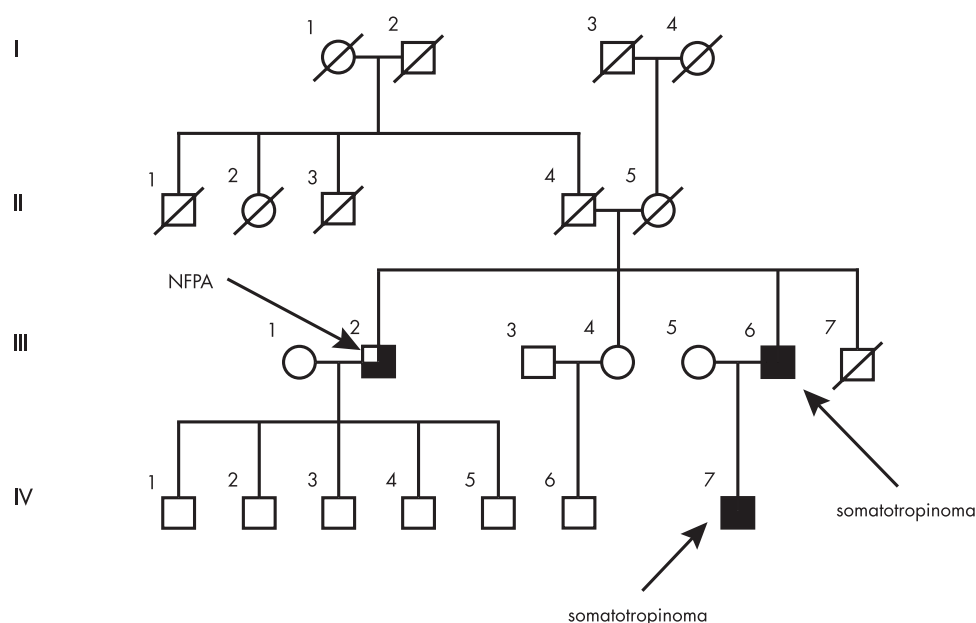


Figure 9. Phenocopy NFPA in the setting of an *AIP* mutation-positive FIPA kindred with acromegaly.

spectively) and an NFPA in case III-2, the brother of case III-6. The tumor size at diagnosis in case III-6 in the 1960s was unknown, although the patient was relatively young (29 yr old) at diagnosis and had suffered from symptoms since adolescence. His son was diagnosed in his early 40s with acromegaly due to a microadenoma. Case III-2 developed a pituitary macroadenoma in his 60s. Although both father and son with somatotropinomas were found to have the p.R271W *AIP* mutation, repeated sequencing of the *AIP* gene in case III-2 was normal. He was diagnosed as having a phenocopy NFPA, thereby markedly curtailing the screening process in generation IV.

### C. Toward integrated genetic screening in pituitary adenomas

Although *AIP* mutations are associated with isolated pituitary tumors, genetic testing cannot be viewed in isolation from syndromic forms of endocrine neoplasia. Indeed, investigation of the patient with a pituitary adenoma should always take account of the possibility of a genetic background and related disease in other tissues. Multiple germline gene mutations are associated with an increased predisposition to pituitary tumor development, but these are usually part of a syndrome displaying additional diagnostic features. The occurrence of multiple individuals with isolated pituitary adenomas in the setting of familial MEN1 and CNC in the absence of other cardinal skin, vascular, or neoplastic signs after a thorough clinical anamnesis is likely to be very rare. Indeed, only 2% of MEN1 patients only had pituitary adenomas, and familial MEN1 cases with only pituitary adenomas are, to our knowledge, unknown (14). However, it is important to note that because approximately 17% of MEN1 patients present with pituitary adenomas, specific efforts must be made to clinically and biochemically search for other disease manifestations. As such, an initial division of pituitary adenoma patients into those with or without any history of syndromic features or family history is a practical first step before considering genetic testing (Fig. 10). Surveillance for MEN1 can follow already agreed guidelines and recommendations, which have been recently and very comprehensively updated to take into account discrimination between MEN1 and other conditions like FIPA (246–248). In familial cases of MEN1, pituitary adenomas are significantly more commonly seen than in sporadic MEN1 (59 *vs.* 34%). As noted above, it is only in exceptional cases that MEN1 is characterized by only a pituitary adenoma in the absence of other cardinal syndromic features (*e.g.*, parathyroid disease). Both FIPA and MEN1 have a large proportion of females with prolactinomas. Pituitary tumors in MEN1 differ significantly from sporadic tumors (including prolactinomas) in terms of more frequently be-

ing macroadenomas (85 *vs.* 42%) (14). Probably related to this larger size, pituitary tumors in MEN1 are also significantly more likely than sporadic tumors to cause symptoms and signs due to local tumor effects. In FIPA, patients are much more likely to present with somatotropinomas (14, 65, 69).

CNC is associated with a typical spectrum of syndromic features, and particular attention should be paid to these, while recalling that pituitary adenomas occur in only a minority of CNC cases, and familial presentation limited only to acromegaly is not known in this condition at this time (46). For patients with pituitary adenomas with other MEN1-like features, but who do not have a *MEN1* mutation, *CDKN1B* mutation screening should be considered (249). However, in the setting of FIPA, *CDKN1B* does not play a clinically relevant role (175). New associations have been made between pituitary adenomas and endocrine neoplasia genes previously not thought to be involved in pituitary tumorigenesis, such as *SDHD* as recently reported by Xekouki *et al.* (250). Investigation of this and other potential associations may expand the number and complexity of testing in the setting of endocrine neoplasia syndromes.

Specifically focusing on the investigation of FIPA, *AIP* testing should be considered in all kindreds, irrespective of pituitary tumor types in the family. In FIPA kindreds that are negative on *AIP* sequencing, deletions have been demonstrated using MLPA, and this method should be considered as a second line of screening. For *AIP* mutation-negative FIPA families (80% of cases), academic research projects are currently under way to discover novel genetic causes, but no current candidates can be recommended for further study in the clinical setting at present. For patients without FIPA or syndromic features suggestive of MEN1, CNC, *etc.*, genetic testing for *AIP* mutations can be considered in a number of situations. Because *AIP* mutations are seen in up to 11% of pituitary macroadenomas diagnosed before the age of 30 and about 20% of those with macroadenomas under the age of 18, *AIP* sequencing should be strongly considered in this readily defined subgroup (108). Also, gigantism is common in *AIP*-mutated patients, and *AIP* testing would seem clinically reasonable in such patients with established gigantism and also in young patients with excessive height gain in association with a pituitary adenoma. There has also been a suggestion that pituitary apoplexy is a feature of *AIP*-mutated pituitary adenomas, reflecting their large size and potentially rapid size expansion; a strict association between an increased risk of apoplexy and *AIP* mutation status remains to be demonstrated. Finally, among patients with somatotropinomas, resistance to medical therapy has been reported for *AIP* mutation-related adenomas (67),

Figure 10.

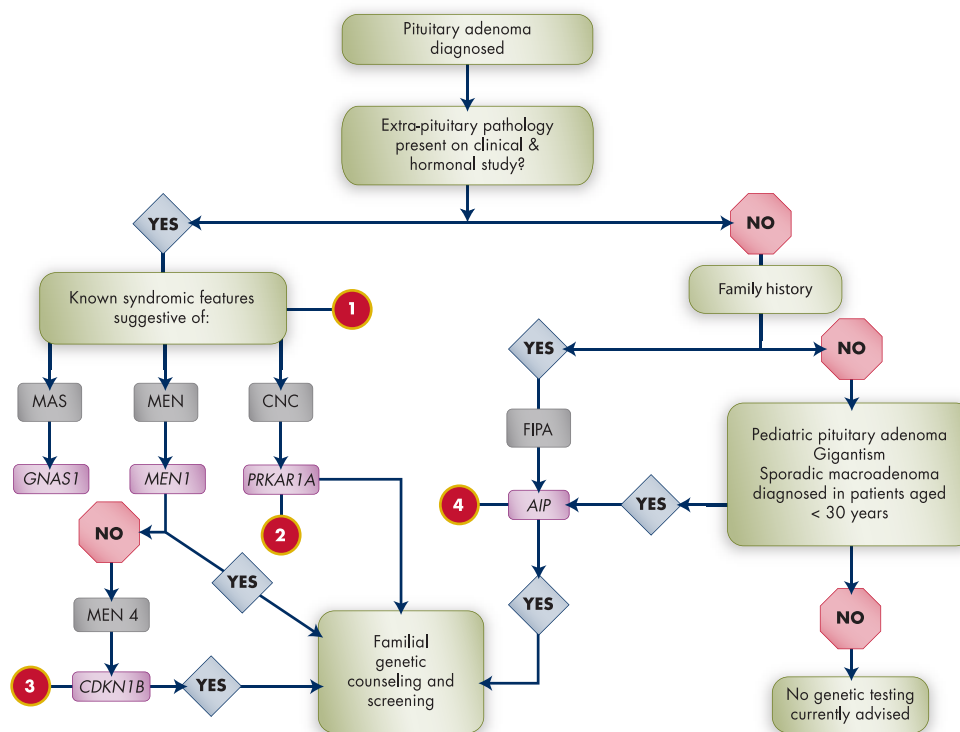


Figure 10. Schematic of suggested clinical decision tree to integrate *AIP* genetic testing into existing testing strategies. The decision tree is based on the presence or absence of typical syndromic features that suggest known diseases such as MEN1, CNC, and McCune Albright syndrome (MAS), which have established genetic testing for known causative genes. Point 1, Established syndromes like MEN1 and CNC are being joined by newer associations of pituitary adenomas with other endocrine tumor types, such as pheochromocytoma in the setting of succinate dehydrogenase subtype gene mutations (250). Ongoing advances in this field will clarify the relative frequency of such associations and the need to integrate testing into standard clinical investigation. Point 2, In CNC, a proportion of patients are negative for *PRKAR1A* mutations, and another locus on chromosome 2 has been suggested (252). Point 3, MEN4 due to *CDKN1B* mutations is a rare but emerging condition with pituitary adenomas as part of the spectrum. *CDKN1B* testing in patients with pituitary adenomas should be limited in the clinical setting to those with associated syndromic features of endocrine or other tumors and negative sequencing for *MEN1* mutations (174, 244). Other rare mutations in cyclin-dependent kinases have also been noted infrequently in MEN1-like conditions, but the study of these remains in the research realm (253). Point 4, In the setting of FIPA, the PAP due to *AIP* germline mutations accounts for about 20% of kindreds. For FIPA kindreds that are *AIP* mutation negative on sequencing, MLPA should be considered to detect more extensive deletions. To date, other genes have not been identified to cause FIPA. Syndromic conditions like MEN1 and CNC do not frequently present as isolated pituitary adenomas in the absence of other features such as hyperparathyroidism. An exception may be young patients with apparently sporadic pituitary macroadenomas, with recent information from Cuny *et al* (254) suggesting that *MEN1* gene sequencing is a valuable investigation in that population. Therefore, in the verified FIPA setting and in younger patients with aggressive pituitary adenomas, *AIP* testing may be considered as the first genetic test to be discussed, as long as MEN1 and CNC are ruled out clinically and by simple biochemical testing (e.g., absence of hypercalcemia or cortisol secretion abnormalities).

which leads to statistically significantly lower hormonal and tumor size responses as compared with *AIP* intact controls (115). Prolactinomas, although less frequent than somatotropinomas in this setting, also appear to be large and relatively resistant to dopamine agonists (115). In patients with sporadic pituitary macroadenomas, resistance to therapy may be another suggestive feature to guide offering genetic testing for *AIP* mutations, although specific *AIP* mutation prevalence studies in resistant populations are awaited.

It is important to note that in cases where an *AIP* mutation is diagnosed in a sporadic case, many families that

agree to genetic testing display asymptomatic mutation carriers. The variable low to moderate penetrance of pituitary adenomas among *AIP* mutation carriers complicates the process of screening because clinically affected patients are in the minority and currently most of our experience is with patients that have already developed macroadenomas. The issue of when to begin clinical and genetic surveillance in relatives of known carriers/affecteds with *AIP* mutations is one that must be informed by the fact that presentation in childhood/adolescence is a typical feature of the illness (90, 101, 104, 106, 108, 115). A patient as young as 6 yr has been diagnosed with an *AIP*

mutation and pituitary macroadenoma, with additional signs and symptoms for the preceding 12–24 months (90). Although screening to date in mutation carriers is still relatively scant, work in larger families has identified carriers with pituitary microadenomas or other abnormalities of maturation (89, 115). These patients remain under surveillance, but information on the rate and characteristics of pituitary tumor evolution is limited and remains an important unanswered clinical question for the future.

For those individuals that are at risk for carrying an *AIP* mutation that has been diagnosed in a relative, the ideal step is to offer genetic screening after appropriate counseling and explanation of the benign nature of pituitary adenomas, the possible benefits of early diagnosis, and the current understanding of the variable penetrance. Genetic screening for a familial *AIP* mutation has the benefit of being able to rule out noncarriers definitively, thereby avoiding unnecessary stress. Given that more than 75% of cases have been diagnosed before the age of 30, screening efforts in new kindreds should ideally be weighted toward children and young adults in the first instance. Mutation carriers or potential carriers that decline initial genetic testing should be offered a thorough clinical assessment by an endocrinologist, accompanied by basal testing of GH/IGF-I and prolactin. Carriers with any signs or symptoms suggestive of a pituitary adenoma or those that express an interest in undergoing imaging should have a baseline pituitary MRI performed. Mutation carriers that are without clinical signs or hormonal abnormalities and have a normal MRI should be followed up, although no minimum period can be recommended based on a lack of evidence. The investigation of potential pediatric/adolescent *AIP* mutation carriers is of particular importance because these patients have added complications in terms of interpreting normal growth spurts and pubertal changes while actively surveying for suggestive endocrine abnormalities. Should genetic testing be permitted by a parent/guardian, this has been performed in children as young as 2 yr of age, with the benefit of being able to reassure parents if the familial mutation is not found. For carriers or those not wishing to have genetic testing performed in their child, care should be taken to actively seek out clinical signs/symptoms suggestive of somatotrope or lactotrope overactivity or tumor impingement on local structures (vision, recurrent headache) and to carefully examine growth velocity. In those with symptoms suggestive of a pituitary adenoma, imaging and hormonal testing should be performed as soon as possible because tumors as large as 35–38 mm have been diagnosed in patients aged 10 or younger.

#### D. Follow-up in the setting of FIPA and *AIP* mutation carriers

There are no established guidelines to outline the recommended follow-up for unaffected *AIP* mutation carriers. For those with no symptoms or evidence of pituitary abnormality, it is important to inform the individual of symptoms that may be suggestive of a new endocrine abnormality and to encourage the subject to return should such symptoms occur. It is probably wise to review the subject after a year, and in the absence of new clinical features, the subject can be returned to their regular physician's care. For pediatric and adolescent carriers, follow-up with the pediatrician for growth or other endocrine disorders is recommended, and rapid referral back to the pediatric endocrinologist upon the appearance of relevant symptoms should be emphasized.

The more challenging situation is the *AIP* mutation carrier that has a microadenoma on MRI and has no clinical signs/symptoms and normal hormonal screening. It is difficult in such cases to determine whether this patient has an incidentaloma that is stable or has an *AIP*-related growing pituitary adenoma. In adult patients, in the absence of new symptoms or hormonal abnormalities, there is probably no need to repeat an MRI until 12 months have passed since the baseline scan. Given the usual early appearance of these tumors, often in childhood/adolescence, and the lack of knowledge about their growth potential, in young patients a follow-up MRI may be more advisable 6 months after diagnosis in *AIP* mutation carriers. Should the tumor remain as a stable microadenoma over that period, then clinical and hormonal follow-up can probably remain in place on an annual or biannual basis as long as no change in endocrine status occurs. In such cases, the usual balance of definitive surgical cure over medical therapy would have to be made according to the individual tumor type and the clinical status of the patient. Follow-up of and decisions regarding intervention in patients with diagnosed *AIP* mutation-related pituitary adenomas does not differ from that of sporadic pituitary adenoma patients.

For FIPA patients without *AIP* mutations, the follow-up recommendations are relatively more difficult to codify in the absence of a causative gene. Efforts should be made to clearly identify patients within the kindred who may be harboring a pituitary adenoma, based on family history and careful anamnesis in collaboration with patients and relatives. Those individuals with specific symptoms or signs of a pituitary adenoma should, like any patient with suggestive symptomatology, undergo relevant hormonal and/or imaging studies. Follow-up management of *AIP*-negative subjects with pituitary adenomas in the setting of FIPA does not at this time differ from those

with sporadic tumors and should, where possible, be guided by international consensus guidelines.

## VI. Future Directions

The advent of FIPA and the discovery of *AIP* as a gene involved in inherited pituitary adenomas have refocused attention on the role of familial and genetic factors in the pituitary adenoma population. However, despite the advances made, approximately 80% of FIPA families remain without a genetic explanation. Ongoing work in *AIP*-negative FIPA families at a genomic level may highlight novel loci and eventually causative genes. In parallel, there may be value in studying the potential role of other endocrine neoplasia-related genes, such as the *SDHx* genes, to discover whether their newly reported links to pituitary tumorigenesis play a role in causation of the FIPA phenotype (250, 251). Because FIPA comprises 2–3% of pituitary adenomas at certain tertiary referral centers (65), improved questioning about family history of pituitary adenomas and other endocrine cancers will be helped by greater awareness of FIPA and the role of *AIP* in causation of pituitary adenomas in families and young patients. With respect to *AIP*, improved understanding of the penetrance related to the PAP induced by *AIP* mutations will be necessary to permit accurate counseling of patients and to improve genetic diagnosis of mutation carriers when tumors are still relatively small. Also, penetrance studies will help to answer whether rare tumor associations seen in FIPA families with *AIP* mutations occur by chance or form a predictable but infrequent association.

Study of the evolution of pituitary adenomas in association with *AIP* mutations from their early stage is necessary to determine what pathway they follow (for example, a hypothetical hyperplasia-microadenoma-macroadenoma progression) and the rate of tumor growth. In this, the potential role of other existing or acquired genetic mutations as cofactors in permitting *AIP* mutation-related tumor development will need to be explored. Of great interest will be the further exploration of *Aip* mutation-related tumorigenesis via the knockout mouse models available, particularly because these studies may relate to identifying the main causative pathway(s) among the legion of current possibilities (dioxin, AHR, PDEs, *etc.*). Such advances may allow for specific therapies to be suggested that could impact positively upon the relatively poor therapeutic responses in these patients.

## Search Strategy

Articles were selected from a PubMed, EMBASE, Web of Science, Google Scholar, and GenBank search for key-words including, *e.g.*, *AIP*, *XAP2*, *ARA9*, *FIPA*, *IFS*, pituitary tumorigenesis, somatotropinoma, *PAP*, and protein interaction. Reference lists in selected articles were also used to broaden the search, and abstract books of recent national and international endocrinology congresses were also consulted.

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