Somatostatin analogues increase AIP expression in somatotropinomas, irrespective of *Gsp* mutations

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Abstract

Germline aryl hydrocarbon receptor interacting protein (*AIP*) gene mutations confer a predisposition to pituitary adenoma (PA), predominantly GH-secreting (GH-PA). As recent data suggest a role for AIP in the pathogenesis of sporadic GH-PA and their response to somatostatin analogues (SSA), the expression of AIP and its partner, aryl hydrocarbon receptor (AHR), was determined by semiquantitative immunohistochemistry scoring in 62 sporadic GH-PA (37 treated with SSA preoperatively). The influence of *Gsp* status was studied in a subset of tumours ($n=39$, 14 Gsp^+) and six GH-PA were available for primary cultures. AIP and AHR were detected in most cases, with a positive correlation between AIP and cytoplasmic AHR ($P=0.012$). Low AIP expression was significantly more frequent in untreated vs SSA-treated tumours (44.0 vs 20.5%, $P=0.016$). AHR expression or localisation did not differ between the two groups. Similarly, *in vitro* octreotide induced a median twofold increase in AIP expression (range 1.2–13.9, $P=0.027$) in GH-PA. In SSA-treated tumours, the AIP score was significantly higher in the presence of preoperative IGF1 decrease or tumour shrinkage ($P=0.008$ and $P=0.014$ respectively). In untreated tumours, low AIP expression was significantly associated with invasiveness ($P=0.028$) and suprasellar extension ($P=0.019$). The only effect of *Gsp* status was a significantly lower nuclear AHR score in Gsp^+ vs Gsp^- tumours ($P=0.025$), irrespective

Key Words
- pituitary adenoma
- aryl hydrocarbon receptor interacting protein
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- acromegaly
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- somatostatin analogues

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of SSA. In conclusion, AIP is involved in the aggressiveness of sporadic GH-PA, regardless of *Gsp* status, and AIP up-regulation in SSA-treated tumours is associated with a better preoperative response, with no clear role for AHR.

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Introduction

The aryl hydrocarbon receptor interacting protein (*AIP*) gene has been identified as a predisposing gene for the development of pituitary adenomas (PA) (Vierimaa *et al.* 2006). *AIP* is a pituitary tumour suppressor gene, with germline inactivating mutations being associated with somatic loss of heterozygosity in the corresponding tumours. A particular preponderance of growth hormone (GH)-secreting PA (GH-PA) in the setting of germline *AIP* mutations (AIP^{mut}) has been reported by clinical studies worldwide (Beckers *et al.* 2013). In our experience, germline AIP^{mut} accounts for 50% of familial isolated PA kindreds with homogeneous somatotropinomas (Daly *et al.* 2007) and 80% of AIP^{mut} patients with PA have GH-PA (Daly *et al.* 2010). AIP^{mut} GH-PA can also present as an apparently sporadic acromegaly or gigantism in young patients, especially in a paediatric context (Beckers *et al.* 2013). When compared with their non-AIP^{mut} counterparts, AIP^{mut} somatotropinomas are commonly more aggressive and more frequently resistant to somatostatin analogues (SSA; Daly *et al.* 2010).

There is a growing body of evidence that AIP down-regulation may contribute to the pathogenesis of sporadic PA, regardless of AIP^{mut} status. Although somatic *AIP* mutations have not been reported to date (Barlier *et al.* 2007, Iwata *et al.* 2007, Jaffrain-Rea *et al.* 2009), AIP expression is frequently reduced in invasive GH-PA (Jaffrain-Rea *et al.* 2009), and loss of AIP immunostaining has been proposed as a marker of tumour aggressiveness in sporadic cases (Kasuki Jomori de Pinho *et al.* 2011). There is also recent evidence that AIP expression is increased by SSA (Jaffrain-Rea *et al.* 2010, Chahal *et al.* 2012) and may predict the post-operative response to SSA in acromegalic patients (Kasuki *et al.* 2012). This suggests that loss of AIP function or expression may contribute to pharmacological resistance in GH-PA.

We have previously observed that *AIP* mutations or down-regulation in PA is frequently accompanied by a reduced expression of its best characterised partner, the aryl hydrocarbon receptor (AHR; Jaffrain-Rea *et al.* 2009). AHR is stabilised in the cytoplasm into a latent AIP/AHR/heat-shock protein 90/p23 complex (Petrulis & Perdrew 2002). Upon activation by exogenous ligands, it translocates to the nucleus and exerts transcriptional effects after heterodimerisation with the aryl hydrocarbon receptor nuclear translocator (ARNT; Beischlag *et al.* 2008). The AHR/ARNT complex mediates the detoxifying effects of AHR and is involved in endocrine disruption (Beischlag *et al.* 2008). Endogenous functions of AHR include the control of apoptosis and cell cycle proliferation (Nguyen & Bradfield 2008) and AHR signalling may up-regulate $p27^{Kip1}$ (Marlowe & Puga 2005), which is also increased by SSA in GH-PA (Ferrante *et al.* 2006, Hubina *et al.* 2006). However, the potential effects of SSA on AHR expression and localisation in GH-PA is unknown.

The best understood genetic event in sporadic GH-PA is the presence of somatic activating mutations of the *GNAS1* (*GNAS*) gene – the *Gsp* oncogene – which induce a constitutive activity of the cAMP pathway, an important target of SSA (Lania *et al.* 2003). As cAMP is an endogenous activator of AHR (Oesch-Bartlomowicz *et al.* 2005) and cAMP signalling can be modulated by AIP through direct interactions with specific phosphodiesterases (PDEs; Bolger *et al.* 2003, de Oliveira *et al.* 2007), *Gsp* may also theoretically affect AIP and/or AHR expression.

We therefore aimed to further evaluate the expression of AIP and AHR in a large series of sporadic GH-PA, taking into account the presence of preoperative SSA treatment and the potential effects of *Gsp* mutations.

Subjects and methods

Patients and samples

A series of surgical samples from 62 GH-PA operated on in patients with sporadic acromegaly was retrospectively studied, including 52 cases from three European centres (Neuromed, Pozzilli and University/Hospital of Padova, Italy; CHU, University of Liège, Belgium) and ten tumours with *Gsp* mutations (Gsp^{+}) provided by additional two centres (University of Aix-Marseilles, France and University of Milan, Italy). Most tumours were collected between 2006 and 2011; archive material collected from 1997 to 2005 was included in order to ensure an appropriate ratio of

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SSA-treated and -untreated tumours and a sufficient number of Gsp^+ samples. The study was approved by local ethics committees. Patients diagnosed before the age of 18 years, with a familial history of acromegaly or with germline *AIP* mutations, were excluded from the study, as well as those who received preoperative therapy with dopamine agonists only. Of the patients, 25 were males and 37 were females, with a mean age of 44.8±12.8 years at surgery (median 44.5 years and range 18–78 years). Patients' clinical, biological and neuroradiological data were recorded. GH, prolactin (PRL) and insulin-like growth factor 1 (IGF1) measurements were obtained for each centre. IGF1 levels were adjusted for age and expressed as percentage of upper normal limit (%ULN). Hormone data were incomplete in 12 cases. Most patients had macroadenomas, as defined by maximal tumour diameter exceeding 10 mm (52 out of 62 cases, 83.9%), a suprasellar extension was recorded in 29 out of 59 cases (49.1%) – not recorded in three cases – and 31 out of 62 cases (50%) were invasive. A total of 37 patients (59.7%) received SSA treatment before surgery (octreotide–LAR 20–40 mg monthly, $n=21$ or lanreotide 60–120 mg monthly, $n=16$), including four patients who also received dopamine agonists for mixed GH/PRL-secreting tumours. Median preoperative treatment duration was 6 months (range 3–108). The pharmacological response was assessed in terms of plasma GH and IGF1 reduction, hormone values at diagnosis and before surgery being available for 33 treated patients to calculate ΔGH and ΔIGF1 as percentages of hormone decrease. Treated patients were then divided into three groups on the basis of preoperative age-corrected IGF1 values (%ULN): group I, controlled (IGF1 normalisation for age, $n=10$); group II, partially controlled (non-normalised IGF1 with ΔIGF1 ≥30% when compared with pretreatment values, $n=13$) and group III, uncontrolled (ΔIGF1 <30% or IGF1 increase when compared with pretreatment values, $n=10$). Data on tumour shrinkage were also available for 26 patients (including one patient with no available ΔIGF1). Immunohistochemistry (IHC) for pituitary hormones was performed in each centre. Cell proliferation was evaluated in 54 cases by Ki67 immunostaining with the monoclonal MIB1 antibody, as described previously (Jaffrain-Rea *et al.* 2002).

Molecular and genetic analysis

Patients gave informed consent for genetic analysis. Direct *AIP* sequencing was performed on leukocyte DNA as previously reported (Daly *et al.* 2007, Occhi *et al.* 2010). A search for activating mutations of the *GNAS1* gene at codons 201 and 227 (*Gsp*) was performed on tumour DNA at each centre, as described previously (Barlier *et al.* 1998, Lania *et al.* 1998, Occhi *et al.* 2011), primers and conditions for *GNAS1* sequencing in the leading centre being available on demand. In a minority of patients lost to follow-up, *AIP* and *GNAS1* sequencing was performed on tumour DNA obtained from paraffin-embedded material (QIAmp DNA FFPE tissue kit, Qiagen). Overall, *AIP* and *GNAS1* sequences could be obtained in 40 and 39 cases respectively, 29 being characterised for both genes. No case had an *AIP* mutation, and no significant difference was retrospectively found between tumours characterised or not for *AIP* sequencing in terms of patient's age, gender, tumour volume, invasiveness and preoperative SSA treatment (data not shown). *Gsp* mutations were present in 14/39 cases (Gsp^+).

Immunohistochemistry

IHC was performed at the University of L'Aquila as described previously (Jaffrain-Rea *et al.* 2009) using a mouse monoclonal anti-AIP antibody at a 1:500 dilution in all cases (clone 35-2, Novus Biologicals LLC, Littleton, CO, USA, distributed by DBA Italia, Milan, Italy) and a polyclonal rabbit anti-AHR antibody at a 1:50 dilution in 53 cases (sc-5579, Santa Cruz Biotechnology, distributed by DBA Italia). Negative controls were performed omitting the primary antibody and normal pituitary (NP) tissue adjacent to PA samples was used as positive controls. Immunostaining for AIP was classified semiquantitatively according to intensity (negative, 0; weak, 1; moderate, 2 and strong, 3) and expression pattern (patchy, 1 and diffuse, 2), with a final score being obtained by multiplying intensity×pattern (range 0–6) (Leontiou *et al.* 2008, Kasuki Jomori de Pinho *et al.* 2011). Low AIP immunostaining (low AIP–IHC) was defined by a semiquantitative score ≤2. AHR immunostaining was also classified semiquantitatively (negative, 0; weak, 1; moderate, 2 and strong, 3) in terms of cytoplasmic AHR (AHR*c*) and nuclear AHR (AHR*n*) localisation. Total AHR (AHR*t*) score was calculated by adding AHR*c* and AHR*n* scores. High AHR*c* and AHR*n* scores were defined as ≥2 and high AHR content (high AHR–IHC) by AHR*t* score ≥4. Photographs of slides were taken using a Zeiss Axioplan 2 microscope (Carl Zeiss Microimaging, Inc., USA) and a Leica DFC 320 digital camera (Leica GmBH, Germany).

In vitro study of GH-secreting PA

The study was previously approved by the local ethics committees at the Universities of Milan and Marseilles

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and informed consent was obtained from the patients. Fresh tissue was obtained by the transsphenoidal route from six untreated GH-PA and enzymatically dissociated in DMEM containing 2 mg/ml collagenase at 37 °C for 2 h, as described previously (Lania *et al.* 2004). Cells from two GH-PA were incubated with or without octreotide (10 nM) for 6, 24 and 48 h and lysed in the presence of protease inhibitors. On the basis of these experiments and due to limited amounts of cells, four additional tumours were studied at baseline and after 24 h of treatment. For each sample, 20 μg proteins were separated on 12% SDS–polyacrylamide gels and transferred to a nitrocellulose filter. Western blotting experiments were performed with the antibodies used for IHC reported hitherto, using a 1:1000 dilution for the anti-AIP antibody and a 1:500 dilution for the anti-AHR antibody respectively and HRP-linked secondary antibodies for protein detection. GAPDH was used as a housekeeping control. The resulting bands were evaluated with the image analysis program NIH Image J (NIH, Bethesda, MD, USA; http://rsbweb.nih.gov/ij).

Statistical analysis

Data are expressed in median (range) and statistical analyses were performed using Statview 5.01 Software for PC (SAS Institute, Cary, NC, USA). Data were analysed by non-parametric analysis, using the Mann–Whitney *U* and Kruskal–Wallis tests for 2 and ≥3 groups' comparisons respectively. The non-parametric Spearman's test was used to correlate AIP and AHR immunoscores. Results are given by ex-aequo corrections for the non-parametric analysis of immunoscores. Distribution of nominal values was compared by the χ^2 test. Multiple logistic regression was performed to evaluate the influence of preoperative SSA where appropriate. In primary cell culture experiments, the Wilcoxon's rank test was used to compare AIP:GADPH and AHR:GADPH ratios in treated and untreated control cells. $P<0.05$ was considered significant.

Results

Study population

In order to look for potential bias in the selection of patients treated by with SSA before surgery, the characteristics of treated and untreated patients and tumours were retrospectively compared (Table 1). Female patients predominated in both groups. Although treated patients tended to be older than untreated patients, the difference was not significant ($P=0.055$). SSA-treated and -untreated groups had similar proportions of subjects with proven normal *AIP* sequencing (including young patients). Plasma GH, PRL and IGF1 (%ULN) levels at diagnosis were similar in both groups. No significant difference existed between the two groups in terms of tumour volume, invasiveness or functional phenotypes. Gsp^+ tumours were also similarly distributed among treated and untreated patients. The significantly lower Ki67 index observed in treated vs untreated tumours ($P=0.002$) was ascribed to the anti-proliferative effect of SSA on somatotropinomas.

Table 1 Comparison of clinical and genetic characteristics in 62 patients affected by sporadic acromegaly according to the presence or the absence of preoperative treatment with somatostatin analogues

	Untreated	Treated	*P*
Patients (*n*)	25	37	
Gender	9 M/16 F	16 M/21 F	0.568
Age (years)	42 (18–64)	49 (21–78)	0.055
Macroadenomas	21/25 (84.0%)	31/37 (83.8%)	0.982
Suprasellar extension	13/23 (56.5%)	16/36 (44.4%)	0.366
Maximal tumour diameter (mm)	13.0 (8.0–40.0) (18)	15.0 (8.0–40.0) (31)	0.901
Invasive tumours	12/25 (48.0%)	19/37 (51.3%)	0.796
GH at diagnosis (ng/ml)	19.6 (4.0–82.0) (23)	22.4 (4.0–107.0) (33)	0.120
IGF1 at diagnosis (%ULN)	203.6 (51.3–814.0) (21)	255.1 (127.0–636.0) (32)	0.155
PRL at diagnosis (ng/ml)	21.5 (12.7–92.5) (21)	15.0 (3.2–253.2) (32)	0.473
Pituitary hormones IHC			0.326
GH	14/25	21/37	–
GH/PRL	6/25	13/37	–
GH/glycoP	5/25	3/37	–
Ki67 (%)	1.3 (0.0–8.0) (21)	0.8 (0.0–3.5) (33)	0.002
AIP genetics	18/25 (72.0%)	22/37 (59.4%)	0.311
Age <30 years old	4/6 (66.6%)	3/4 (75.0%)	–
Gsp^+	5/16 (31.2%)	9/23 (39.1%)	0.614

Owing to the retrospective character of the study, some data were missing. For continuous variables with missing values, the number of available cases is indicated within parentheses for each item.

General immunohistochemical findings

Examples of AIP and AHR immunostaining in four representative cases and in a NP control are shown in Fig. 1. Some degree of AIP immunostaining was observed in all but two cases (96.8%). Diffuse cytoplasmic immunostaining was the most frequent pattern and low AIP expression was observed in 17 out of 62 cases (27.4%). Preoperative SSA was found to significantly influence AIP expression. Low AIP–IHC was twice as frequent in

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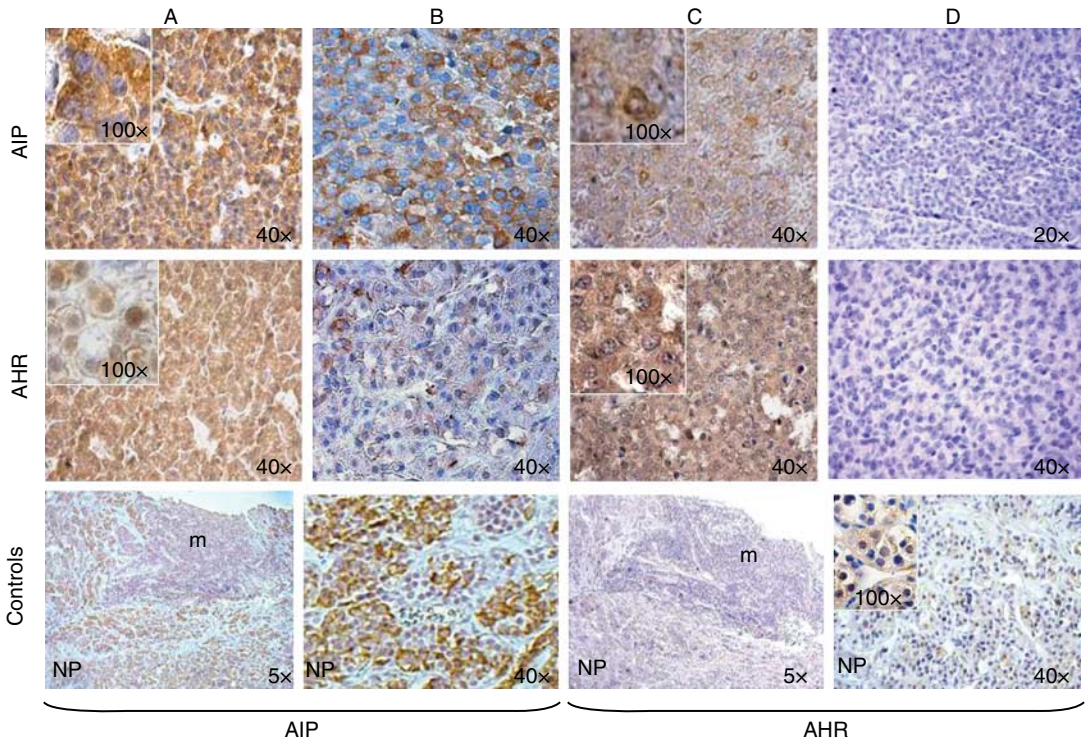

Figure 1

AIP and AHR immunostaining in sporadic GH-secreting adenomas. Immunostaining for AIP (upper panel) in four somatotroph adenomas (A, B, C and D), with the corresponding AHR immunostaining (lower panel). (A) A pure GH-secreting microadenoma treated with SSA before surgery, showing high AIP and AHR expression, with cytoplasmic (AHR*c*) and nuclear AHR (AHR*n*) localisation; (B) a pure, enclosed, GH-secreting, macroadenoma showing patchy AIP and AHR*c* expression; (C) a mixed, invasive, Gsp^+ GH/PRL-secreting macroadenoma treated with SSA before surgery, showing diffuse AIP immunostaining with a subset of highly positive cells and exclusive AHR*c* immunostaining; (D) a huge, invasive, GH-secreting macroadenoma showing negative immunostaining for either AIP or AHR. The lowest panel (controls) shows AIP and AHR immunostaining in the normal pituitary (NP) tissue adjacent to a pituitary microadenoma (m, a microprolactinoma), where both AIP and AHR were barely detectable.

untreated vs SSA-treated GH-PA (44.0 vs 20.5%, $P=0.016$). A similar trend was seen taking only those with proven normal *AIP* status, although the difference did not reach significance (44.4 vs 22.7%, $P=0.140$). Hence, low AIP-expressing GH-PA were characterised in the whole series and in untreated cases taken as a subgroup (Table 2).

Low AIP–IHC was associated with disease aggressiveness. All low AIP–IHC tumours but one were macroadenomas. Suprasellar extension was more frequent in low AIP–IHC vs high AIP–IHC tumours ($P=0.0003$), and this was confirmed in untreated cases ($P=0.019$). Low AIP–IHC tumours also tended to be more invasive than those retaining AIP expression, although the difference reached significance in untreated tumours only ($P=0.028$). A higher Ki67 index was found in invasive vs non-invasive GH-PA in the untreated group only (2.5% (1.0–8.0) vs 1.0% (0.0–3.0), $P=0.043$). Accordingly, a significant negative correlation was found between the AIP score and the Ki67 index in the whole series ($P=0.011$) and in untreated tumours ($P=0.036$), but not in SSA-treated tumours (Table 3). Significantly higher preoperative plasma GH, IGF1 (%ULN) and Ki67 index values were observed in low AIP–IHC vs high AIP–IHC tumours ($P=0.006$, $P=0.022$ and $P=0.019$ vs high AIP–IHC respectively), with similar non-significant trends in the untreated group (Table 2). Introducing preoperative SSA treatment as a covariant for each parameter, a significant negative association was confirmed between low AIP–IHC and higher Ki67 values ($P=0.047$), with a similar trend for higher preoperative plasma GH ($P=0.065$), but not for IGF1.

Some AHR*c* immunostaining was also observed in most tumours with available data (50 out of 53 cases; 94.3%), including 29 cases (54.7%) that had a high AHR*c* score. AHR*n* was detected in nearly half of the studied cases (26 out of 53, 49.1%), of which 18 (34.0%) had a high AHR*n* score. Correlations between AIP and AHR immunoscores are shown in Table 3. Overall, a significant positive correlation was found between the AHR*n* and AHR*c* scores

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Table 2 Factors influencing AIP–IHC in a series of 62 sporadic somatotropinomas and in a subgroup of 25 untreated cases

	All sporadic somatotropinomas ($n=62$)			**Untreated only** ($n=25$)		
	High AIP–IHC	Low AIP–IHC	***P***	High AIP–IHC	Low AIP–IHC	***P***
Patients (*n*)	45 (72.6%)	17 (27.4%)		14 (56.0%)	11 (44.0%)	
Gender	20 M/25 F	5 M/12 F	0.282	6 M/8 F	3 M/8 F	0.420
Age (years)	48 (18.0–78.0)	42 (21.0–59.0)	0.172	42.5 (18.0–64.0)	41.0 (21.0–53.0)	0.460
Macroadenomas	36/45 (77.8%)	16/17 (94.1%)	0.177	11/14 (78.6%)	10/11 (90.9%)	0.404
Maximum diameter (mm)	15.0 (8.0–40.0)	20.5 (8.0–40.0)	0.039	12.0 (8.0–40.0)	22.0 (8.0–37.0)	0.111
Suprasellar extension	15/43 (34.9%)	14/16 (87.5%)	0.0003	4/12 (33.3%)	9/11 (81.8%)	0.019
Invasive tumours	20/45 (44.4%)	11/17 (64.7%)	0.150	4/14 (28.6%)	8/13 (61.5%)	0.028
Preoperative GH (ng/ml)	5.6 (0.6–104.9)	24.0 (1.2–82.0)	0.006	11.9 (3.1–60.5)	25.0 (4.6–32.0)	0.156
Preoperative IGF1 (%ULN)	152.4 (38.5–611.6)	226.0 (75.3–334.0)	0.022	158.8 (51.3–611.6)	221.2 (159.1–283.0)	0.189
Preoperative PRL (ng/ml)	10.6 (3.8–174.0)	15.1 (6.7–80.0)	0.374	20.7 (7.6–92.5)	22.1 (6.7–55.8)	0.850
Hyperprolactinemia ($\geq$30 ng/ml)	12/45 (26.7%)	8/17 (47.1%)	0.125	4/14 (28.6%)	6/11 (54.5%)	0.187
IHC						
GH	27/45	8/17	0.297	9/14	5/11	0.607
GH/PRL	14/45	5/17		3/14	3/11	
Other mixed	4/45	4/17		2/14	3/11	
Ki67 (%)	1.0 (0.0–5.0)	2.0 (0.0–8.0)	0.019	1.1 (0.4–5.0)	2.5 (0.0–8.0)	0.130
Gsp$^+$	11/29 (37.9%)	3/10 (30.0%)	0.652	3/9 (33.3%)	2/7 (28.6%)	0.838
Preoperative SSA treatment	31/45 (68.9%)	6/17 (35.3%)	0.016	–	–	–
Preoperative SSA treatment duration (months)	7.5 (3.0–108.0)	6.0 (4.5–38.0)	0.851	–	–	–

IHC, immunohistochemistry; low and high AIP–IHC, AIP immunostaining score $\leq$2 and $\geq$3 respectively; *Gsp*$^+$, tumours with somatic *Gsp* mutations.

($P=0.004$) whereas AIP correlated significantly with AHR*c* ($P=0.012$) and AHR*t* ($P=0.049$), but not with AHR*n*. Correlations between AIP and AHR approached significance in untreated tumours ($P=0.05$ for AHR*t* and $P=0.077$ for AHR*c*). By contrast, AHR*n* strongly correlated with AHR*c* in treated tumours only ($P=0.0015$).

Searching for factors able to influence AHR content, regardless of its intracellular localisation, we further analysed high AHR–IHC tumours, which accounted for 32.1% of the whole series (17/53). In high AHR–IHC tumours, microadenomas were more frequent (29.4 vs 8.3%, $P=0.045$), suprasellar extension was less frequent (25.0 vs 60.0%, $P=0.02$) and pure GH-secreting PA predominated over mixed secreting GH-PA (76.5 vs 44.4%, $P=0.029$) when compared with low AHR–IHC. By contrast, no significant influence of gender, tumour invasiveness or preoperative hormone profile was observed. The AHR*t* score and the characteristics of high AHR–IHC tumours were not statistically influenced by preoperative SSA treatment (Table 4).

Table 3 Correlations between AIP and the Ki67 and AHR scores in sporadic somatotropinomas

	All cases ($n=53$)		**Untreated** ($n=23$)		**Treated** ($n=30$)	
	P	ρ	*P*	ρ	*P*	ρ
AIP vs Ki67	0.011*	−0.35	0.036*	−0.47	0.362	−0.16
AIP vs AHR*t*	0.048*	0.27	0.050	0.41	0.341	0.18
AIP vs AHR*c*	0.012*	0.35	0.077	0.38	0.101	0.30
AIP vs AHR*n*	0.478	0.10	0.497	0.14	0.667	0.08
AHR*c* vs AHR*n*	0.004*	0.40	0.236	0.25	0.001*	0.59

AHR*t*, total AHR score; AHR*c*, cytoplasmic AHR score; AHR*n*, nuclear AHR score. Results are given for ex-aequo correction.
*Significant *P* values (<0.05).

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Table 4 Characterisation of high AHR–IHC sporadic somatotropinomas

	All sporadic somatotropinomas ($n=53$)			**High AHR*t*–IHC** ($n=17$)		
	High AHR*t*–IHC	Low AHR*t*–IHC	***P***	Untreated	Treated	***P***
Patients (*n*)	17/53 (32.1%)	36/53 (67.9%)		6/23 (26.1%)	11/30 (36.7%)	0.413
Gender	6 M/11 F	15 M/21 F	0.658	3 M/3 F	3 M/8 F	0.345
Age (years)	40 (21.0–52.0)	45 (18.0–78.0)	0.172	36.0 (21.0–51.0)	48.0 (28.0–57.0)	0.145
Macroadenomas	12/17 (70.6%)	33/36 (91.7%)	0.045	4/6 (66.7%)	8/11 (72.7%)	0.793
Maximum diameter (mm)	15.0 (8.0–40.0)	15.0 (8.0–40.0)	0.502	20.0 (9.0–40.0)	15.0 (8.0–31.0)	0.497
Suprasellar extension	4/16 (25.0%)	21/35 (60.0%)	0.020	2/6 (33.3%)	2/10 (20.0%)	0.551
Invasive tumours	8/17 (47.1%)	19/36 (52.8%)	0.697	3/6 (50.0%)	5/11 (45.4%)	0.858
Preoperative GH (ng/ml)	6.9 (1.0–44.6)	8.3 (0.6–104.9)	0.672	15.7 (4.3–29.0)	6.0 (1.0–44.5)	0.075
Preoperative IGF1 (%ULN)	159.1 (52.6–576.0)	182.2.0 (38.5–338.9)	0.561	172.0 (142.9–576.0)	144.8 (52.6–352.0)	0.346
Preoperative PRL (ng/ml)	14.3 (3.8–50.6)	13.2 (4.2–174.0)	0.282	20.0 (9.0–33.0)	8.5 (3.8–56.0)	0.161
Hyperprolactinemia ($\geq$30 ng/ml)	4/17 (23.5%)	14/36 (38.9%)	0.270	2/6 (33.3%)	2/11 (18.2%)	0.488
IHC			0.092			
GH	13/17	16/36		5/6	8/11	0.738
GH/PRL	3/17	15/36		1/6	2/11	
Other mixed	1/17	5/36		0/6	1/11	
Ki67 (%)	0.8 (0.0–6.0)	0.8 (0.0–8.0)	0.887	1.2 (0.5–6.0)	0.5 (0.0–3.1)	0.050
Gsp$^+$	2/9 (22.2%)	11/22 (50.0%)	0.155	0/2 (0.0%)	2/7 (28.6%)	0.91
Preoperative SSA treatment	11/17 (64.7%)	19/36 (52.8%)	0.413	–	–	–
Preoperative SSA treatment duration	6.2 (4.0–23.0)	7.5 (3.0–108.0)	0.381	–	–	–

IHC, immunohistochemistry; low and high AHR*t*–IHC, AHR total score <4 and ≥4 respectively; *Gsp*$^+$, tumours with somatic *Gsp* mutations.

Relationship between AIP/AHR expression and the clinical response to SSA

In order to evaluate the relationship between AIP/AHR expression and the effect of preoperative SSA treatment, treated patients were divided into controlled, partially controlled and uncontrolled based on preoperative IGF1 (%ULN) values. Results are summarised in Table 5.

By definition, preoperative IGF1 (%ULN) significantly differed among the three groups ($P<0.0001$). Differences in disease control were related to the effect of treatment, as plasma GH and IGF1 (%ULN) were similar at diagnosis. Accordingly, ΔIGF1 ($P=0.0004$) significantly differed among the three groups, with a similar but not significant trend for ΔGH ($P=0.082$). Uncontrolled cases had significantly lower ΔIGF1 ($P=0.0007$) and ΔGH ($P=0.031$) than controlled and partially controlled tumours taken together. Tumour shrinkage was reported in a subset of tumours (7/26, 26.7%).

The proportion of low AIP–IHC tumours and the AIP score significantly differed among the three groups ($P=0.006$ and $P=0.014$ respectively). In particular, uncontrolled tumours had a significantly lower AIP expression than controlled and partially controlled tumours taken together (low AIP–IHC in 50 vs 4.3% and median AIP score 2.5 vs 4.0, $P=0.0003$ and $P=0.008$ respectively). High AIP–IHC was observed in all tumours showing preoperative shrinkage, contrasting with low AIP–IHC in 6 out of 19 tumours in the absence of shrinkage (31.6%). Accordingly, the AIP score was significantly higher in the presence (4.5 (3–5)) than in the absence (3.0 (1–5)) of tumour shrinkage ($P=0.014$). In contrast, no significant difference in AHR expression was found that was attributable to the effect of preoperative SSA treatment in terms of IGF1 normalisation or tumour shrinkage.

The relationship between AIP–IHC and the response to SSA was also studied in tumours with proven normal *AIP* sequences. Half had received preoperative SSA (20/40 cases), of which seven showed a partial and seven a complete hormone response (i.e. 14 out of 20 responders), and tumour shrinkage was reported in 2 out of 15 cases. As shown in Fig. 2, the AIP score was significantly higher in the presence of a partial or complete hormone response ($P=0.008$ vs uncontrolled tumours) and in the presence of preoperative hormone shrinkage ($P=0.046$). AHR immunostaining was

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Table 5 Relationship between AIP/AHR expression and the preoperative response to somatostatin analogues in 33 sporadic somatotropinomas

	Controlled	Partially controlled	Uncontrolled	P_1[a]	P_2[a]
Patients (*n*)	10	13	10		
Gender	3 M/7 F	7 M/6 F	5 M/5 F	0.493	0.730
Age (years)	52.0 (30.0–72.0)	38.0 (21.0–61.0)	44.0 (26.0–78.0)	0.411	0.891
Macroadenomas	8/10 (80%)	10/13 (76.9%)	10/10 (100%)	0.272	0.109
Suprasellar extension	4/10 (40%)	6/13 (46.1%)	6/10 (60%)	0.478	0.238
Invasive	5/10 (50%)	7/13 (53.8%)	6/10 (60%)	0.902	0.678
GH at diagnosis (ng/ml)	13.0 (4.0–57.9)	44.0 (6.9–107.0)	24.1 (4.8–75.0)	0.234	0.827
IGF1 at diagnosis (%ULN)	269.5 (127.0–636.0)	279.0 (158.0–418.6)	239.4 (187.0–393.9)	0.775	0.580
Pretreatment duration (months)	10.0 (4.0–108.0)	5.0 (3.0–21.0)	6.0 (4.5–23.0)	0.303	0.887
Preoperative GH (ng/ml)	3.2 (0.6–7.0)	5.3 (0.6–55.0)	19.0 (1.1–104.9)	0.053	0.028
Preoperative IGF1 (%ULN)	75.3 (38.5–104.0)	165.6 (131.0–278.0)	226.1 (144.8–352.0)	<0.0001	<0.0001
ΔGH (%)	−75.6 (−97.9; −46.1)	−65.6 (−97.1; −2.0)	−37.9 (−98.6; +66.7)	0.082	0.031
ΔIGF1 (%)	−66.5 (−88.6; −29.8)	−35.6 (−76.0; −29.6)	−27.9 (−13.1; +38.2)	0.0004	0.0007
Preoperative tumour shrinkage	3/8 (37.5%)	2/7 (28.6%)	1/10 (10%)	0.376	0.181
Ki67 (%)	0.5 (0.0–1.0)	0.3 (0.0–3.0)	0.8 (0.0–3.5)	0.408	0.212
High AIP–IHC	9/10 (90%)	13/13 (100%)	5/10 (50%)	0.006	0.002
AIP score	4.0 (1.0–6.0)	4.0 (3.0–5.0)	2.5 (1.0–5.0)	0.014	0.008
High AHR–IHC	4/10 (40.0%)	2/8 (25.0%)	4/10 (40.0%)	0.756	0.724
AHR*t* score	2.5 (1.0–6.0)	2.0 (1.0–4.0)	3.0 (1.0–6.0)	0.747	0.588
AHR*c* score	2.0 (1.0–3.0)	2.0 (1.0–3.0)	2.0 (1.0–3.0)	0.627	0.757
AHR*n* score	0.0 (0.0–3.0)	0.0 (0.0–2.0)	1.0 (0.0–3.0)	0.642	0.203

P_1, three groups' comparison by Kruskal–Wallis test; P_2, two groups' comparison (controlled and partially controlled taken together vs uncontrolled) by Mann–Whitney *U* test.
[a]For the comparison of immunoscores, results are given for ex-aequo correction.

unrelated to the outcome of treatment, although a trend towards a higher AHR*c* score in controlled and partially controlled tumours was observed when compared with uncontrolled tumours ($P=0.07$) (data not shown).

In vitro effect of octreotide on GH-PA

In order to search for a direct effect of octreotide on AIP and AHR expression in GH-PA, primary cultures from six GH-PA – one Gsp^+ and five Gsp^- – were treated with 10 nM octreotide *in vitro*. Data are illustrated in Fig. 3. Two GH-PA (both Gsp^-) were suitable for time-dependent experiments, showing a significant increase in AIP expression after 24 h of treatment. The remaining tumours were studied at baseline and after 24 h of treatment. Measurable levels of AIP were detected in all tumours, whereas in two cases, no reliable quantification of AHR could be obtained. A variable but significant increase in AIP expression was observed in all cases (median 2.03-fold; range 1.2–13.9, $P=0.027$), whereas AHR expression was increased in a single Gsp^- tumour only (median 1.2-fold, range 1.0–2.8, *P*, NS). The Gsp^+ tumour showed a 2.9-fold increase in AIP expression and no increase in AHR.

Influence of *Gsp* mutations on AIP and AHR expression

The characteristics of Gsp^+ and Gsp^- somatotropinomas are summarised in Table 6. No significant difference was observed at diagnosis between the two groups. Among tumours treated with SSA preoperatively (9 Gsp^+ and 14 Gsp^-), Gsp^+ tumours tended to be associated with a higher reduction in plasma GH values and a higher rate of shrinkage before surgery ($P=0.09$ vs Gsp^- for both parameters). Significantly, lower Ki67 values were also observed in Gsp^+ when compared with Gsp^- tumours in SSA-treated cases ($P=0.006$).

No significant effect of *Gsp* status was observed on AIP expression. In particular, as observed in unselected GH-PA, a lower AIP score was observed in Gsp^+ tumours in the presence of a suprasellar extension ($P=0.034$ vs intrasellar Gsp^+ tumours, data nor shown). The only effect of *Gsp* status was a significantly lower AHR*n* score in Gsp^+ tumours ($P=0.025$ vs Gsp^- tumours), suggesting cytoplasmic retention of AHR in Gsp^+ GH-PA. This finding was confirmed in the presence of preoperative SSA treatment ($P=0.031$ vs treated Gsp^- tumours). In contrast, preoperative SSA treatment was associated with a significant increase in AHR expression in Gsp^- tumours ($P=0.013$ for

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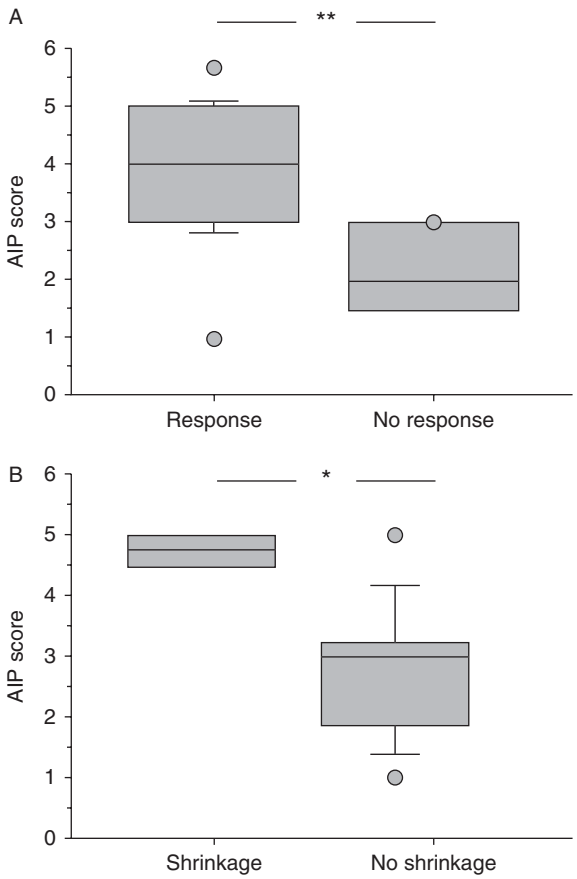

Figure 2
Variations in the AIP immunoscore according to the preoperative response to somatostatin analogues in 20 sporadic somatotropinomas (normal AIP sequence subgroup). The AIP score was significantly higher in the presence of a partial or complete hormone response (**P=0.008, panel A) and in the presence of preoperative shrinkage (*P=0.046, panel B).

AHR*c* and P=0.037 for AHR*t* vs untreated *Gsp*$^-$ tumours respectively), with no significant change in its nuclear localisation.

Discussion

This extensive series of tumours from patients with sporadic acromegaly provides substantial new information on the effects of SSA on the expression of AIP and its best characterised molecular partner, AHR. In addition, it evaluates for the first time the influence of somatic *Gsp* mutations on such parameters.

Data obtained across the whole series are consistent with previous reports showing AIP down-regulation in aggressive somatotropinomas (Jaffrain-Rea *et al.* 2009, Kasuki Jomori de Pinho *et al.* 2011). However, tumour volume and proliferative activity were found to have a stronger impact on AIP expression than invasive features. AIP loss was significantly associated with suprasellar extension and higher Ki67 values regardless of preoperative SSA treatment, but it was significantly associated with invasiveness in untreated cases only. This indicates that preoperative pharmacological treatment introduces an important limitation in the use of AIP immunostaining as a marker of invasiveness in somatotropinomas. This is similar to the effect of SSA pretreatment on the Ki67

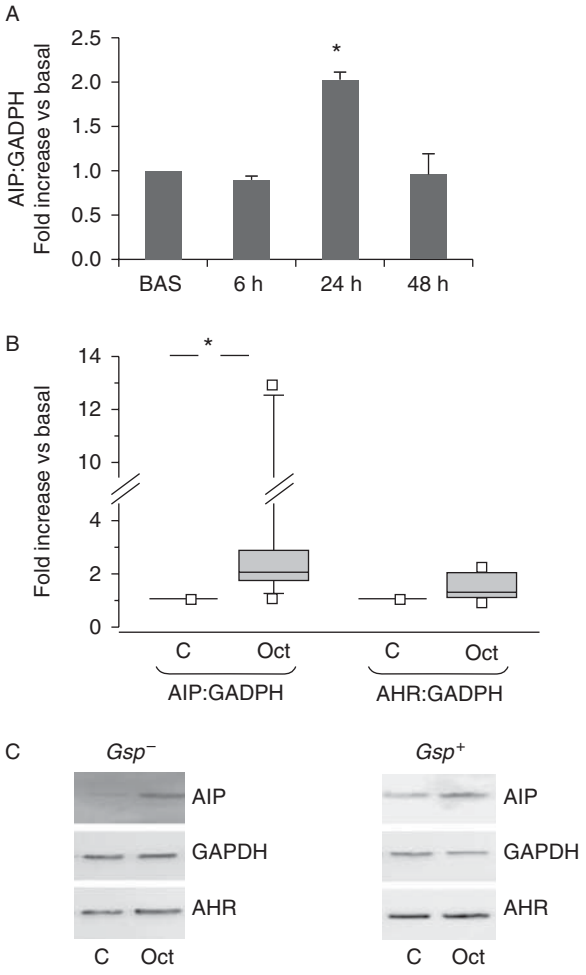

Figure 3
In vitro effect of octreotide treatment on AIP and AHR expression for GH-secreting adenomas. Western blotting experiments were performed after protein extraction from primary cultures of six GH-secreting adenomas, incubated in the presence or in the absence of 10 nM octreotide. AIP:GADPH and AHR:GADPH ratios were obtained by densitometry of the relative bands. (A) A maximal increase in AIP expression was observed at 24 h in two GH-PA treated for up to 48 h (P=0.002 vs basal expression). (B) Shown are pooled data obtained for GH-PA after 24 h of octreotide treatment for AIP (right panel, n=6) and AHR (left panel, n=4) protein expression. Overall, a significant increase in AIP, but not in AHR, expression was observed (P=0.027). (C) Examples of western blotting results obtained at 24 h for *Gsp*$^-$ (right panel) and for *Gsp*$^+$ (left panel) tumours. A significant increase in AIP, but not in AHR, was present in both cases.

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Table 6 Clinical characteristics and AIP/AHR expression in 39 sporadic somatotroph adenomas with or without *Gsp* mutations

	Gsp$^+$	*Gsp*$^-$	*P*[a]
Tumours (*n*)	14	25	
Gender	6 M/8 F	9 M/16 F	0.673
Age (years)	51.5 (30.0–72.0)	45.0 (21.0–61.0)	0.147
Macroadenomas	13/14 (92.9%)	19/25 (76.0%)	0.188
Suprasellar extension	5/13 (38.5%)	13/23 (56.5%)	0.298
Maximum diameter (mm)	14.0 (8.0–30.0) (10)	15.0 (8.0–40.0) (20)	0.680
Invasive	5/14 (35.7%)	13/25 (52.0%)	0.328
GH at diagnosis	22.5 (4.0–56.9) (14)	13.5 (4.0–107.0) (20)	0.916
IGF1 at diagnosis (%ULN)	239.9 (51.3–678.0) (13)	283.5 (96.1–814.0) (20)	0.127
PRL at diagnosis	20.1 (6.7–152.8) (12)	22.0 (3.2–66.0) (20)	0.951
Preoperative SSA	9/14 (64.3%)	14/25 (56.0%)	0.614
ΔGH (%)[b]	−77.6 (−95.2; −30.6) (8)	−56.0 (−94.4; −2.0) (12)	0.093
ΔIGF1 (%)[b]	−29.9 (−84.5; −25.7) (6)	−43.4 (−79.8; −20.0) (10)	0.605
Ki67 (%)			
All	0.5 (0.0–3.0) (11)	1.2 (0.0–5.0) (22)	0.061
Treated	0.0 (0.0–0.5) (7)	1.2 (0.0–3.1) (13)	0.006
Untreated	2.3 (1.0–3.0) (4)	1.3 (0.0–5.0) (9)	0.589
Low AIP–IHC			
All	3/14	7/25	0.652
Treated	1/9	2/14	0.825
Untreated	2/5	5/11	0.838
AIP score			
All	4.0 (2.0–5.0)	4.0 (0.0–6.0)	0.765
Treated	4.0 (2.0–5.0)	4.0 (2.0–6.0)	0.897
Untreated	3.0 (2.0–5.0)	3.0 (0.0–5.0)	0.645
AHR*t* score			
All	2.0 (0.0–5.0)	2.5 (0.0–6.0)	0.259
Treated	2.0 (1.0–5.0)	4.0 (2.0–6.0)*	0.095
Untreated	2.0 (0.0–2.0)	2.0 (0.0–4.0)	0.517
AHR*c* score			
All	2.0 (0.0–3.0)	2.0 (0.0–3.0)	0.415
Treated	2.0 (1.0–3.0)	2.0 (1.0–3.0)*	0.999
Untreated	1.5 (0.0–2.0)	1.0 (0.0–2.0)	0.705
AHR*n* score			
All	0.0 (0.0–2.0)	2.0 (0.0–3.0)	0.025
Treated	0.0 (0.0–2.0)	2.0 (0.0–3.0)	0.031
Untreated	0.0 (0.0–1.0)	1.0 (0.0–2.0)	0.227

SSA, somatostatin analogues; *Gsp*$^+$, Gsp mutation; *Gsp*$^-$, no Gsp mutation.
*$P<0.05$ vs untreated *Gsp*$^-$ adenomas.
[a]For the comparison of immunoscores, all results are given for ex-aequo correction.
[b]Treated cases only.

index, which can be significantly reduced by the anti-proliferative effects of SSA in GH-PA (Losa *et al.* 2001, Jaffrain-Rea *et al.* 2002, this study). Overall, low AIP expression was also associated with higher preoperative GH/IGF1 levels. Although this could, in part, be due to the lower proportion of low AIP–IHC tumours treated by SSA preoperatively, a trend towards higher preoperative GH levels in low AIP–IHC tumours was seen after correction for preoperative treatment on the whole series and in untreated tumours. Taken together, these findings indicate that low AIP–IHC tumours share common characteristics with *AIP*^{mut} GH-PA (Daly *et al.* 2010). Notably, the higher percentage of GH/PRL adenomas reported in *AIP*^{mut} PA has not been observed among low AIP–IHC somatotropinomas (Kasuki *et al.* 2012, this study). The mechanisms of *AIP* down-regulation in non-*AIP*^{mut} somatotropinomas have been poorly investigated, but overexpression of the miR-107 has been recently proposed (Trivellin *et al.* 2012).

Dysregulation of AHR expression has been reported in a number of tumours (Harper *et al.* 2006, Dietrich & Kaina 2010) and reduced AHR (Jaffrain-Rea *et al.* 2009) and ARNT (Heliövaara *et al.* 2009, Raitila *et al.* 2010) expression have been observed in *AIP*^{mut} PA. In this study, only 32% of sporadic GH-PA displayed a high AHR content. We found these tumours to be smaller and to include a higher proportion of pure GH-secreting PA than those displaying a low AHR content, suggesting that AHR down-regulation may also occur during the evolution of sporadic GH-PA. A significant correlation was found between AIP and AHR*c*, further supporting a role for AIP in the stabilisation of AHR in PA (Jaffrain-Rea *et al.* 2009). This may be attenuated by preoperative SSA treatment, potentially suggesting that the stability of the AIP/AHR complex may be influenced by SSA or that SSA differentially affect AIP and AHR expression in GH-PA. By contrast, despite AHR signalling being potentially enhanced by AIP (Petrulis & Perdrew 2002), AHR*n* was unrelated to AIP, irrespective of SSA treatment. Thus, the potential influence of AIP on AHR signalling in sporadic GH-PA remains unclear.

Data from a large series of sporadic GH-PA with proven normal *AIP* sequences in the majority of cases support recent evidence that AIP expression is increased by preoperative SSA treatment (Jaffrain-Rea *et al.* 2010, Chahal *et al.* 2012). Notably, similar results were obtained whether lanreotide (Jaffrain-Rea *et al.* 2010, Chahal *et al.* 2012, this study) or octreotide (Jaffrain-Rea *et al.* 2010, this study) was used for the preoperative treatment of acromegalic patients. In contrast, no change in *AIP* expression was observed in two *AIP*^{mut} cases operated before and after treatment (Jaffrain-Rea *et al.* 2010). Supporting recent findings in GH_3 cells (Chahal *et al.* 2012), this study provides the first evidence for a variable but significant up-regulation of AIP expression by octreotide in human GH-PA *in vitro*, with a median twofold increase. A significant relationship was

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also found between AIP immunostaining and the outcome of pre-surgical SSA treatment, both in terms of hormonal control and tumour shrinkage, further indicating AIP to be an important mediator of SSA in GH-PA. Indeed, the AIP score was significantly lower in uncontrolled tumours when compared with partially and fully controlled tumours and significantly higher in the presence of preoperative shrinkage. In particular, none of the tumours showing preoperative shrinkage had low AIP immunostaining, contrasting with 30% of those showing no effect of SSA on tumour volume. These results echo those obtained in a large series of *AIP*^{mut} acromegalic patients, in which the median shrinkage with SSA was highly statistically significantly lower than in control acromegalic patients (0 vs 41%, $P<0.000001$; Daly *et al*. 2010). In this study, no difference was found according to the degree of disease control obtained at the time of surgery (IGF1 normalisation or reduction by $\geq$30% compared with baseline values). Owing to the retrospective and multicentre characteristics of our study, we might have been unable to distinguish between these two groups. Preoperative SSA treatment was not standardised, so that differences in the degree of response obtained before surgery did not necessarily reflect differences in SSA sensitivity. Additional factors may also be involved in the modulation of AIP by SSA. Chahal *et al*. (2012) found a positive correlation between AIP expression and IGF1 changes in female acromegalics only. A similar non-significant trend was present in our series (data not shown), but the higher proportion of female cases could have introduced some bias and no gender specificity could be shown. However, the unusual male predominance in *AIP*^{mut} somatotropinomas (Daly *et al*. 2010) and reported interactions between AIP, AHR and steroid receptors (Beischlag *et al*. 2008), in particular oestrogen receptor α (Matthews & Gustafsson 2006, Cai *et al*. 2011), suggest potential variations in AIP expression or function according to patient's gender and the steroid milieu, which deserve further investigation.

Because the cyclin kinase inhibitor p27^{Kip1} can be induced by SSA (Ferrante *et al*. 2006) and by AHR signalling (Marlowe & Puga 2005), we proposed the hypothesis that AHR itself could be a target of SSA in GH-PA. However, no significant difference in AHR content or in its nuclear localisation was observed between SSA-treated vs -untreated tumours, or according to the effect of treatment. Although there was some evidence that AHR*c* immunostaining could be higher in SSA-treated tumours, this was also unrelated to the effect of treatment and might be due to the higher AIP expression observed in treated GH-PA. Unexpectedly, a strong correlation was found between AHR*n* and AHR*c* in treated tumours. This may reflect the complex regulation of nucleocytoplasmic shuttling of AHR, which involves several exogenous and endogenous activators (Beischlag *et al*. 2008, Nguyen & Bradfield 2008). Overall, these data argue against a significant role for AHR in the pharmacological response to SSA in GH-PA.

Most of the pharmacological effects of octreotide and lanreotide on GH-PA are mediated by somatostatin receptor (SSTR) subtypes 2 and 5 (Ben-Shlomo & Melmed 2010). AIP immunostaining was identified as a predictive factor of post-operative SSA sensitivity independent of SSTR2 (Kasuki *et al*. 2012) and a slightly higher SSTR5 expression was observed in *AIP*^{mut} tumours (Chahal *et al*. 2012), suggesting that AIP-related differences in SSA sensitivity are not due to important changes in SSTRs. Potential variations in the expression of receptors reported to negatively affect the pharmacological response to SSA, such as truncated variants of SSTR5 (Durán-Prado *et al*. 2010, 2012) or the dopamine receptor D2R (Zatelli *et al*. 2005), have not been reported yet. An alternative molecular link between AIP expression and SSA signalling is *ZAC1*, a tumour suppressor gene involved in the anti-proliferative action of octreotide (Theodoropoulou *et al*. 2006), whose expression correlates with tumour shrinkage and IGF1 changes in treated GH-PA (Theodoropoulou *et al*. 2009). *ZAC1* has been recently proposed to act downstream of AIP in GH_3 cells (Chahal *et al*. 2012). However, potential correlations between AIP and *ZAC1* expression in human GH-PA remain to be further explored.

A relationship between AIP, AHR and cAMP signalling, the best characterised pathway in GH-PA and an important target of SSA, is suggested by experimental evidence indicating that i) cAMP is a non-ligand activator of AHR able to induce transcriptional responses different from exogenous ligands (Oesch-Bartlomowicz *et al*. 2005), ii) nucleocytoplasmic shuttling of AHR induced by cAMP is inhibited by PDE2, which is stabilised by AIP (de Oliveira *et al*. 2007) and iii) AIP interactions with PDE4A5 can be disrupted by *AIP* mutations (Leontiou *et al*. 2008). There is also very recent evidence that AIP reduces forskolin-induced cAMP signalling in GH_3 cells, although forskolin did not influence AIP expression (Formosa *et al*. 2013). However, intracellular cAMP concentrations have not been reported in the presence of *AIP* abnormalities in GH-PA and the potential effect of cAMP signalling on AIP expression in these tumours is unknown. Because *Gsp* mutations induce a constitutive activation of the cAMP/PKA pathway, we studied AIP and AHR expression

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according to *Gsp* status. In agreement with most reports, no specific phenotype was observed in Gsp^+ tumours, although they tended to respond better than Gsp^- tumours to preoperative SSA treatment (Barlier *et al.* 1998, Lania *et al.* 2003). Accordingly, treated Gsp^+ tumours had significantly lower Ki67 than their Gsp^- counterpart. AIP expression was similar in Gsp^+ and Gsp^- tumours. Because only large Gsp^+ tumours had a low AIP expression, down-regulation of AIP appears as a late event in Gsp^+-related tumorigenesis. Notably, no *Gsp* mutations were found in a small series of AIP^{mut} GH-PA, further suggesting that these are independent pathogenetic events (Angelini *et al.* 2010). Preoperative SSA treatment had no differential effect on AIP expression in Gsp^+ and Gsp^- tumours, and the effect of octreotide on AIP expression was unremarkable in the Gsp^+ tumour studied *in vitro*. In contrast, some effect of *Gsp* status could be found on AHR expression and localisation. In Gsp^- tumours, preoperative SSA was associated with a significantly higher AHR content and AHR was increased by octreotide in one of three Gsp^- tumours studied *in vitro*. Therefore, SSA may induce AHR in a subset of Gsp^- GH-PA. Unexpectedly, AHR*n* expression was lower in Gsp^+ vs Gsp^- GH-PA, regardless of AHR content, suggesting cytoplasmic retention of AHR in Gsp^+ tumours. A potential explanation is that Gsp^+ tumours activate regulatory mechanisms that operate to counteract the increase in cAMP concentration (Lania *et al.* 2003, Pertuit *et al.* 2009), among which is an increased PDE activity, which might in turn inhibit AHR*n* shuttling. In addition, cAMP signalling involves different subcellular compartments, which may differentially regulate endocrine functions and AHR activation in somatototroph cells. Indeed, cytoplasmic retention of AHR was also observed in treated Gsp^+ tumours. The biological implications of these findings remain to be further investigated.

In conclusion, this study further supports a role for AIP down-regulation in the pathogenesis of sporadic acromegaly, with low AIP-expressing tumours sharing phenotypic features with AIP^{mut} somatotropinomas. Overall, AIP down-regulation is associated with a reduced AHR*c* expression, with no significant effect on its nuclear localisation. AHR, but not AIP, appears to be differentially regulated according to *Gsp* status. In contrast, AIP, but not AHR, is an important mediator of SSA in GH-PA.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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