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AIP and *MEN1* mutations and AIP immunohistochemistry in pituitary adenomas in a tertiary referral center

Adrian F. Daly[1*], David A. Cano[2*], Eva Venegas-Moreno[2], Patrick Petrossians[1], Elena Dios[2], Emilie Castermans[3], Alvaro Flores-Martínez[2], Vincent Bours[3], Albert Beckers[1**], Alfonso Soto-Moreno[2**]

[1]Department of Endocrinology, Centre Hospital Universitaire de Liège, Liège Université, 4000 Liège, Belgium. [2]Unidad de Gestión de Endocrinología y Nutrición. Instituto de Biomedicina de Sevilla (IBiS), Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Spain. [1]Department of Human Genetics, Centre Hospital Universitaire de Liège, Liège Université, 4000 Liège, Belgium

* these authors contributed equally to the work; ** these authors oversaw the work equally

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Addresses for Correspondence:

Albert Beckers MD, PhD,
Chief, Department of Endocrinology,
Centre Hospitalier Universitaire de Liège,
Domaine Universitaire du Sart Tilman,
4000 Liège, Belgium.

Alfonso Soto Moreno MD, PhD,
Chief, Department of Endocrinology,
Hospital Universitario Virgen del Rocío,
Sevilla, Spain.

AIP, FIPA, acromegaly somatostatin analog, resistance, syndrome

Abstract

Background: Pituitary adenomas have a high disease burden due to tumor growth/invasion and disordered hormonal secretion. Germline mutations in genes such as MEN1 and AIP are associated with early onset of aggressive pituitary adenomas that can be resistant to medical therapy.

Aims: We performed a retrospective screening study using published risk criteria to assess the frequency of AIP and MEN1 mutations in pituitary adenoma patients in a tertiary-referral center.

Methods: Pituitary adenoma patients with pediatric/adolescent onset, macroadenomas occurring ≤30 years of age, familial isolated pituitary adenoma (FIPA) kindreds, and acromegaly or prolactinoma cases that were uncontrolled by medical therapy were studied genetically. We also assessed whether immunohistochemical staining for AIP (AIP-IHC) in somatotropinomas was associated with somatostatin analogs (SSA) response.

Results: Fifty-five patients met the study criteria and underwent genetic screening for AIP/MEN1 mutations. No mutations were identified and large deletions/duplications were ruled out using MLPA. In a cohort of sporadic somatotropinomas, low AIP-IHC tumors were significantly larger ($p=0.002$) and were more frequently sparsely-granulated ($p=0.046$) than high AIP-IHC tumors. No significant relationship between AIP-IHC and SSA responses was seen.

Conclusions: Germline mutations in AIP/MEN1 in pituitary adenoma patients are rare and the use of published risk criteria did not identify cases in a large tertiary-referral setting. In acromegaly, low AIP-IHC was related to larger tumor size and more frequent sparsely-granulated subtype but no relationship with SSA-responsiveness was seen. The genetics of aggressive, treatment-resistant and familial pituitary adenomas remain largely unexplained and screening criteria could be significantly refined.

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Introduction

Clinically apparent pituitary adenomas are present in about 1:1000 of the general population in Europe; the most frequent sub-types are prolactinomas, non-secreting adenomas and somatotropinomas, while Cushing's disease and thyrotropinomas are rarer (1, 2, 3). Treatment of pituitary adenomas varies according to pituitary adenoma sub-type. Responses to therapy are variable due to heterogeneity among patient profiles and tumor characteristics. For instance, acromegaly patients may be resistant to somatostatin analogs (SSA) that target the somatostatin receptor subtype 2 (SST2), while a small proportion of prolactinoma patients may not respond to labeled doses of dopamine agonists (DA). Hence, multimodal therapy involving neurosurgery, medical therapy and radiotherapy can be needed to treat pituitary adenomas (4, 5, 6, 7).

There is an increased likelihood of aggressive pituitary adenoma characteristics (early age at diagnosis, large tumor size, increased invasiveness) in association with a number of germline genetic mutations. Of these, the *aryl hydrocarbon receptor interacting protein* (*AIP*) gene and the *MEN1* gene have been widely studied in the clinical setting. Germline *MEN1* mutations lead to multiple endocrine neoplasia type 1 (MEN1), which is characterized by tumors occurring in the parathyroids, enteropancreatic endocrine tissues and anterior pituitary (8). *MEN1* mutations can be associated with early onset and relatively difficult to treat pituitary adenomas (9, 10, 11). Germline *AIP* mutations (*AIP*mut) or deletions generally predispose to acromegaly, usually presenting as familial isolated pituitary adenomas (FIPA) (12). Notably, *AIP*mut-associated somatotropinomas occur at a significantly younger age and are larger and more extensive than non-*AIP*mut control cases (13). These characteristics lead to a high rate of gigantism among *AIP*mut affected patients (14). *AIP*mut-associated acromegaly patients have a significantly worse response to treatment with SST2-specific SSA compared with *AIP* wild-type acromegaly controls, both in terms of smaller IGF-1 decreases and less tumor shrinkage. In acromegaly patients without *AIP*mut it has also been suggested that AIP immunohistochemical

score in somatotropinomas is a good indicator of whether patients were SST2-specific SSA responders (15, 16).

Screening studies in the general clinical population of pituitary adenomas are not particularly useful as *AIP*muts are rare (0-4% positive cases) (17, 18, 19). Several recommendations have been made regarding the ideal characteristics of patients to refer for *AIP*mut testing, including pituitary gigantism patients, FIPA families, pediatric pituitary adenoma patients and those with pituitary macroadenomas (particularly acromegaly), occurring ≤30 years of age (20, 21, 22). Given the characteristic resistance to SST2-specific SSA in *AIP*mut acromegaly, it has been suggested that such patients might be informative for specific screening. Oriola *et al* reported that 8% of acromegaly patients who had failed surgery and SSA had *AIP* gene variants (23). To address the practicality of these suggested screening factors in the clinical setting, we analyzed *AIP* and *MEN1* status in a cohort of pituitary adenoma patients from a large regional referral population. We also assessed the relationship between tumoral immunohistochemical staining for AIP, disease characteristics and SST2-specific SSA hormonal responses in sporadic acromegaly patients.

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Patients and Methods.

This was a single center, retrospective study performed in patients from the Department of Endocrinology, Hospital Universitario Virgen del Rocío, Seville, Spain. Patients diagnosed at any time with a pituitary adenoma were eligible, up to a cut-off date of July 2017.

Data on each patient included sex, date of birth, age at diagnosis, tumor size (maximum diameter), tumor classification (micro-or macroadenomas), treatment (surgery, medical therapy, radiotherapy) and the magnitude of hormonal responses to treatment with SSA (including % reduction in IGF-1 from baseline; GH levels on an oral glucose tolerance test), where relevant.

Inclusion criteria: We undertook a retrospective analysis of the patient population treated for pituitary adenomas who were in follow-up at the study center (n=903).

We identified individuals that fell into the following categories:

1. Somatotropinomas and prolactinomas that were hormonally resistant to medical treatment.

Patients with documented acromegaly, defined as a failure to suppress GH following an oral glucose tolerance test, an age/sex corrected IGF-1 level above the upper limit of the normal range and a pituitary tumor identified on magnetic resonance imaging (MRI) at baseline. Lack of hormonal control (SSA resistance) was defined as an IGF-1 above the upper limit of normal for age and sex, and a non-suppressed GH following an oral glucose load following at least three months of treatment with octreotide or lanreotide at their maximum labeled/tolerated dose in the pre-operative or adjuvant setting. Patients with prolactinomas had to have serum prolactin levels that were chronically elevated above the upper limit of normal in association with a macroadenoma on MRI. Lack of hormonal control (DA resistance) was defined as per Molitch (17) as a failure to achieve normalization of serum prolactin at the highest labeled dose of cabergoline (2 mg/week). Resistance to medical therapy with SSA or DA in terms of tumor shrinkage was not included as a criterion in this study.

2. Early-onset pituitary adenomas.

a) Patients with pituitary tumors that occurred at or before 18 years of age (pediatric pituitary tumors). Pituitary tumors could be of any clinical subtype and of any diameter, as long as tumor was confirmed on MRI at diagnosis; this subgroup also included pituitary gigantism patients.

b) Patients with MRI-confirmed pituitary macroadenomas that occurred (first symptoms or diagnosed) ≤30 years of age.

3. FIPA kindreds.

Patients that had one or more related family members with a pituitary adenoma on clinical history in the absence of MEN1 or other syndromes.

Genetic studies

Genetic analyses of the *AIP* and *MEN1* genes were performed using leukocyte derived DNA as described previously (24). In addition to sequence changes, all patients underwent studies to screen for exon-level or whole gene deletions or duplications using multiplex ligation dependent probe amplification (MLPA) kit P244 (SALSA P244 Probemix, MRC-Holland) according to the manufacturer's instructions. In one patient with an *AIP* sequence variant DNA was extracted from the pituitary adenoma to test for loss of heterozygosity (LOH) at the *AIP* locus. Sequence variations were assessed and graded according to the American College for Medical Genetics guidelines. In the case where class 3 (variant of unknown significance), class 4 (likely pathogenic) and class 5 (pathogenic) sequence changes were identified, related family members underwent clinical screening for disease features and where appropriate, were offered genetic testing. Patients provided informed consent for the study, which was approved by the Ethics Committees of the CHU de Liège and the Hospital Universitario Virgen del Rocío.

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Immunohistochemistry for AIP

We undertook a specific study of immunohistochemistry for AIP (AIP-IHC) in a series of 51 somatotropinomas operated on at the Hospital Universitario Virgen del Rocío, Seville, Spain. These 51 patients comprise part of a cohort of patients described in Venegas-Moreno *et al* (25). All acromegaly patients included in this study were surgically pre-treated with SSA (octreotide or lanreotide) for at least two months, following the usual clinical practice in our hospital. IHC was performed using a mouse monoclonal anti-AIP antibody (1:500 dilution; NB100-127 (B35-2), Bio-Techne R&D Systems S.L.U., Madrid, Spain) as described in (16, 26, 27). A semi-quantitative score for AIP staining intensity was applied: 0 = negative; 1 = weak; 2 = moderate; 3 = strong. This was multiplied by a score for expression patterning of 1 = patchy and 2 = diffuse to provide a final score ranging from 0 to 6. A low overall AIP immunostaining result was defined by a semi-quantitative AIP-IHC score ≤2, whereas high AIP-IHC was defined as a score of ≥3. For Ki-67 quantification, we counted at least 1000 cells in an area with the highest cell density. Results are expressed as the percentage of tumor cells with positive nuclei of the total number of cells. Cytokeratin CAM5.2 characteristics and staining pattern were used to classify somatotropinomas as sparsely or densely granulated tumors. The densely granulated tumors had a diffuse perinuclear CAM5.2 staining pattern in >70% of tumor cells, while sparsely granulated adenomas had a paranuclear and spherical pattern in >70% of cells.

Results

Patient characteristics

As noted in Figure 1, from a total population of 903 pituitary adenoma patients, 67 met the inclusion criteria for the genetic study and 55 of these underwent genetic testing. Details of the patient population are shown in Table 1. Among the 55 participants, there were eight FIPA families; seven were two-member families, and one was a three-member acromegaly-prolactinoma kindred. There were 12 pediatric pituitary adenoma patients, most of whom had Cushing's disease, while one had gigantism. Fifteen patients had a pituitary macroadenoma that presented ≤30 years of age, 17 patients had SSA-resistant acromegaly and three had DA-resistant prolactinomas. The median age at diagnosis of the group was 27 years (range 10-62 years) and most patients were female (n=39). All but five of the patients had macroadenomas; four microadenomas occurred in children with Cushing's disease aged 12-15 years at diagnosis and one was in a FIPA patient.

Genetic results

The screening study was undertaken to assess whether patients in the proposed high-risk criteria group had *AIP* and *MEN1* mutations/deletions, but no pathological genetic variants were found in *AIP* or *MEN1* in any of the 55 patients. Similarly, on MLPA no deletions of *AIP* or *MEN1* or their individual exons were found. Three subjects were heterozygous for the p.D172D *AIP* variant (rs2276020), and one had the p.D44D variant (rs11822907); both variants are considered benign or likely benign in nature. One patient had the p.A299V (rs148986773) change in *AIP*, which has been reported previously in clinical studies. It is considered more likely to be benign, and based on tumor DNA studies, we conformed that there was no loss of heterozygosity (LOH) at the *AIP* locus, which further supports this non-pathological classification. Family screening demonstrated the p.A299V change in an asymptomatic parent and in a sibling. There was one *MEN1* variant found in one pediatric patient with Cushing's disease, p.R171Q (rs607969), although this too is considered as benign/likely benign.

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Immunohistochemistry of AIP in sporadic acromegaly

A total of 51 somatotropinomas from sporadic acromegaly patients were analyzed. These patients were not selected according to tumoral or other disease characteristics and their baseline clinical features are shown in Table 2. Forty-five tumors were macroadenomas. Nine of the adenomas displayed both GH and prolactin expression while the remaining 42 were purely GH-secreting tumors. Representative images of AIP immunohistochemistry in normal pituitary and in somatotropinomas with different semiquantitative AIP-IHC scores are shown in Figure 2. All GH-producing tumors displayed some degree of AIP immunoreactivity. Thus, none of the patients were classified as score 0. Twenty-four GH-producing tumors exhibited low AIP-IHC scores (≤2). Tumor size was significantly greater in the low AIP-IHC group (median=25 mm [IQR, 15-35.8]) as compared with the high AIP-IHC patients (median=15 mm [IQR, 10-20.3]; P=0.002). No other statistically significant differences in gender, age and GH or IGF-1 levels at diagnosis were observed between low and high AIP-IHC patient groups (Table 2).

Reliable data to determine the response to SSA was available for 39 patients at three months of treatment (26 before surgery and 13 as adjuvant therapy) and for 35 patients at six months of treatment (18 before surgery and 17 as adjuvant therapy). As there were no differences in the magnitudes of response to SSA between patients treated preoperatively or adjuvantly (25), we analyzed all of the SSA response data as a group at three months and then at six months. No differences in percentage reduction IGF-1 were observed between the low and high AIP-IHC groups after either three or six months of SSA treatment (Figure 2).

Discussion

In this study we assessed the prevalence of germline mutations of *AIP* and *MEN1* in a focused group of 55 patients with familial and sporadic isolated pituitary adenomas from a large tertiary referral center in Seville, Spain. The group was selected based on published criteria about patients that had a high likelihood of *AIP* and *MEN1* mutations. The study cohort was young overall (median age 27 years) with large pituitary adenomas (median maximum diameter 22 mm) and included eight new FIPA families with 2-4 affected members. Despite this focused selection, none of the 55 patients had germline mutations in *AIP* or *MEN1* and no cases of *AIP*/*MEN1* deletions were found.

The results of the current study, while at first glance "negative", do provide important information. When individual clinical centers are considering screening programs for pituitary adenoma patients, the relative importance of different proposed criteria need to be weighed. As in the current study, many new FIPA families can be identified at large tertiary referral centers, once the family history is specifically explored. Most such kindreds will be *AIP*mut negative, as about 80-85% of FIPA families remain genetically unexplained. Pediatric pituitary adenoma series report *AIP*mut rates of 11-20% (28, 29, 30, 31). Most pediatric *AIP*mut-related pituitary adenomas are somatotropinomas with occasional prolactinomas or non-functioning tumors. Our pediatric cohort showed no *AIP*mut, which is probably because most had Cushing's disease, which is only very rarely associated with pathological *AIP* mutations (29). In pediatric and adolescent patients with *AIP*mut-related pituitary adenomas, a typical presentation is with pituitary gigantism. Indeed, *AIP*mut are the single most important cause of pituitary gigantism, explaining 29% of cases, followed by X-linked acrogigantism (X-LAG) syndrome (10%) and McCune Albright syndrome (5%) (14). In the current cohort there was only one young patient with pituitary gigantism and he was negative for not only *AIP/MEN1* mutations but also did not have X-LAG syndrome on array comparative genome hybridization (data not shown).

There is considerable uncertainty about how to best define "young-onset" adult pituitary adenoma, with age cutoffs of 30 and 40 years having been proposed in the past (19, 30, 31).

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While Preda *et al* found a low rate (approximately 3%) of *AIP*mut in a prospective, single—center study of patients aged < 40 years, we reported a higher rate of nearly 12% among an international group of sporadic macroadenoma patients aged <30 years at diagnosis (19, 30). The contrast between the current results and that of our previous multicenter study may be explained by the relatively more severe patient profile of the *AIP*mut-positive patients identified in our previous study (30). While in the Seville sporadic cohort the median age was 27 years and the median tumor diameter was 23 mm, in our international study the median age at diagnosis (18 years) and maximal tumor diameter (39 mm) were indicative of more severe disease. It may be that in order to direct screening, the general age of potential adult patients should be revised downwards to below 30 years at disease onset/diagnosis and that only patients with extensive and/or invasive macroadenomas should initially be considered for *AIP*mut analysis.

The topic of standardized screening criteria for *AIP* mutations was considered recently by Caimari *et al* (32). Analyzing data from the Korbonits group, they devised a risk stratification assessment for genetic screening that confirmed a number of factors such as young age at onset (including gigantism), FIPA, macroadenomas and GH excess (all $P \leq 0.001$). Young age at onset (19-30 years) alone was also an independent risk factor ($P=0.015$). This stratification system points to certain extremely high-risk categories such as FIPA cases with macroadenomas occurring up to 18 years of age. In the absence of either FIPA, a macroadenoma or an age up to 18 years, the risk fell markedly in that stratification system. In the case where only moderate risk of an *AIP*mut is present, individual patient characteristics become important. We agree that in such instances, it is vital to take an individualized approach so as not to discount aggressive cases of prolactinoma, non-functioning adenomas or apoplexy cases. No general risk stratification is foolproof, however, as shown by our current study: despite meeting the criteria for high risk of *AIP*mut like acromegaly with macroadenomas in FIPA, no *AIP*mut casesvwere seen.

This study addressed whether adding the criteria of resistance to medical therapy with first-generation SSA in acromegaly patients, or maximum labeled dose cabergoline in three

prolactinoma patients could improve the identification of *AIP* or *MEN1* mutations. Resistance to first generation SSA is an established characteristic of *AIP*mut related acromegaly (13). This may be caused by interference with important mediators of SST2 function, such as Gai2 or ZAC1 (33, 34). Oriola *et al* previously reported a separate Spanish cohort of acromegaly patients with SSA resistance and noted a rate of *AIP*mut approaching 8% (23). The current study suggests that even in a population of acromegaly patients with macroadenomas, the addition of resistance to octreotide and lanreotide does not improve detection rates for *AIP* or *MEN1* mutations. Given the identification of cabergoline resistant prolactinoma patients with *AIP* and *MEN1* mutations in previous international studies (10, 13, 35), we also screened for this criterion in the Seville population but only three patients were identified and none carried an *AIP* or *MEN1* mutation/deletion.

Previous studies have reported that decreased tumoral AIP expression might be associated with poor response to first-generation SSA treatment of somatotropinomas, although the results are inconsistent (15, 16, 26, 33, 36). We did not find any such relationship between AIP IHC and response to SSA treatment using the same commercial AIP antibody and scoring system as in those previous studies. A possible explanation for the discrepancy among studies is that all the patients included in our study received SSA pre-treatment while waiting for surgery. Pre-treatment with octreotide/lanreotide in acromegaly is associated with increased AIP protein expression (26, 33). Chahal *et al* did not find an overall correlation between SSA response and AIP IHC in pretreated patients (33). Jaffrain-Rea *et al* reported significantly higher pre-operative GH and IGF-1 levels in a group of 67 acromegaly patients with low tumoral AIP staining; this difference was, however, not seen among a subgroup (n=25) of patients who had not received preoperative SSA treatment (26). Furthermore, the only significant differences between low and high AIP IHC staining in non-SSA pretreated acromegaly patients in that study were in terms of increased invasiveness and suprasellar extension associated with low AIP staining. These inter-study differences reflect an imperfect correlation between AIP IHC and hormonal SSA responses. It should be noted that AIP IHC is quite variable in somatotropinomas, even among populations with germline *AIP*mut, and is a poor tool for

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screening for possible *AIP*mut cases in a pathological setting (36). Given the fact that AIP-IHC results could be biased or influenced by SSA pretreatment, as noted above, studies with well-balanced groups of SSA pretreated and non-pretreated acromegaly patients would be helpful to clarify the role of the effect of SSA pretreatment on AIP-IHC. Subsequent studies could also explore the role of AIP-IHC in predicting control of acromegaly with SSA under combined hormonal and tumor shrinkage criteria.

More consistently than predicting hormonal effects in acromegaly, low AIP IHC does seem to correlate with tumor aggressiveness, invasion and extension in somatotropinomas (16, 26). This echoes our finding of significantly larger tumor size in low versus high AIP-IHC acromegaly groups. We also found that sparsely granulated adenomas were significantly more frequent in the low AIP-IHC group. Sparsely granulated adenomas have previously been shown to be associated with lower responses to octreotide/lanreotide and better responses to pasireotide and they predominate in *AIP*mut cases (27, 37, 38). In sporadic acromegaly it is difficult to know whether the relationship between low AIP-IHC and more frequent sparsely granulated tumors is a cause or an effect. Specific studies to fully explain the means by which *AIP*mut cause somatotrope tumorigenesis will hopefully cast some light on this issue.

In conclusion, our understanding of the genetics of pituitary adenomas is expanding quickly and many targets for screening are emerging. Due to this rapid progress, it is difficult to devise concrete guidelines for genetic testing in pituitary adenoma populations, although both expert recommendations and risk stratification models are helpful. While many pituitary adenoma patients with *AIP* and *MEN1* mutations with have been identified in the literature, the current study underlines that in the vast majority of FIPA and sporadic cases, no genetic cause is known. Furthermore, the addition of other potential aggressive characteristics, such as, SSA resistance may not improve the ability to discriminate groups of patients at high risk of *AIP* and *MEN1* mutations in the tertiary referral setting. AIP-IHC is a promising pathological marker for somatotropinoma growth and invasion, although this study suggests that its role in predicting hormonal responses to SSA in acromegaly responses appears to be limited. We show that even when focusing on patients with the highest risk, such as, pituitary gigantism, FIPA kindreds

with acromegaly, and pediatric-onset patients with large aggressive macroadenomas, many supposedly high-risk patients proved to be negative for germline *AIP* and *MEN1* genetic pathology, indicating that other novel genetic factors probably remain to be identified.

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Legends

Figure 1. Disposition of study subjects according to screening characteristics.

Figure 2. Immunohistochemical detection of AIP in somatotropinomas. Representative image of AIP immunohistochemistry in normal pituitary (A) and GH-secreting adenomas showing low (B; diffuse, weak) and high AIP expression (C; patchy, strong). Scale bar: 50 μm in C for A and B. (D) Comparison of IGF-1 percent reduction after three months of SSA treatment in tumors with low or high AIP-IHC expression. (E) Comparison of IGF-1 percent reduction after six months of SSA treatment with low or high AIP-IHC expression. Data points represent values for each individual patient. Mean and standard error (SEM) values are shown.

Table 1. Demographic, clinical and genetic characteristics of the study population. AIP: aryl hydrocarbon receptor interacting protein; DA: dopamine agonist; FIPA: familial isolated pituitary adenoma; MacroAd: macroadenoma; MEN1: multiple endocrine neoplasia type 1; MicroAd: microadenoma; MLPA: multiplex ligation dependent probe assay; SSA: somatostatin analog; WT: wild-type.

Table 2. Baseline characteristics of 51 acromegaly patients studied using AIP immunohistochemistry.

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Patient number	Tumor type	Age disease onset (yr)	Max. tumor diameter (mm)	Micro/ Macroadenom	Surgery	RTx	*AIP* status	*MEN1* status
1	FIPA (2-member homogeneous prolactinoma)	60	15	MacroAd	No	No	WT; MLPA normal	WT; MLPA normal
2	FIPA (2-member homogeneous prolactinoma)	13	27	MacroAd	No	no	p.D172D; polymorphism; MLPA normal	WT; MLPA normal
3	FIPA (2-member homogeneous acromegaly)	35	15	MacroAd	Yes	No	p.D172D; polymorphism; MLPA normal	WT; MLPA normal
4	FIPA (2-member heterogeneous TSHoma/non-	17	6	MicroAd	No	No	WT; MLPA normal	WT; MLPA normal
5	FIPA (2-member heterogeneous	61	11	MacroAd	Yes	No	WT; MLPA normal	WT; MLPA normal
6	FIPA (2-member homogeneous non-	46	26	MacroAd	Yes	No	WT; MLPA normal	WT; MLPA normal
7	FIPA (4-member heterogeneous	18	19	MacroAd	Yes	Yes	WT; MLPA normal	WT; MLPA normal
8	FIPA (2-member heterogeneous	26	15	MacroAd	Yes	No	WT; MLPA normal	WT; MLPA normal
9	Pediatric Pituitary adenoma (non functioning)	10	10	MacroAd	No	No	WT; MLPA normal	WT; MLPA normal
10	Pediatric Pituitary adenoma (Cushing)	18	12	MacroAd	Yes (2)	No	WT;MLPA normal	p.R171Q (rs607969) benign/likely benign
11	Pediatric Pituitary adenoma (Cushing)	12	6	MicroAd	Yes (2)	No	WT; MLPA normal	WT; MLPA normal
12	Pediatric Pituitary adenoma (Cushing)	15	2	MicroAd	Yes	No	WT; MLPA normal	WT; MLPA normal
13	Pediatric Pituitary adenoma (Cushing)	16	10	MacroAd	Yes	No	WT; MLPA normal	WT; MLPA normal
14	Pediatric Pituitary adenoma (non functioning, silent	16	15	MacroAd	Yes	No	WT; MLPA normal	WT; MLPA normal
15	Pediatric Pituitary adenoma (Cushing)	10	25	MacroAd	Yes (2)	No	WT; MLPA normal	WT; MLPA normal
16	Pediatric Pituitary adenoma (Cushing)	11	3	MicroAd	Yes	No	WT; MLPA normal	WT; MLPA normal
17	Pediatric Pituitary adenoma (Cushing)	15	5	MicroAd	No	No	WT; MLPA normal	WT; MLPA normal
18	Pediatric Pituitary adenoma (Cushing)	15	11	MacroAd	Yes	No	p.D44D; polymorphism; MLPA normal	WT; MLPA normal
19	Pediatric Pituitary adenoma (non functioning, silent	17	37	MacroAd	Yes (4)	Yes	p.A299V (c.896 C>T); likely benign VUS; No	WT; MLPA normal
20	Pediatric pituitary adenoma (gigantism)	12	23	MacroAd	Yes	No	WT; MLPA normal	WT; MLPA normal
21	Acromegaly, resistant to SSA	37	15	MacroAd	Yes	Yes	WT; MLPA normal	WT; MLPA normal
22	Acromegaly, resistant to SSA	40	25	MacroAd	Yes	Yes	WT; MLPA normal	WT; MLPA normal
23	Acromegaly, resistant to SSA	39	12	MacroAd	Yes	Yes	WT; MLPA normal	WT; MLPA normal
24	Acromegaly, resistant to SSA	58	15	MacroAd	Yes	No	WT; MLPA normal	WT; MLPA normal
25	Acromegaly, resistant to SSA	25	30	MacroAd	Yes	Yes	WT; MLPA normal	WT; MLPA normal
26	Acromegaly, resistant to SSA	47	37	MacroAd	Yes	Yes	WT; MLPA normal	WT; MLPA normal
27	Acromegaly, resistant to SSA	62	18	MacroAd	Yes	Yes	WT; MLPA normal	WT; MLPA normal
28	Acromegaly, resistant to SSA	41	50	MacroAd	Yes	Yes	WT; MLPA normal	WT; MLPA normal
29	Acromegaly, resistant to SSA	60	20	MacroAd	Yes	No	WT; MLPA normal	WT; MLPA normal
30	Acromegaly, resistant to SSA	58	18	MacroAd	Yes	No	WT; MLPA normal	WT; MLPA normal
31	Acromegaly, resistant to SSA	41	40	MacroAd	Yes	Yes	WT; MLPA normal	WT; MLPA normal
32	Acromegaly, resistant to SSA	42	20	MacroAd	Yes	No	WT; MLPA normal	WT; MLPA normal
33	Acromegaly, resistant to SSA	25	22	MacroAd	Yes (2)	Yes	WT; MLPA normal	WT; MLPA normal
34	Acromegaly, resistant to SSA	31	38	MacroAd	Yes (2)	Yes	WT; MLPA normal	WT; MLPA normal
35	Acromegaly, resistant to SSA	40	70	MacroAd	Yes (2)	Yes	WT; MLPA normal	WT; MLPA normal
36	Acromegaly, resistant to SSA	40	37	MacroAd	Yes	Yes	WT; MLPA normal	WT; MLPA normal
37	Acromegaly, resistant to SSA	20	25	MacroAd	Yes	No	WT; MLPA normal	WT; MLPA normal
38	Prolactinoma, resistant to DA	32	40	MacroAd	Yes	No	WT; MLPA normal	WT; MLPA normal
39	Prolactinoma, resistant to DA	34	27	MacroAd	Yes	Yes	WT; MLPA normal	WT; MLPA normal
40	Prolactinoma, resistant to DA	50	30	MacroAd	Yes	No	WT; MLPA normal	WT; MLPA normal
41	Sporadic macroadenoma onset ≤ 30 years	28	23	MacroAd	Yes	Yes	WT; MLPA normal	WT; MLPA normal
42	Sporadic macroadenoma onset ≤ 30 years (Non	23	20	MacroAd	Yes	No	p.D172D; polymorphism; MLPA normal	WT; MLPA normal
43	Sporadic macroadenoma onset ≤ 30 years (Non	28	30	MacroAd	Yes	No	WT; MLPA normal	WT; MLPA normal
44	Sporadic macroadenoma onset ≤ 30 years	28	25	MacroAd	Yes	Yes	WT; MLPA normal	WT; MLPA normal
45	Sporadic macroadenoma onset ≤ 30 years (Non	25	25	MacroAd	Yes (3)	No	WT; MLPA normal	WT; MLPA normal
46	Sporadic macroadenoma onset ≤ 30 years (Non	28	22	MacroAd	Yes	No	WT; MLPA normal	WT; MLPA normal
47	Sporadic macroadenoma onset ≤ 30 years	27	13	MacroAd	Yes	No	WT; MLPA normal	WT; MLPA normal
48	Sporadic macroadenoma onset ≤ 30 years (Non	23	42	MacroAd	Yes (2)	No	WT; MLPA normal	WT; MLPA normal

Patient number	Tumor type	Age disease onset (yr)	Max. tumor diameter (mm)	Micro/ Macroadenom	Surgery	RTx	*AIP* status	*MEN1* status
49	Sporadic macroadenoma onset ≤ 30 years (Non	22	30	MacroAd	Yes (3)	Yes	WT; MLPA normal	WT; MLPA normal
50	Sporadic macroadenoma onset ≤ 30 years	24	20	MacroAd	Yes	No	WT; MLPA normal	WT; MLPA normal
51	Sporadic macroadenoma onset ≤ 30 years	30	30	MacroAd	Yes	No	WT; MLPA normal	WT; MLPA normal
52	Sporadic macroadenoma onset ≤ 30 years	23	11	MacroAd	Yes (2)	No	WT; MLPA normal	WT; MLPA normal
53	Sporadic macroadenoma onset ≤ 30 years	30	30	MacroAd	Yes (3)	Yes	WT; MLPA normal	WT; MLPA normal
54	Sporadic macroadenoma onset ≤ 30 years	19	40	MacroAd	Yes (2)	Yes	WT; MLPA normal	WT; MLPA normal
55	Sporadic macroadenoma onset ≤ 30 years	16	33	MacroAd	Yes (2)	Yes	WT; MLPA normal	WT; MLPA normal

Table 1

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Characteristics	Low AIP-IHC	High AIP-IHC	*P*-value
Sex (number, male/female)	11/13	15/12	0.488
Age at diagnosis (years, median, IQR)	37 (32.5-42.5)	40 (31-48)	0.515
Maximum tumor diameter at diagnosis (mm, median, IQR)	25 (15-35.8)	15 (10-22.3)	**0.002**
GH at diagnosis (ng/ml, median, IQR)	20.5 (9.9-44.3)	22.5 (8.4-40)	0.852
IGF-1 at diagnosis (%ULN, median, IQR)	280 (238-343)	228 (182-311)	0.163
Treatment duration (months, median, IQR)	6 (2-10.5)	5.5 (2.8-11.5)	0.718
Ki-67 index (%, median, IQR)	0.4 (0.3-1)	0.2 (0.1-1)	0.143
GH-producing histological subtypes (number, sparsely/densely granulated)	12/7	9/18	**0.046**

Data are presented as medians with interquartile ranges (IQR). ULN, upper limit of normal for age- and gender-matched IGF-1 levels.

Table 2.

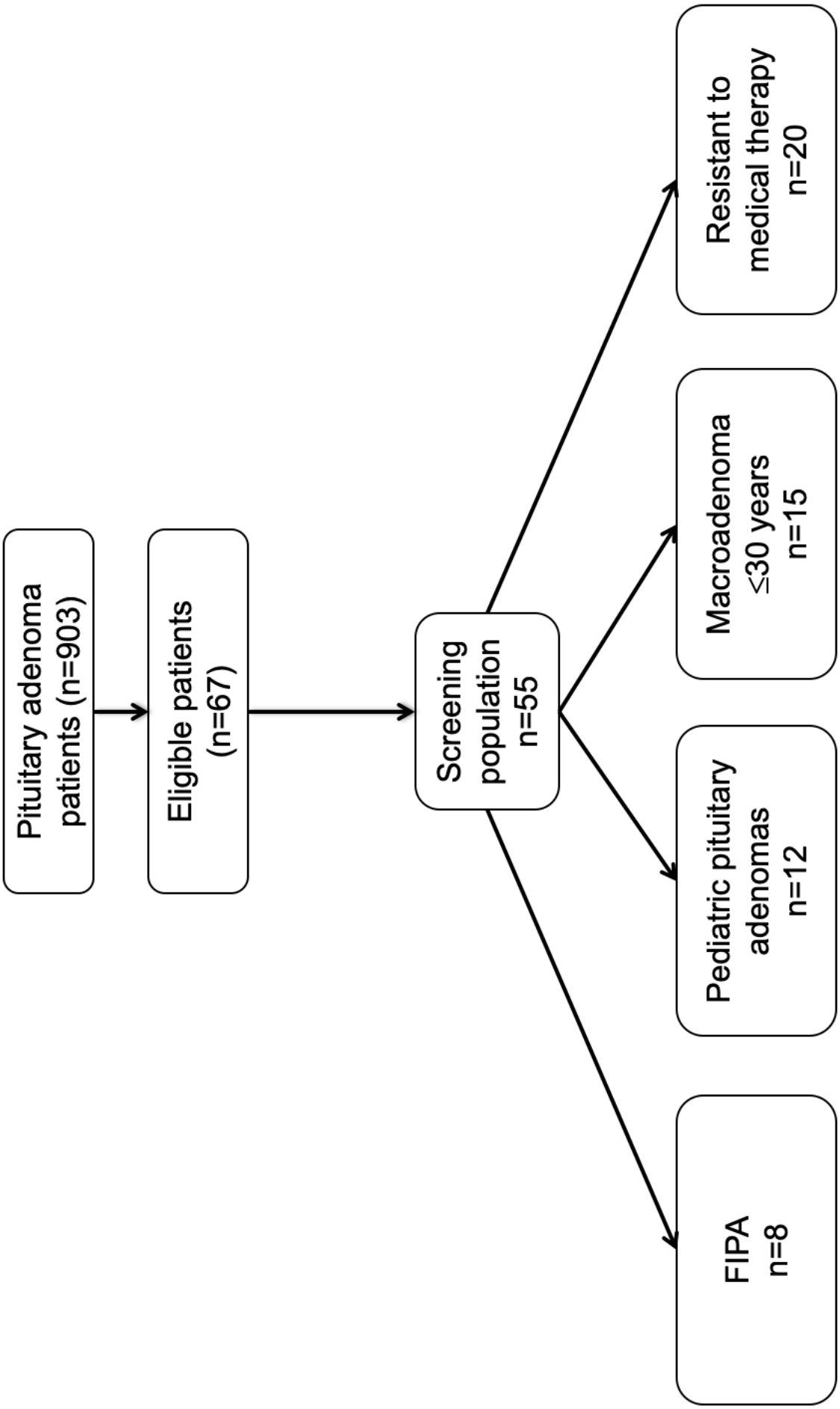

Figure 1

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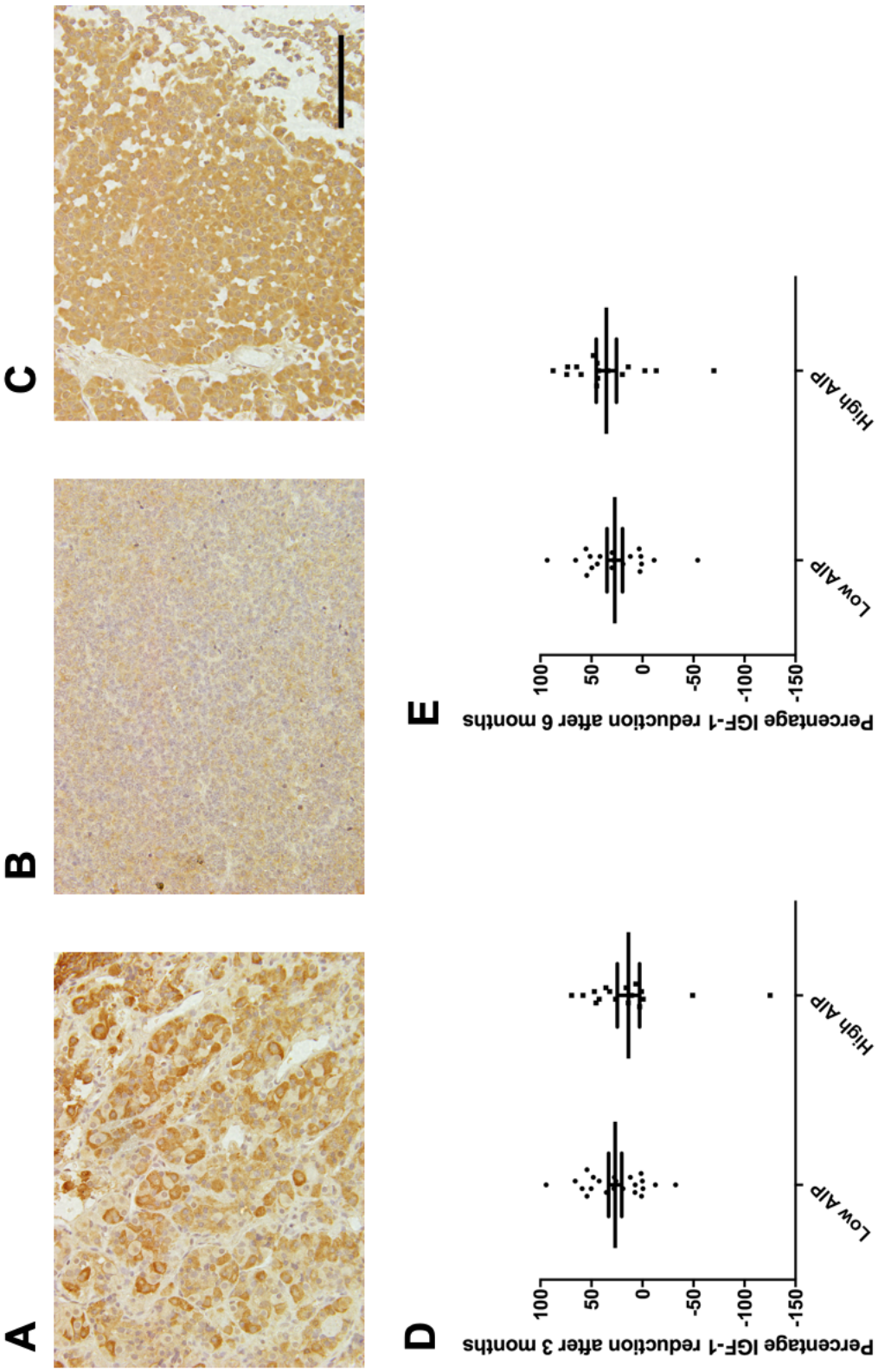

Figure 2