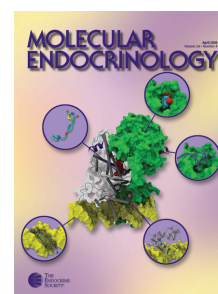


## Clinical Characteristics and Therapeutic Responses in Patients with Germ-Line AIP Mutations and Pituitary Adenomas: An International Collaborative Study

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## Clinical Characteristics and Therapeutic Responses in Patients with Germ-Line *AIP* Mutations and Pituitary Adenomas: An International Collaborative Study

Adrian F. Daly,\* Maria A. Tichomirowa,\* Patrick Petrossians,\* Elina Heliövaara, Marie-Lise Jaffrain-Rea, Anne Barlier, Luciana A. Naves, Tapani Ebeling, Auli Karhu, Antti Raappana, Laure Cazabat, Ernesto De Menis, Carmen Fajardo Montañana, Gerald Raverot, Robert J. Weil, Timo Sane, Dominique Maiter, Sebastian Neggers, Maria Yaneva, Antoine Tabarin, Elisa Verrua, Eija Eloranta, Arnaud Murat, Outi Vierimaa, Pasi I. Salmela, Philippe Emy, Rodrigo A. Toledo, Maria Isabel Sabaté, Chiara Villa, Marc Popelier, Roberto Salvatori, Juliet Jennings, Ángel Ferrandez Longás, José Ignacio Labarta Aizpún, Marianthi Georgitsi, Ralf Paschke, Cristina Ronchi, Matti Valimäki, Carola Saloranta, Wouter De Herder, Renato Cozzi, Mirtha Guitelman, Flavia Magri, Maria Stefania Lagonigro, Georges Halaby, Vinciane Corman, Marie-Thérèse Hagelstein, Jean-François Vanbellinchen, Gustavo Barcelos Barra, Anne-Paule Gimenez-Roqueplo, Fergus J. Cameron, Françoise Borson-Chazot, Ian Holdaway, Sergio P. A. Toledo, Günter K. Stalla, Anna Spada, Sabina Zacharieva, Jerome Bertherat, Thierry Brue, Vincent Bours, Philippe Chanson, Lauri A. Aaltonen, and Albert Beckers<sup>†</sup>

**Context:** *AIP* mutations (*AIPmut*) give rise to a pituitary adenoma predisposition that occurs in familial isolated pituitary adenomas and less often in sporadic cases. The clinical and therapeutic features of *AIPmut*-associated pituitary adenomas have not been studied comprehensively.

**Objective:** The objective of the study was to assess clinical/therapeutic characteristics of *AIPmut* pituitary adenomas.

**Design:** This study was an international, multicenter, retrospective case collection/database analysis.

**Setting:** The study was conducted at 36 tertiary referral endocrine and clinical genetics departments.

**Patients:** Patients included 96 patients with germline *AIPmut* and pituitary adenomas and 232 matched *AIPmut*-negative acromegaly controls.

**Results:** The *AIPmut* population was predominantly young and male (63.5%); first symptoms occurred as children/adolescents in 50%. At diagnosis, most tumors were macroadenomas (93.3%); extension and invasion was common. Somatotropinomas comprised 78.1% of the cohort; there were also prolactinomas ( $n = 13$ ), nonsecreting adenomas ( $n = 7$ ), and a TSH-secreting adenoma. *AIPmut* somatotropinomas were larger ( $P = 0.00026$ ), with higher GH levels ( $P = 0.00068$ ), more frequent extension ( $P = 0.018$ ) and prolactin cosecretion ( $P = 0.00023$ ), and occurred 2 decades before controls ( $P < 0.000001$ ). Gigantism was more common in the *AIPmut* group ( $P < 0.000001$ ). *AIPmut* somatotropinoma patients underwent more surgical interventions ( $P = 0.00069$ ) and had lower decreases in GH ( $P = 0.00037$ ) and IGF-I ( $P = 0.028$ ) and less tumor shrinkage with somatostatin analogs ( $P < 0.00001$ ) vs. controls. *AIPmut* prolactinomas occurred generally in young males and frequently required surgery or radiotherapy.

**Conclusions:** *AIPmut* pituitary adenomas have clinical features that may negatively impact treatment efficacy. Predisposition for aggressive disease in young patients, often in a familial setting, suggests that earlier diagnosis of *AIPmut* pituitary adenomas may have clinical utility. (*J Clin Endocrinol Metab* 95: E373–E383, 2010)

**P**ituitary adenomas occur relatively frequently and the prevalence of clinically apparent pituitary adenomas is one in 1064–1289 of the general population (1, 2). Although almost universally benign, pituitary tumors are associated with a heavy clinical burden due to a combination of local compressive symptoms, the systemic effects of hormonal hypersecretion, and the need for neurosurgery, chronic medical therapy, or radiotherapy. Hence, the molecular pathophysiology underlying pituitary adenoma formation has been the subject of extensive research.

Mutations in multiple oncogenes and tumor suppressor genes have been associated with a role in pituitary tumorigenesis (3). The best characterized of these include *gsp*, *PTTG*, and *MEG3* among others (3–5). These are generally noted as somatic mutations in tumor specimens after surgery. In contrast, very few germline genetic mutations that are implicated in inherited pituitary tumor risk are known. Multiple endocrine neoplasia (MEN) type 1 and Carney complex (CNC) are the best-described familial pituitary tumor syndromes (6, 7). MEN4 is a newer, rare MEN1-like syndrome caused by germline mutations in the *CDKN1B* gene (8). MEN1 and CNC can be screened for genetically to identify at-risk carriers and potentially diagnose tumors, pituitary or others, at an earlier stage. However, the molecular pathophysiology of pituitary adenomas is less clear in many other families, such as kindreds with familial isolated pituitary adenomas (FIPA) (9).

Recently interest has turned to the identification of new genes associated with familial pituitary adenomas. In 2006 Vierimaa *et al.* (10) reported that mutations in the

aryl hydrocarbon receptor interacting protein gene (*AIP*) conferred a pituitary adenoma predisposition in familial pituitary adenoma kindreds in Finland and Italy. Since then, extensive studies have identified many *AIP* mutations (*AIPmut*) in familial and sporadic pituitary adenomas (11–17). *AIPmut* account for 15% of FIPA kindreds (50% of those with homogeneous familial somatotropinomas) and are associated with somatotropinomas, prolactinomas, nonsecreting adenomas, and rare cases of Cushing disease (11, 12, 18).

To date, studies have concentrated largely on the issue of *AIPmut* prevalence among various patient populations. There have been indications of relatively aggressive disease features in pituitary adenoma patients with *AIPmut* (10, 11, 17, 19), but clinical aspects have not been studied specifically in a standardized fashion. Therefore, we undertook a standardized, comprehensive analysis of a large international cohort of patients with *AIPmut* and pituitary adenomas to determine the demographic, hormonal, radiological, and therapeutic characteristics of these patients.

## Subjects and Methods

This was an international collaborative study to determine the clinical characteristics and responses to therapy in patients with *AIPmut*-associated pituitary adenomas. The collaboration involved 36 centers in Belgium, Finland, France, Italy, Spain, Germany, Bulgaria, The Netherlands, Brazil, Argentina, the United States, Australia, New Zealand, and Lebanon. This study included pituitary adenoma patients without MEN1, MEN4, or

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CNC that were diagnosed with *AIPmut* from 2006 to 2009 and were originally diagnosed with pituitary adenomas between January 1, 1970, and December 31, 2009. The only selection criterion was a willingness to undergo genetic studies after provision of informed consent, and the population was not otherwise selected by uniform criteria such as age, sex, tumor type/characteristics, or responses to therapy (1727 patients consented to take part). *AIP* genetic studies were performed using leukocyte DNA extracted from peripheral blood as described by Vierimaa *et al.* (10); multiplex ligation-dependent probe amplification studies were performed as described previously (13, 20). Normal population genetic databases were assessed for the presence of *AIP* polymorphisms. All patients and controls (see below) provided informed written consent for genetic testing at their center in their local language, and the study was approved by the Ethics Committee of the Centre Hospitalier Universitaire of Liège.

Clinical and therapeutic data were collected *de novo* using standardized data collection under predefined criteria for all patients at all participating study centers (see Supplemental Material, published on The Endocrine Society's Journals Online web site at <http://jcem.endojournals.org>). Anonymized patient information on demographics, diagnosis, genetics, hormonal profiles at diagnosis, and radiological criteria were collected. Therapeutic responses for each patient after neurosurgery, somatostatin analog (SSA) therapy, radiotherapy, dopamine agonists, and pegvisomant were collected and tabulated. Long-term responses to therapy were collated for patients treated for 12 months or longer after initial treatment and included information on hormonal, clinical, and radiological disease status; treatment modalities used; and the presence of hypopituitarism. Tumor size was measured as the maximum diameter on computed tomography or magnetic resonance imaging and tumors were classified accordingly as microadenomas (<10 mm) or macroadenomas ( $\geq 10$  mm); giant adenomas were tumors measuring 40 mm or greater in maximum diameter. Information on extrasellar extension and invasion of surrounding structures were also collected in all available instances from radiological reports or from surgical notes. Diagnosis of gigantism was verified in patients with current/previous evidence of abnormal, progressive, and excessively rapid growth velocity for age, a height greater than 2 SD scores above normal for their population (*i.e.* > 95th percentile) and height greatly (>5 cm) in excess of the calculated mid-parental height in the absence of constitutional tall stature (21–23).

Long-term disease control criteria ( $\geq 12$  months of follow-up after therapy) were defined according to tumor type. In all cases tumor size had to be stable without growth or expansion. For patients with somatotropinomas, control at last follow-up was defined as the absence of clinical activity, an age/sex-appropriate IGF-I that was at the upper limit of normal (ULN) or less for the assay used and a valid random GH level less than 1 ng/ml at last follow-up. In prolactinoma cases, serum prolactin had to be at the ULN or less for the assay used. For nonsecreting-adenomas, disease control was defined as long-term tumor size stability; in thyrotropinoma cases, patients had to be symptom free and have serum TSH, T<sub>4</sub>, and T<sub>3</sub> levels within normal limits.

### Control population

Previous studies reported that *AIPmut* are predominantly associated with somatotropinomas (10, 11, 12, 17, 19, 20). A suitable control population database of *AIPmut*-negative somatotropinoma patients was developed *de novo* to compare demo-

graphic, clinical, and therapeutic features. The control database comprised 298 non-MEN1, non-CNC acromegaly patients from the collaborating study centers. All patients had normal germline *AIP* gene sequences. Anonymized demographic, clinical, and therapeutic data and long-term outcomes were collected on control patients using identical criteria used for the *AIPmut* patients. To minimize potential bias due to variations in treatment practice among centers and over time, the control group was stratified according to decade of diagnosis and geographic region (northern Europe, southern Europe/Mediterranean, North America, South America, and Oceania). Control patients were then randomly extracted to match the *AIPmut* group in terms of decade of diagnosis and geographic region to give a proportion of three or more control cases for each *AIPmut* case. This final stratified control group used for comparative purposes consisted of 232 *AIPmut*-negative somatotropinoma patients.

Predefined comparisons between the *AIPmut* and the control group were performed on the following disease and treatment characteristics: gender ratio, ages at diagnosis and at first symptoms, tumor size and classification, proportion of patients with extrasellar extension and invasion, GH and IGF-I levels at baseline, prolactin cosecretion at baseline, treatment characteristics (number/type of surgery, use of radiotherapy, hormonal and radiological responses to medical therapies), proportions of patients with controlled and active disease, disease control as a function of cumulative therapies, and frequency of hypopituitarism among patients with controlled and active disease.

### Statistics

Continuous data were represented as medians and ranges. Because data were nonnormally distributed, comparisons were made using a nonparametric test (Wilcoxon's signed rank test). For count data, values were placed in a contingency table and compared with a  $\chi^2$  test. Where continuous data were plotted as density graphs, a kernel density approximation was computed using a Gaussian kernel, and bandwidth was calculated using Silverman's rule of thumb. The kernel density was finally plotted as a continuous curve. All statistical analyses were performed using the R statistical package, version 2.7.0 (R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria; <http://www.r-project.org>).

## Results

### Study population

The study population comprised 96 patients with *AIPmut* and pituitary adenomas. There were 43 separate *AIP* mutations; 54.2% of patients had mutations leading to premature stop codons causing protein truncation, whereas a further 31.3% had missense mutations. Most patients presented in FIPA kindreds (59.4%), 10.4% had a familial *AIPmut* and no known relatives with pituitary adenomas, and 29 patients (30.2%) were apparently sporadic cases.

### Clinical characteristics

Demographic and clinical features of the *AIPmut* cohort and each tumor subgroup are shown in Table 1. The



**TABLE 1.** Demographics, tumor phenotypes and tumor characteristics in 96 patients with *AIP* mutations and pituitary adenomas

	All (n = 96)	Somatotropinoma (n = 75)	Prolactinoma (n = 13)	Non-secreting adenoma (n = 7)	TSH-secreting adenoma (n = 1)
Sex (% male)	61 males/35 females (63.5%)	46 males/29 females (61.3%)	10 males/3 females (76.9%)	4 males/3 females (57.1%)	1 male/0 females (100%)
Age at diagnosis (yr)	23.0 (8.0–74.0)	22.5 (8.0–60.0)	22.0 (12.0–39.0)	31.0 (12.0–74.0)	39.0
Age at first symptoms (yr)	18.0 (4.0–67.0)	17.8 (4.0–50.0)	18.0 (12.0–39.0)	31.0 (12.0–74.0)	39.0
Delay in diagnosis (yr)	2.0 (0.0–19.0)	2.0 (0.0–19.0)	0.0 (0.0–6.0)	0.0 (0.0–7.0)	0.0
Maximum tumor diameter (mm)	25.0 (6.0–85.0)	22.5 (7.0–60.0)	31.0 (6.0–85.0)	27.5 (14.0–35.0)	30.0
Macroadenoma (%)	93.3	93.1	92.3	100	100
Extrasellar extension (%)	79.5	65.1	91.7	85.7	0.0
Invasion (%)	56.3	51.7	69.2	57.1	0.0

Extrasellar extension was defined as clearly visible superior or lateral extension of the tumor beyond the sellar borders on radiological imaging or at surgery. Invasion was defined as radiological, surgical, or pathological evidence of the presence of pituitary tumor tissue invading or penetrating the structures forming the normal border of the pituitary gland. Age at diagnosis, age at first symptoms, delay in diagnosis, and maximum tumor diameter are presented as median (ranges).

population was predominantly male (63.5%) and the age at diagnosis was young. The median age at first symptoms of 18.0 yr indicates half the patients were children or adolescents at clinical onset. Tumors were overwhelmingly macroadenomas (93.3%), were large (12 were giant adenomas), and 56.3% had invaded local structures at diagnosis. No statistically significant differences existed between characteristics in male and female patients with *AIPmut*.

## Analyses by tumor type

### Somatotropinomas

Somatotropinomas were the predominant tumor type associated with *AIPmut* with 75 patients (78.1% of the cohort); 34 separate mutations were noted. The *AIPmut* somatotropinoma group (Table 2) was mainly male (61.3%), and there was a significantly higher male to female ratio than controls ( $P = 0.027$ ). The median age at first symptoms was 20.5 yr earlier in *AIPmut* somatotropinoma patients *vs.* controls ( $P < 0.000001$ ); first symptoms occurred as children or adolescents in 52.2% of the *AIPmut* cohort as compared with only 4.3% of controls (Fig. 1). Similarly, the *AIPmut* cohort was diagnosed nearly 2 decades before mutation-negative controls ( $P < 0.000001$ ; Fig. 1). Gigantism was significantly more frequent in the *AIPmut* cohort than controls ( $P < 0.000001$ ); all 24 patients with gigantism in the *AIPmut* group were males as compared with five of 15 patients with gigantism in the control group who were female.

Median maximum tumor diameter was larger ( $P = 0.00026$ ; Fig. 2), and the proportion of patients with macroadenomas was higher in the *AIPmut* group *vs.* controls ( $P = 0.026$ ; Table 2); 9.3% of tumors were giant adenomas in the *AIPmut* group as compared with 1.3% among controls. There was a higher frequency of extrasellar extension ( $P = 0.018$ ) and a trend toward more frequent invasion of local structures in the *AIPmut* cohort *vs.* controls. Larger tumor size in the *AIPmut* group was associated with significantly higher median levels of GH at diagnosis than controls ( $P = 0.00068$ ; Fig. 3); median IGF-I levels did not differ ( $P = 0.48$ ). Cosecretion of GH and prolactin was nearly twice as frequent in the *AIPmut* group as in controls ( $P = 0.00023$ ).

Treatment of patients was multimodal in 61.3 and 66.4% of the *AIPmut* cohort and control cases, respectively. The proportions of patients that received various combinations of different treatment modalities were the same in both groups. The median duration of follow-up after diagnosis was similar in the two groups [*AIPmut*: 9.0 yr (range 1.0–38.5 yr); control: 9.5 yr (range 0.5–34.5 yr)]. Follow-up periods after diagnosis and after treatment did not differ between the *AIPmut* and control populations.

**TABLE 2.** Comparisons between clinical characteristics at diagnosis of *AIPmut*-associated and non-*AIPmut* somatotropinoma groups

	Somatotropinoma group		P value
	<i>AIPmut</i> (n = 75)	Control (n = 232)	
Sex ratio (male/female)	1.6	0.87	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 cosecretion (%)	56.1	28.9	0.00023
Gigantism (%)	32.0	6.5	<0.000001

Extrasellar extension was defined as clearly visible superior or lateral extension of the tumor beyond the sellar borders on radiological imaging or at surgery. Invasion was defined as radiological, surgical, or pathological evidence of the presence of pituitary tumor tissue invading or penetrating the structures forming the normal border of the pituitary gland. Age at diagnosis, age at first symptoms, delay in diagnosis, maximum tumor diameter, and GH and IGF-I levels at diagnosis are presented as median (ranges).

Among 71 *AIPmut* somatotropinoma patients with more than 12 months of follow-up, control was achieved in 50 cases (70.4%) and acromegaly remained active in 21 cases (26.8%). The long-term disease control rate was higher in control patients (182 of 226; 80.5%), but this was not statistically significant ( $P = 0.06$ ). Among the patients with a higher cumulative treatment burden (three or more distinct modalities), long-term disease control rates were significantly poorer in the *AIPmut* group *vs.* controls [15 of 27 (55.6%) *vs.* 63 of 76 (82.9%), respectively;  $P = 0.01$ ].

Similar proportions of patients had pituitary neurosurgery in the *AIPmut* (87.3%) and control groups (80.5%); reoperation was significantly more frequent in the *AIPmut* group than the controls (21.9 *vs.* 5.5%, respectively;  $P = 0.00069$ ). There was a trend toward more frequent use of radiotherapy in the *AIPmut* group than in controls (41.4 *vs.* 24.7%, respectively;  $P = 0.15$ ). Percentage reductions in GH and IGF-I were similar for primary, pre-, and postoperative SSA use within each group. In the *AIPmut* group (n = 38), the median SSA-induced reductions in GH [40.0% (range 0.0–99.0%)] and IGF-I [47.4% (range 0.0–83.4%)] were significantly lower than those seen in the 164 control patients treated with long-term SSA [GH: 75.0% (range 0.0–99.0%);  $P = 0.0004$ ; IGF-I: 56.0% (range 0.0–100.0%);  $P = 0.028$ ]. The median magnitude of tumor shrinkage achieved with SSA was significantly higher in the control group [median 41.1% (range 0.0–95.0%)] *vs.* *AIPmut* patients [0.0% (range 0.0–90.0%);  $P < 0.000001$ ]. The disease control rates achieved with SSA in the *AIPmut* and control groups, respectively, were as follows: primary treatment (one of six *vs.* 17 of 32); preoperative (one of six *vs.* six of 16) and postoperative (nine of 26 *vs.* 51 of 84). Concomitant radiotherapy use was similar among patients who were controlled *vs.* those

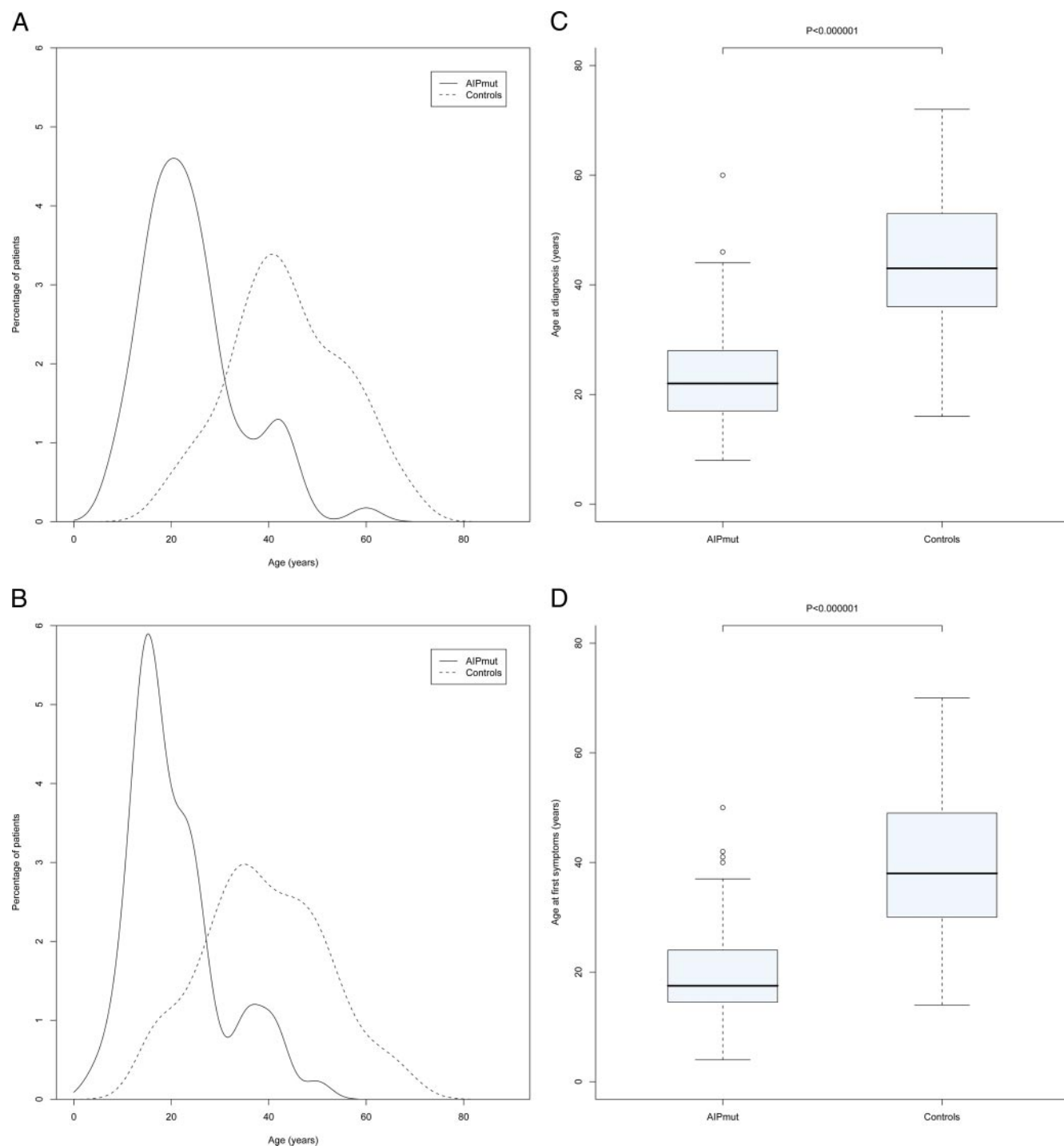
not controlled by SSA in the two groups. Four cases, all in the *AIPmut* group, had complete postoperative SSA resistance with increasing GH/IGF-I levels; tumor expansion during SSA therapy occurred in three of these cases. Unlike in the *AIPmut* group in which three of four patients were uncontrolled by pegvisomant therapy, all 19 control acromegaly patients who received pegvisomant had controlled IGF-I levels at follow-up.

The frequency of hypopituitarism was similar in the *AIPmut* and control groups (22.5 *vs.* 25.2%), but the *AIPmut* group had a significantly higher number of deficient axes than controls patients ( $P < 0.000001$ ).

### Prolactinomas

There were 13 patients with *AIPmut* and prolactinomas in the cohort, nine of whom have not been reported previously. Seven patients came from FIPA kindreds, two had familial mutations without other known affected family members, and four were apparently sporadic cases. Most patients were male (76.9%; Table 1). Patients had young median ages at first symptoms (18.0 yr) and diagnosis (22.0 yr), and median prolactin levels at diagnosis were high (2520.0 ng/ml; range 74.0–60,000.0 ng/ml). Median maximum tumor diameter was large (31.0 mm; range 6.0–85.0 mm), 12 of 13 tumors were macroadenomas, 11 of these had extrasellar extension and nine were invasive at diagnosis.

All but one patient received primary dopamine agonist therapy, which was associated with reductions from baseline in prolactin of 50–99%. Initial normalization of prolactin secretion occurred in five cases (maximum cabergoline dose 2.5 mg/wk); one further patient later developed secondary dopamine agonist resistance and tumor growth despite high-dose cabergoline (7 mg/wk). Two transsphenoidal surgeries



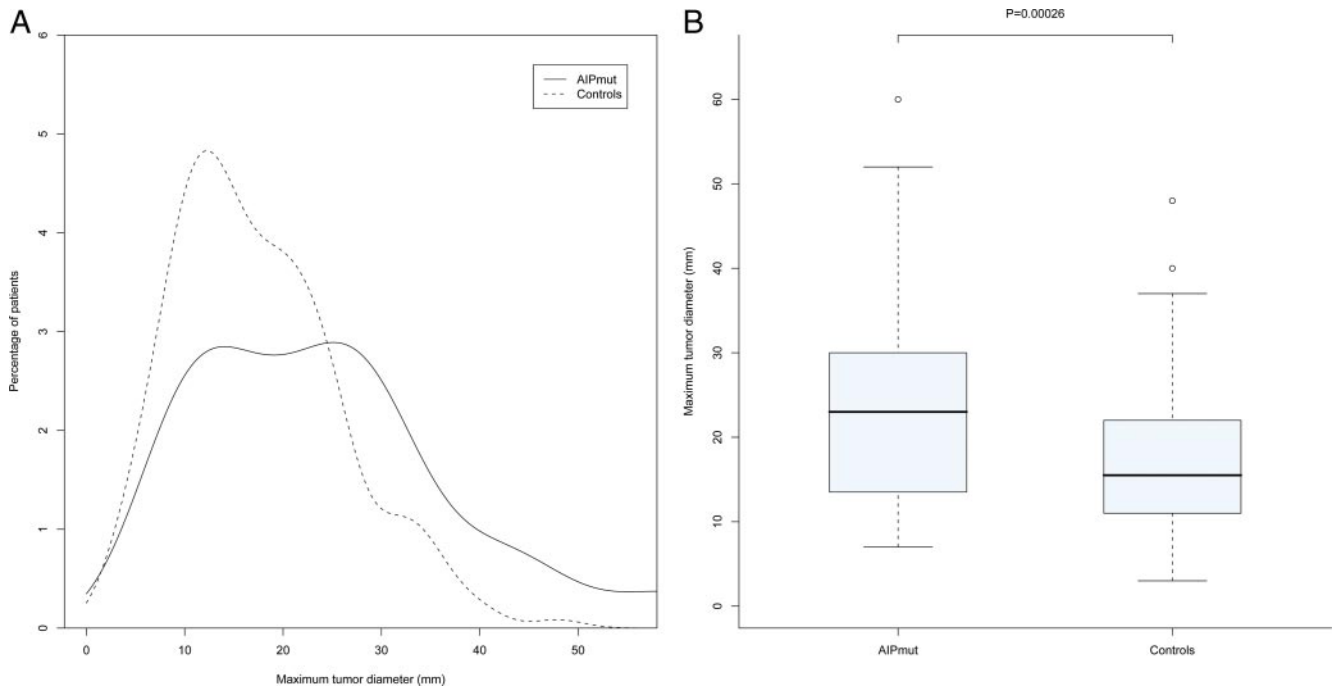
**FIG. 1.** Significantly younger age at diagnosis (A and C) and age at first symptoms (B and D) in somatotropinoma patients with *AIPmut* (n = 71) and control somatotropinoma patients (n = 232). A and C, Frequency plot curves of ages at diagnosis and first symptoms for the two groups. B and D, Box and whisker plots in which the box represents the 25th and 75th percentiles, and the dark line within the box is the median. The whiskers represent the extremes of data that lie one box length distance above and below the 25th and 75th centiles, respectively.

plus radiotherapy was needed to achieve disease control. Six patients (50%) were initially uncontrolled with dopamine agonists and underwent surgery, one of whom underwent three transsphenoidal and one transcranial interventions plus radiotherapy, whereas another two patients had two surgical interventions each. Radiotherapy was eventually undertaken in three operated patients. Long-term control of

prolactin secretion was achieved in eight of 13 patients (61.5%) and two patients developed hypopituitarism.

### Nonsecreting adenomas

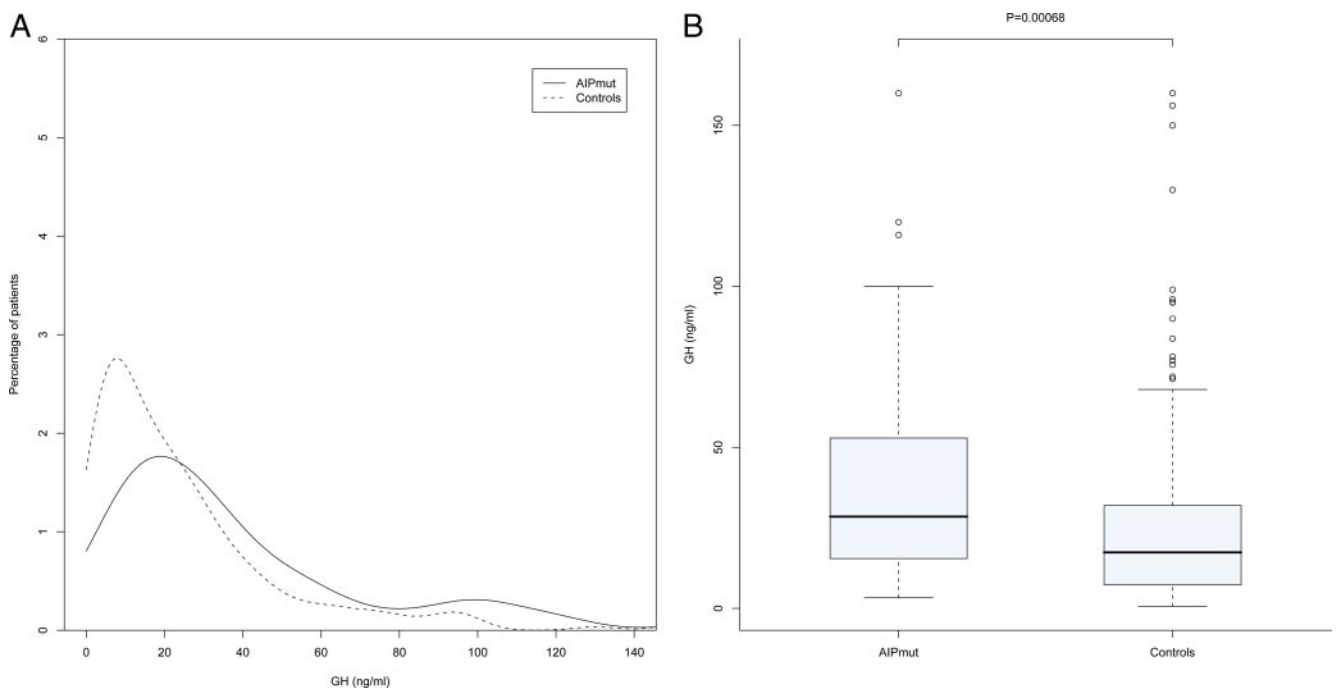
Seven nonsecreting pituitary adenomas occurred in patients (four males, three females) with *AIPmut*, and all came from FIPA kindreds. The median age at diagnosis



**FIG. 2.** Significantly greater maximum tumor diameter in somatotropinoma patients with *AIPmut* (n = 71) and control somatotropinoma patients (n = 232). A, A frequency plot curve. B, Box and whisker plot in which the *box* represents the 25th and 75th percentiles, and the *dark line within the box* is the median. The *whiskers* represent the extremes of data that lie one box length distance above and below the 25th and 75th centiles, respectively.

was younger than commonly described for this disease [31.0 (range 12.0–74.0 yr)] (24). All tumors were macroadenomas, six had suprasellar extension and four were invasive at diagnosis. Two patients presented with pituitary

apoplexy. All patients had mildly elevated prolactin at diagnosis, and in three patients who received dopamine agonists, two achieved normal prolactin (no tumor shrinkage). At diagnosis, one patient had hypogonadism and one had



**FIG. 3.** Significantly greater GH level at diagnosis in somatotropinoma patients with *AIPmut* (n = 71) and control somatotropinoma patients (n = 232). A, A frequency plot curve. B, Box and whisker plot in which the *box* represents the 25th and 75th percentiles, and the *dark line within the box* is the median. The *whiskers* represent the extremes of data that lie one box length distance above and below the 25th and 75th centiles, respectively.



hypofunction of the cortisol, thyroid, and gonadal axes, which did not resolve after therapy. Six patients underwent surgery and one patient who underwent a transcranial approach received radiotherapy due to a large remnant. Long-term control of tumor size was achieved in all cases.

### **TSH-secreting adenoma**

A 39-yr-old male patient presented with a 6-month history of tachycardia and breathing difficulties in association with elevated T<sub>3</sub> and T<sub>4</sub> levels, a normal TSH level, and had a noninvasive pituitary macroadenoma on magnetic resonance imaging. No other hormonal abnormalities were noted at diagnosis. The patient twice underwent transsphenoidal surgery, but the tumor regrew on both occasions within less than 1 yr. A missense *AIPmut* (I257V) was discovered; family screening identified the same mutation in the unaffected mother and brother. After the second tumor recurrence, the patient was treated with octreotide long-acting repeatable 20 mg/month, which resulted in a hormonal normalization but no change in the residual tumor size.

### **Genotype-phenotype relationships**

There were no statistical differences in terms of the clinical or therapeutic characteristics among patients with different types of *AIPmut* (truncating, frameshift mutations, missense mutations, intronic mutations, or in-frame deletions). The characteristics of the three most frequent *AIP* mutations, Q14X (n = 13), R304X (n = 8), and R271W (n = 7), did not differ from the group as a whole.

## **Discussion**

In this study we report the clinical and therapeutic features in 96 patients with germline *AIPmut* and anterior pituitary adenomas in the setting of FIPA and sporadic disease (10–13, 16, 20, 39, 41, 47, and Supplemental Material Refs. 1 and 2); 41 patients are reported for the first time. This study is the first to apply standardized data collection methods to an extensive international *AIPmut* cohort to assess clinically relevant patient and disease characteristics, including responses to therapy.

The spectrum of anterior pituitary tumors associated with *AIPmut* now includes all clinical subtypes. Nearly 80% of patients with *AIPmut* present with somatotropinomas, and more than half cosecrete GH and prolactin. A third of somatotropinoma patients in the *AIPmut* group had gigantism. A further 13.5% of patients had prolactinomas, whereas nonsecreting pituitary adenomas are clearly also a feature of the *AIPmut* spectrum. The *AIPmut* TSH-secreting tumor is the first to be reported; because

these are rare tumors (<1% of all pituitary tumors), it remains to be seen whether *AIPmut* is frequent in this setting (25). Cushing disease is a very rare association with *AIPmut*, with only two cases in the literature and none in the current series (12, 18).

The reason for the predominance of somatotropinomas among patients with *AIPmut* is unclear; however, this subgroup has specific features compared with a well-matched international *AIPmut*-negative control somatotropinoma group. *AIPmut*-associated somatotropinomas had first symptoms and were diagnosed 20 yr earlier as compared with controls and were significantly larger, more frequently extensive and had a greater frequency of prolactin hypersecretion. In addition, *AIPmut* status was also associated with significantly higher levels of GH secretion at baseline *vs.* controls. These features also appeared to impact the therapeutic responses, with poorer disease outcomes in the *AIPmut* group. Large, extensive, and invasive macroadenomas and high GH secretion are associated with a lower rate of control with primary neurosurgery; hence, the significantly higher rate of reoperation in the *AIPmut* cohort is not surprising (26). In addition, SSA therapy was associated with significantly lower decreases from baseline in GH and IGF-I in the *AIPmut* group, whereas tumor shrinkage was also significantly less pronounced than in controls. A trend toward more frequent use of radiotherapy and the failure of pegvisomant to control IGF-I in three of four individuals in the *AIPmut* group (as compared with 19 of 19 controlled pegvisomant-treated sporadic patients) lends further evidence to *AIPmut* patients forming a challenging part of the therapeutic spectrum in acromegaly. This is supported by the finding that *AIPmut* somatotropinoma patients who received similarly high ( $\geq 3$ ) cumulative numbers of therapies had significantly lower rates of long-term disease control as compared with the somatotropinoma control group. The reason for the poorer responses to SSA is not known and is a compelling topic for further study, particularly because SSA receptor expression and the activity of vital determinants of SSA function like the ZAC1 (zinc finger protein which regulates apoptosis and cell cycle arrest) (27), in *AIPmut* somatotropinoma cells remain unknown. Practically it may be that large tumor size and the relatively poor SSA responses in such cases might warrant a tumor debulking approach to favor eventual control with SSA (28, 29).

Gigantism is an integral but very rare component of the acromegaly disease spectrum, with little more than 100 cases reported (30–34). Gigantism may occur exceptionally in other conditions like MEN1, CNC, or McCune-Albright syndrome (30). In contrast, the current results suggest that gigantism is a frequent finding among patients

with *AIPmut*, with 32% of all *AIPmut* somatotropinomas having gigantism, which contrasts strongly with 6.5% among controls. This latter figure in the non-*AIPmut* control group is itself suggestive that gigantism may not be as rare as previously thought. Gigantism occurred in a familial setting in 63% of *AIPmut* cases in this series, although there were nine apparently sporadic giants. In contrast, Leontiou *et al.* (17) found no *AIPmut* cases among seven sporadic giants, although gigantism did appear to occur frequently among their FIPA kindreds. The likely explanation for the high frequency of gigantism in the setting of *AIPmut* is due to the common features of large somatotropinomas secreting high levels of GH that become symptomatic predominantly before epiphyseal closure.

A pronounced gender imbalance was seen in the *AIPmut* cohort with about two thirds of patients being male. This gender imbalance was marked in the prolactinoma group, which was 76.9% male. These patients had large tumors, half of which were not controlled by dopamine agonists, and some were difficult to control with multiple surgeries and radiotherapy. Male sex is known to be associated with a higher rate of aggressive or treatment-resistant prolactinomas, and *AIPmut* status might explain a proportion of such cases (35, 36). Overall, the male preponderance among this series differs markedly from the pituitary disease characteristics in MEN1, in which the gender balance is reversed (69% female) (37). This difference may be due to the fact that prolactinomas comprise 62% of pituitary tumors in MEN1 and are 2.5 times more frequent in women (37). Interestingly, prolactinomas in MEN1 patients are, like the *AIPmut* cases, also comparatively difficult to treat. CNC is, in general, a disease with a strong female preponderance (63%) (38). Although acromegaly is a recognized phenotypic component of CNC, it is relatively uncommon, occurring in only 42 patients (12%) in the largest series, making valid comparisons with *AIPmut* patients difficult.

The penetrance of the pituitary adenoma predisposition conferred by *AIPmut* remains an unresolved question. Based on current figures (there are >100 asymptomatic *AIPmut* carriers related to patients in this study), the penetrance of pituitary adenoma among FIPA kindreds with *AIPmut* is 15–45%. This incomplete penetrance stands in direct contrast to MEN1 and CNC. Because most *AIPmut*-related pituitary adenomas (87.5%) present before the age of 40 yr, many younger *AIPmut* carriers will require extended follow-up to definitively determine penetrance. Current penetrance rates suffer from various sources of bias, such as small kindreds with limited availability for genetic and clinical evaluation, in addition to apparently sporadic patients in whom family *AIP* genetic

testing was not possible. True *de novo* sporadic cases have been identified (18). As demonstrated by large, apparently extensive multigenerational families in Finland, Italy, and elsewhere, mutation founders may have lived in the distant past (10, 39–41); this suggests that *AIPmut* status does not greatly impair biological fitness, unlike in more aggressive genetic tumor syndromes (42). It remains to be determined whether some *AIPmut* confer a lower disease penetrance than others.

Another important feature is the almost uniformly early age at onset and the rapid growth characteristics in *AIPmut* pituitary adenomas. The fact that more than half of patients present already with extensive pituitary macroadenomas as children or adolescents suggest that *AIPmut* status confers a predisposition to rapid tumor growth, a point underlined by the short time from first symptoms to diagnosis (2.0 yr). It is unclear whether loss of the wild-type *AIP* allele in pituitary tissue itself leads to pituitary adenoma development or whether this somatic second hit permits rapid expansion of preexisting nests of abnormal cells (*e.g.* hyperplastic zones). It remains to be determined whether other modulating factors exist that can alter the development of pituitary adenomas among *AIPmut* carriers. Furthermore, *AIPmut*-related disease in humans remains limited to a pituitary adenoma phenotype, which contrasts strongly with other genetic causes of pituitary adenomas (MEN1, MEN4, CNC), which tend to affect multiple tissues. Pituitary data from *Ara9* knockout mice models have not yet been reported (43, 44). Few data are available on the molecular effects of *AIPmut* in the pituitary itself, and it remains unclear whether the primary mechanism governing tumorigenesis is via the aryl hydrocarbon receptor (AhR), down-regulation of AhR nuclear translocator (45), interactions with phosphodiesterases (17), or via RET-survivin (46). Recent immunohistochemical data indicate that AIP expression is high in somatotropinomas and nonsecreting tumors (17, 47). In somatotropinomas, however, significantly lower AIP immunostaining occurs in invasive as compared with noninvasive cases. Furthermore, AIP immunostaining was abolished in only a minority of *AIPmut* pituitary adenomas (46). It may be that decreases in AIP immunostaining is a feature of aggressiveness in somatotropinomas, irrespective of mutation status.

## Conclusions

*AIPmut* status is associated with the development of anterior pituitary adenomas, and all pituitary tumor phenotypes have now been described, usually in a familial setting. *AIPmut*-related pituitary adenomas are generally large and expansive, and more than half are invasive at diagnosis. Patients are predominantly male and young,

with half of cases presenting during childhood or adolescence. Somatotropinomas are encountered most frequently (nearly 80%) and gigantism is notably frequent. *AIPmut*-associated somatotropinomas are significantly larger, more commonly extensive, occur at a younger age, secrete higher levels of GH, and have more frequent prolactin cosecretion than matched acromegalic patients without *AIPmut*. *AIPmut* somatotropinomas require repeat surgery significantly more often than controls, whereas hormonal and tumor responses to SSA are significantly lower than controls; an increased risk of hypopituitarism is seen in the *AIPmut* cohort. *AIPmut*-related prolactinomas appear also to have aggressive and difficult-to-treat clinical characteristics.

These results suggest that improving outcomes among *AIPmut*-associated pituitary tumors might require earlier diagnosis at the microadenoma or enclosed macroadenoma stage. This adds impetus to exploring the most appropriate way to identify *AIPmut* patients, which might be aided by considering genetic screening only in FIPA kindreds and young patients with large tumors (48).

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