Pasireotide responsiveness in acromegaly is mainly driven by somatostatin receptor subtype 2 expression

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Published in:

The Journal of clinical endocrinology and metabolism. 2019;104:915-924.

ABSTRACT

Background: The response to first-generation somatostatin receptor ligands (SRLs) treatment in acromegaly correlates with expression of somatostatin receptor subtype 2 (SST_2). However, pasireotide shows the highest binding affinity for SST_5. It has been suggested that in acromegaly SST_5 expression is better at predicting the response to pasireotide LAR (PAS-LAR) treatment than SST_2 expression.

Aim: To investigate in active acromegaly patients whether response to SRL treatment correlates to PAS-LAR treatment, and to what extent SST_2 and SST_5 expression are correlated to response to PAS-LAR treatment.

Methods: We included 52 patients from a cohort that initially received SRL treatment, followed by SRL and pegvisomant combination treatment, and finally PAS-LAR treatment. The long-term response to PAS-LAR was evaluated using a PAS-LAR score. In 14 out of 52 patients, somatotroph adenoma tissue samples were available to evaluate SST_2 and SST_5 expression using a previously validated immunoreactivity score (IRS).

Results: The percentage IGF-I (x ULN) reduction which was observed after SRL treatment correlated with PAS-LAR response score during follow-up ($r = 0.40$, $P = 0.003$, $n = 52$). After exclusion of SRL pretreated patients, SST_2 IRS was positively correlated to PAS-LAR score ($r = 0.58$, $P = 0.039$, $n = 9$), while SST_5 IRS showed no relation ($r = 0.35$, $P = 0.36$, $n = 9$).

Conclusions: In a cohort of patients partially responsive to SRLs, the IGF-I lowering effects of PAS-LAR treatment correlated with the effect of SRLs treatment and seemed to be mainly driven by SST_2 expression instead of SST_5.

INTRODUCTION

Acromegaly is a severe systemic condition most commonly caused by a somatotroph adenoma that secretes excessive levels of growth hormone (GH) and insulin-like growth factor I (IGF-I), leading to increased mortality and morbidity (1). Treatment modalities that normalize GH and IGF-I levels restore normal life expectancy (2). This goal can be achieved pharmacologically, both by inhibiting pituitary GH secretion and blocking peripheral GH action.

First-generation long-acting somatostatin receptor ligands (SRLs, octreotide and lanreotide) represent the cornerstone for medical treatment of acromegaly. The biochemical response to SRL treatment has been consistently shown to be positively correlated to somatostatin receptor subtype 2 (SST_2) protein expression on the adenoma (3-7).These compounds inhibit pituitary GH secretion by preferential binding with high affinity to SST_2. However, biochemical normalization of GH and IGF-I can only be achieved in about 40% of patients. Therefore, the majority of patients are partially or even completely resistant to SRLs. An effective treatment option to normalize IGF-I levels in partially resistant patients is the addition of the GH receptor antagonist PEGV to SRLs. A recent study from our group showed that patients using SRLs and PEGV combination treatment had a lower SST_2 expression at time of surgery compared with medically naïve patients (8). The required PEGV dose to achieve IGF-I normalization was inversely correlated to SST_2 expression, but not to SST_5 expression (8).

Pasireotide long-acting release (PAS-LAR) is a novel multireceptor somatostatin analogue, which binds with high affinity to all SSTR subtypes but SST_4. In contrast to octreotide, pasireotide shows high subnanomolar affinity to SST_5 (9). In vitro studies have shown that pasireotide modulates somatostatin receptor trafficking and phosphorylation in a distinct manner from octreotide (10, 11), inducing less SST_2 internalization, phosphorylation and ß-arrestin recruitment than octreotide. In medically naïve acromegaly patients PAS-LAR has demonstrated superior efficacy in reducing IGF-I levels over octreotide LAR, while the effect on GH reduction was superimposable (12). This latter observation was also recently confirmed *in vitro* (13). We recently reported the 24-weeks results of the Pasireotide LAR and Pegvisomant (PAPE) study (14). This prospective open-label conversion study assessed the efficacy and safety of PAS-LAR alone or in combination with PEGV in acromegaly patients controlled with SRLs and PEGV combination treatment (14). Switching to PAS-LAR resulted in a significant PEGV dose reduction, but also a higher incidence of diabetes mellitus (14).

It is assumed that the efficacy of a given somatostatin receptor ligand is directly correlated to the SSTR subtype binding profile and the pattern of SSTR expression in the somatotroph adenoma (5, 6). However, although guidelines do not report specific recommendations so far, it is generally assumed that octreotide and lanreotide are more effective when SST_2 is highly and predominantly expressed, while pasireotide is more effective when SST_5 is the predominant subtype and SST_2 is absent or poorly expressed (15). The aims of the present study were therefore: 1) to investigate whether the IGF-I response after SRL treatment correlates to the IGF-I response after PAS-LAR treatment; 2) to investigate to what extent SST_2 and SST_5 immunoreactivity are correlated to responsiveness to PAS-LAR treatment in somatotroph adenomas.

MATERIALS AND METHODS

Patients and somatotroph adenoma tissue selection

Data collection of acromegaly patients was performed at the Erasmus MC Pituitary Center in Rotterdam. We initially started with a cohort of 61 acromegaly patients who received PAS-LAR treatment during their participation in the PAPE study (Figure 1) (14).

All these patients have previously been treated with SRLs, followed by SRL and PEGV combination therapy. Cabergoline was used in 7 patients in combination with SRLs, and in two patients during the PAPE study (14). After exclusion of patients that received postoperative radiotherapy (n = 7), and patients that received SRL treatment less than 4 months (n = 2), 52 patients remained and were finally included in the study cohort. In total 19 out of these 52 patients previously underwent neurosurgery. Reasons for surgery included adenomas with reasonable chance for cure such as (intrasellar) microadenomas, or macroadenomas with risk of visual impairment.

We selected only those patients with sufficient adenoma tissue available to perform immunohistochemistry (IHC). One patient underwent a second surgery during follow-up. For clarity, in this latter case we analyzed only the tissue sample of the first surgery. From the 14 remaining somatotroph adenoma tissue samples included for IHC analysis (SSTR subcohort), 10 tissue samples have been stained previously (8), while 4 cases were newly stained (figure 1). We retrospectively collected data on medical history and clinical response to first-generation SRLs. Prospective data on the PEGV dose and IGF-I levels were used from the PAPE study (14). The PAPE study was registered with ClinicalTrials.gov, number NCT02668172. All patients were included after written informed consent.

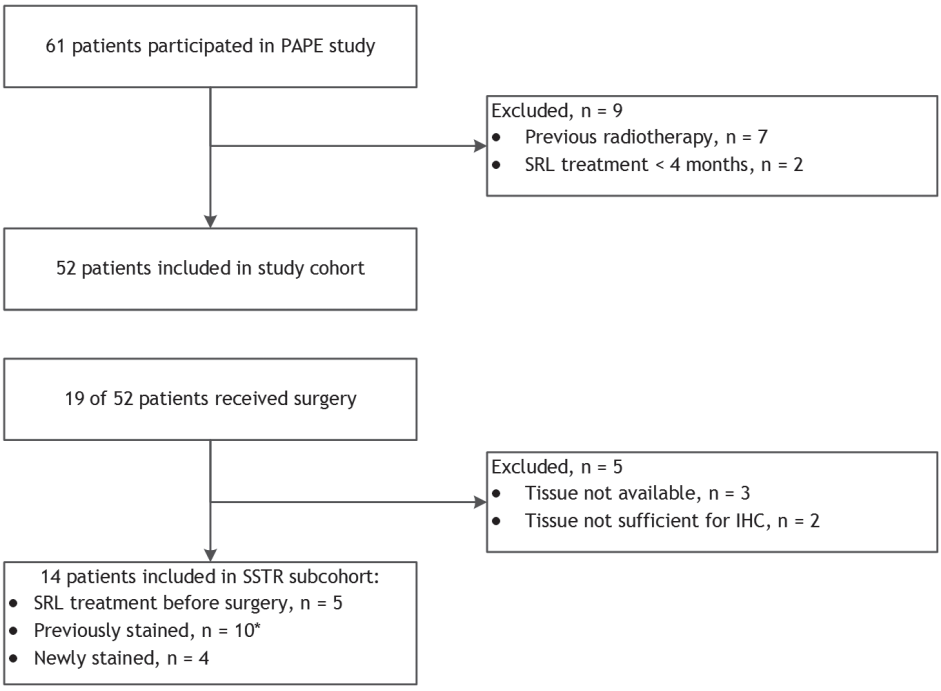

Figure 1. Flowchart of the selection procedure for the study cohort and the somatotroph adenoma tissue samples included in the SSTR subcohort.
All patients eventually received SRL and PEGV combination treatment, and were switched to pasireotide LAR treatment during the PAPE study (14). SRL = first-generation long-acting somatostatin receptor ligands; PEGV = pegvisomant; PAPE = pasireotide LAR and pegvisomant study; SSTR = somatostatin receptor subtype; IHC = immunohistochemistry.

Outcomes

Response to SRL treatment was defined as IGF-I x age-adjusted upper limit of normal (IGF-I x ULN), and as percentage of IGF-I suppression after at least 4 months SRL treatment. In patients that underwent surgery, post-operative IGF-I levels after at least 3 months were considered. Response to PAS-LAR during the PAPE study was divided into short-term and long-term response. Short-term treatment response was defined as IGF-I levels (x ULN) at 24 weeks (i.e. after 3 injections of PAS-LAR 60 mg). During the extension phase from 24 until 48 weeks, both the PAS-LAR dose and PEGV dose were titrated according to a protocol to achieve IGF-I levels within the normal range. Therefore, the long-term response to PAS-LAR was based on a composite "PAS-LAR treatment response score" (PAS-LAR score) in order to fully capture the effect of PAS-LAR taking into account PEGV dose reduction, discontinuation and eventually PAS-LAR dose reduction. The PAS-LAR score comprised five categories representing the difference in PEGV dose and PAS-LAR dose at week 48 versus baseline (week number 0): 0 = PEGV dose reduction 0-33% (in combination with PAS-LAR 60 mg), 1 = PEGV dose

reduction 33-66% (+ PAS-LAR 60 mg), 2 = PEGV dose reduction 66-100% (+ PAS-LAR 60 mg), 3 = PEGV treatment discontinued and PAS-LAR dose reduced to 40 mg, 4 = PEGV treatment discontinued and PAS-LAR dose reduced to 20 mg every 4 weeks. 100% PEGV dose reduction corresponds to PAS-LAR 60 mg monotherapy. Higher PAS-LAR score corresponds to a better response to PAS-LAR treatment.

IGF-I assays

Total IGF-I serum concentrations during the PAPE study were measured by the immunometric IDS-iSYS assay (Immunodiagnostic Systems Limited; Boldon, United Kingdom; intraassay coefficient of variation (CV) 8.1%, interassay CV 2.1%) (16). Total IGF-I serum concentrations before and after SRL treatment were measured using different assays: Immulite 2000 assay, a solid-phase, validated enzyme-labelled chemiluminescent immunometric assay (DPC Biermann GmbH/Siemens, Fernwald, Germany; intraassay variability of 2–5%, interassay variability of 3–7%), the immunometric IDS-iSYS assay (Immunodiagnostic Systems Limited; Boldon, United Kingdom; intraassay coefficient of variation (CV) 8.1%, interassay CV 2.1%), and two different radioimmunoassays (Diagnostic Systems Laboratories, Webster, Tex., USA, intraassay CV 3.9%, interassay CV 4.2%), and Medgenix Diagnostics, Fleurus, Belgium; intraassay coefficient of variation (CV) 6.1%, interassay CV 9.9%). Total IGF-I was interpreted according to the sex and age-dependent ranges used in accordance with previous reports (17, 18). Because different IGF-I assays were used over time, IGF-I levels were expressed as upper limits of normal (ULN), and not as the absolute values.

Immunohistochemistry

Somatotroph adenoma tissues were stained for hematoxylin and immunostained for SST_2 and SST_5. Formalin-fixed paraffin-embedded tumor samples were cut into sequential 4-µm-thick sections and deparaffinized and stained using a fully automated Ventana BenchMark ULTRA stainer (ref: 790-2208, Ventana, Tucson, Ariz., USA) according to the manufacturers' instructions at the Pathology Department. Binding of peroxidase-coupled antibodies was detected using 3,39-diaminobenzidine as a substrate, and the sections were counterstained with hematoxylin. The rabbit monoclonal anti-SSTR2 antibody clone UMB-1 (SS-8000, BioTrend, Köln, Germany) was used at a dilution of 1:50, and the rabbit monoclonal anti-SSTR5 antibody clone UMB-4 at a dilution of 1:400 (ab109495, Abcam, Cambridge, UK). Normal pancreatic tissue served as a positive control for both SST_2 and SST_5 staining. For negative controls, the primary antibody was omitted. Immunostaining of the adenomas was scored semiquantitatively based on an immunoreactivity scoring system (IRS). The IRS is calculated by the product of the percentage of positive stained cells (0: 0%; 1: <10%; 2: 10–50%; 3: 51–80%; 4: 80%) and the staining intensity (0: no staining; 1: weak staining;

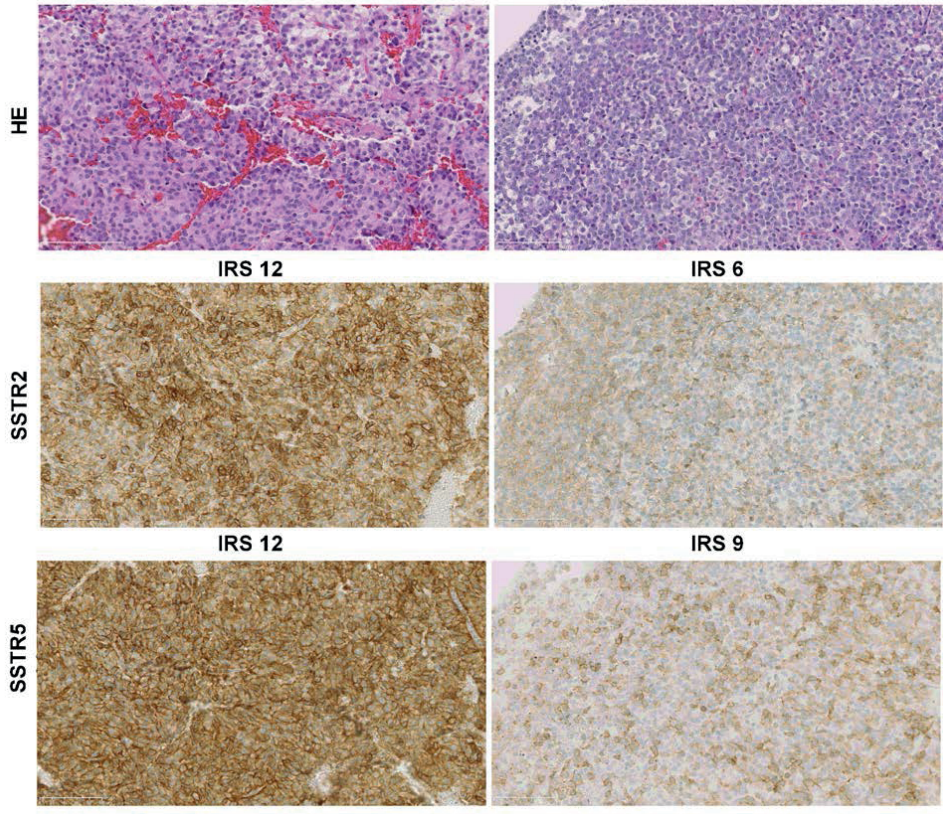

Figure 2. SST_2 and SST_5 protein expression of somatotroph adenomas scored by the immunoreactivity score (IRS).
HE = Haematoxylin and eosin; SSTR2 = somatostatin receptor subtype 2; SSTR5 = somatostatin receptor subtype 5, IRS = immunoreactivity score.

2: moderate staining; 3: strong staining) (19). The IRS ranges between 0 and 12. The newly stained somatotroph adenoma tissue samples were scored by two independent investigators (A.M. and E.C.) based on the histopathological description of the sample provided by the pathologist. Both investigators were blinded to each other's findings and the patients' characteristics. Figure 2 shows two representative cases.

Statistical methods

Categorical data were represented as observed frequencies and percentages. Continuous data were represented as mean and 95% confidence interval (CI) or median and range. The Kolmogorov-Smirnov and the Shapiro-Wilk test were used to test normality of variables. If assumption of normal data distribution was met, the paired t-test was used. For non-normally distributed variables the Wilcoxon signed-rank test was used. Results of correlation analyses were represented as Spearman's Rank correlation

coefficients (r). We considered *P*-values < 0.05 (two-tailed) to be statistically significant. Statistical analyses were performed with SPSS version 24 (IBM SPSS Statistics for Windows, Armonk, N.Y., USA) and graphs were drawn using GraphPad Prism version 6 for Windows (GraphPad Software, San Diego, California, USA).

RESULTS

Patient characteristics and treatment modalities

Characteristics of the 52 patients included in the study cohort are presented in Table 1. After initial treatment with SRLs, most patients (84.6%) had IGF-I levels above 1.2 x the ULN. All patients continued to receive SRL and PEGV combination treatment with a median PEGV dose of 100 mg/week (IQR 60–160).

Table 1. Patient characteristics entire cohort

Characteristics	Patients (n = 52)
Age (median, range)	53.5 (26 – 80)
Female patients (n, %)	22 (42.3%)
Macroadenomas (n, %)	44 (84.6%)
Previous surgery (n, %)	19 (34.6%)
SRL treatment prior to surgery (n, %)	5 (9.6%)
SRL treatment duration (months, mean, 95% CI)	11.2 (8.2 – 14.2)
IGF-I before SRL therapy (x ULN, mean, 95% CI)	3.14 (2.80 – 3.49)
IGF-I after SRL therapy (x ULN, mean, 95% CI)	2.13 (1.82 – 2.45)
IGF-I ≤ 1.2 x ULN after SRL monotherapy (n, %)	13 (27.1%)
Weekly PEGV dose (mg, mean, 95% CI)	137 (101–172)

IGF-I levels are shown before initiation of SRL monotherapy and after ≥ 4 months SRL monotherapy. PEGV dose during SRL and PEGV combination therapy at baseline of PAPE study.

Table 2 reports the characteristics of patients that were included in the SSTR subcohort (n = 14). All these patients harboured a macroadenoma. 5 out of 14 patients had received SRL treatment prior to surgery, [4 achieved initial IGF-I normalization (< 1.2 x ULN)] and the remaining nine patients were drug-naïve before surgery. During follow-up, all 14 patients received SRL and PEGV combination therapy (median PEGV dose 100 mg/week (IQR 80–145)). After 24 weeks, the median PEGV dose was 45 mg/week (IQR 23–75) and decreased to 0 mg/week (IQR 0–70) after 48 weeks. Three out of 14 (21.4%) patients were on PAS-LAR monotherapy after 24 weeks, increasing to 8 patients (57.1%) after 48 weeks. More in detail, in two patients PAS-LAR dose was reduced to 40 mg and in two other patients to 20 mg monotherapy every 4 weeks.

Table 2. Patients' characteristics of the SSTR subcohort

Patient number	Sex, Age (y)	SRL pre-treatment	IGF-I (ULN) after SRL	Percentage of IGF-I reduction after SRL	IGF-I (ULN) after PAS-LAR 24 weeks	Baseline PEGV dose (mg/week)	48 weeks PEGV dose (mg/week)	48 weeks percentage PEGV dose reduction	48 weeks PAS-LAR dose (mg)	48 weeks PAS-LAR score	SST_2 (IRS)	SST_5 (IRS)
1	M, 80	No	0,72	65,9	,90	80	0	100	40	3	6	12
2	F, 37	No	1,67	48,5	1,05	80	0	100	60	2	12	12
3	M, 57	No	1,48	42,7	1,28	80	50	37.5	60	1	8	1
4	M, 38	No	1,77	18,2	1,78	700	540	22.9	60	0	1	12
5	F, 36	Yes	3,83	15,5	1,11	100	50	50	60	1	1	0
6	F, 36	No	3,17	7,1	2,91	400	400	0	60	0	6	9
7	M, 61	Yes	1,51	54,2	,79	80	0	100	60	2	12	12
8	F, 71	No	2,21	5,5	1,24	120	60	50	60	1	2	12
9	M, 51	No	3,14	21,2	1,16	120	0	100	60	2	9	12
10	M, 53	Yes	1,89	44,9	1,14	100	100	0	60	0	12	12
11	F, 56	No	0,76	74,6	,39	20	0	100	20	4	12	8
12	F, 46	Yes	1,00	62,6	,49	100	0	100	20	4	9	4
13	M, 46	Yes	1,85	19,4	,49	70	0	100	60	2	12	6
14	F, 69	No	0,61	60,0	,72	220	0	100	40	3	9	12

Detailed description of patients' general characteristics, IGF-I levels after SRL and PAS-LAR treatment, and the SST_2 and SST_5 immunoreactivity score (IRS). In addition, PEGV doses are shown during SRL and PEGV combination treatment at baseline and after switching to PAS-LAR treatment at 48 weeks. The PAS-LAR score takes into account both the achieved PEGV dose reduction and PAS-LAR dose reduction at 48 weeks compared with baseline.

In the study cohort (n = 52), at 24 weeks, the median PEGV dose was 45 mg/week (IQR 30-80) and 10 of 52 (19.2%) patients were on PAS-LAR monotherapy. At 48 weeks, the median PEGV dose decreased to 40 mg/week (IQR 0 - 90) and 25 of 52 (48.1%) patients were on PAS-LAR monotherapy.

Protein expression of SST_2 and SST_5

The median SST_2 IRS was 9 (IQR 5-12), the median SST_5 IRS was 12 (IQR 5.5-12), and the median SST_2/SST_5 ratio was 1.0 (IQR 0.6 - 1.8). We did not find a statistically significant difference in SST_2 and SST_5 expression between medically naïve (n = 9) and SRL pre-treated (n = 5) patients (P = 0.31 and P = 0.25, respectively). More in detail, median SST_2 IRS was 12 (IQR 5 - 12) in pre-treated patients and 8 (4 - 10.5) in the naïve ones, while median SST_5 IRS was respectively 6 (2- 12) and 12 (8.5 - 12) in SRL pre-treated and naïve patients.

The relation between SSTR immunoreactivity and response to SRL treatment is shown in the supplemental data (page 22) (20). In line with previous findings, the percentage IGF-I reduction after SRL treatment was positively correlated to SST_2 IRS, while an inverse trend was observed between SST_2 IRS and IGF-I (x ULN) levels after SRL treatment. The PEGV dose at baseline during the PAPE study was inversely correlated to the SST_2 IRS.

Relationship between response to SRL treatment and PAS-LAR treatment in study cohort

We observed a significant positive correlation between IGF-I (x ULN) levels after SRL treatment and IGF-I levels after 24 weeks PAS-LAR treatment (r = 0.50, P = 0.0002, n = 52, 3A). However, no relation was observed between the percentage IGF-I reduction after SRL treatment and after 24 weeks PAS-LAR treatment (r = 0.026 P = 0.85, n = 52) (Supplemental Figure 1A) (20). With respect to response to PAS-LAR after 48 weeks, IGF-I (x ULN) levels after SRL treatment showed a strong inverse correlation with the PAS-LAR score (r = −0.53, P = 0.0006, n = 52, Figure 3B). Moreover, the percentage IGF-I (x ULN) reduction after SRL treatment was positively correlated to the PAS-LAR score (r = 0.40, P = 0.003, n = 52, Figure 3C) as well. We also observed a significant relationship between IGF-I (x ULN) levels after SRL treatment and after 48 weeks PAS-LAR treatment (r = 0.30, P = 0.028, n = 52) (Supplemental Figure 1B) (20).

Relationship between response to SRL treatment and PAS-LAR treatment in SSTR subcohort

In the SSTR subcohort, we observed a positive correlation between IGF-I (x ULN) levels after SRL treatment and after 24 weeks PAS-LAR treatment (r = 0.58, P = 0.029,

n = 14, Figure 4A), while no corelation was observed with percentage IGF-I reduction (Supplemental Figure 1C) (20). Higher SST_2 IRS was correlated with lower IGF-I levels (x ULN) after 24 weeks PAS-LAR treatment (r = −0.61, *P* = 0.020, n = 14, Figure 4B), while SST_5 IRS did not show any relation to IGF-I levels (r = 0.16, *P* = 0.58, n = 14, Figure 4C).

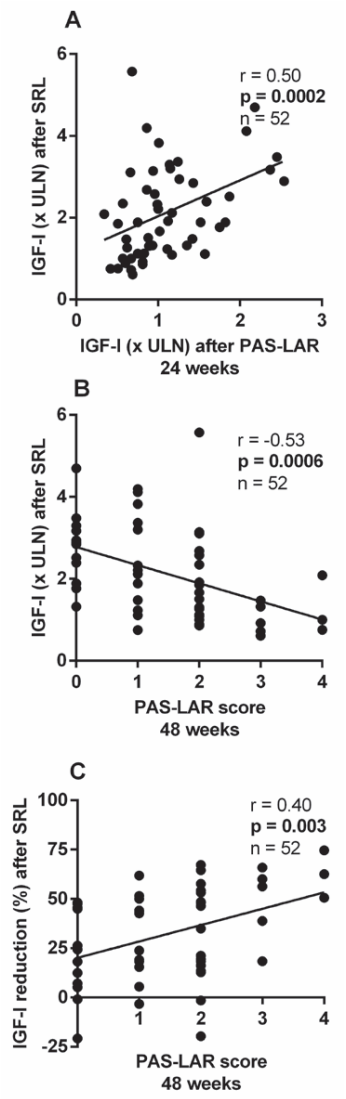

Figure 3. Relation between response to SRL treatment and response to PAS-LAR treatment in the study cohort. IGF-I (x ULN) levels after SRL treatment were correlated to IGF-I (x ULN) levels after PAS-LAR treatment at 24 weeks (3A), and inversely correlated to the PAS-LAR score at 48 weeks (3B). The percentage IGF-I (x ULN) reduction after SRL treatment was positively correlated to the PAS-LAR score at 48 weeks (3C).

With respect to the 48 weeks PAS-LAR response, the PAS-LAR score at 48 weeks was inversely correlated to IGF-I (x ULN) levels after SRL treatment (r = −0.71, *P* = 0.005, n = 14, Figure 5A), and positively correlated to the percentage IGF-I reduction after SRL treatment (r = 0.80, *P* = 0.001, n = 14, Figure 5B). Furthermore, IGF-I (x ULN) levels

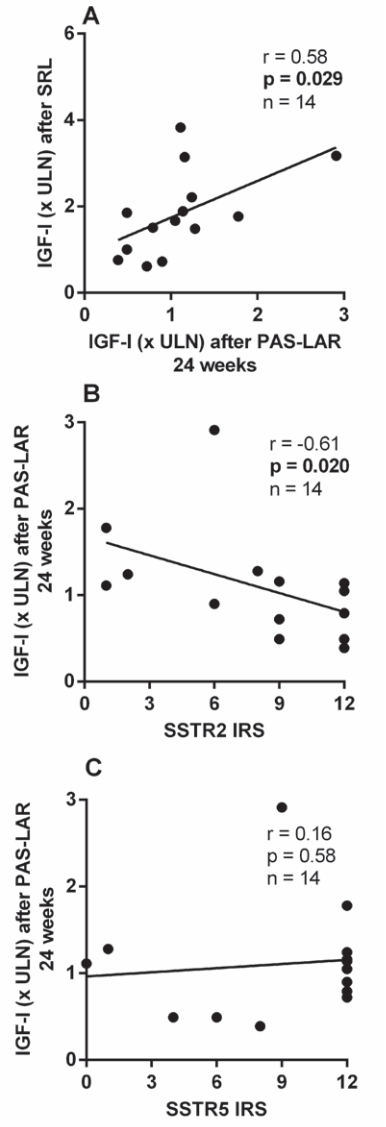

Figure 4. Relation between response to SRL treatment and the response to PAS-LAR treatment at 24 weeks in the SSTR subcohort. IGF-I (x ULN) levels after PAS-LAR treatment at 24 weeks were correlated to IGF-I levels after SRL treatment (4A), and inversely correlated to SST_2 expression (4B), but not to SST_5 expression (4C).

after SRL treatment were correlated to IGF-I (x ULN) levels after PAS-LAR treatment at 48 weeks (r = 0.58, P = 0.031, n = 14, Supplemental Figure 1D) (20).

We observed a trend, although not statistically relevant, for a direct correlation between SST_2 IRS and the PAS-LAR score (r = 0.41, *P* = 0.14, n = 14, Figure 5C), and no relation was found between SST_5 IRS and PAS-LAR score (r = −0.073, *P* = 0.80, n = 14, Figure 5D). Interestingly, considering only those patients naïve to SRL treatment before surgery (n = 9), the correlation between SST_2 IRS and the PAS-LAR score (r = 0.69, *P* = 0.039, n = 9) was statistically significant, while this was not the case for SST_5 IRS.

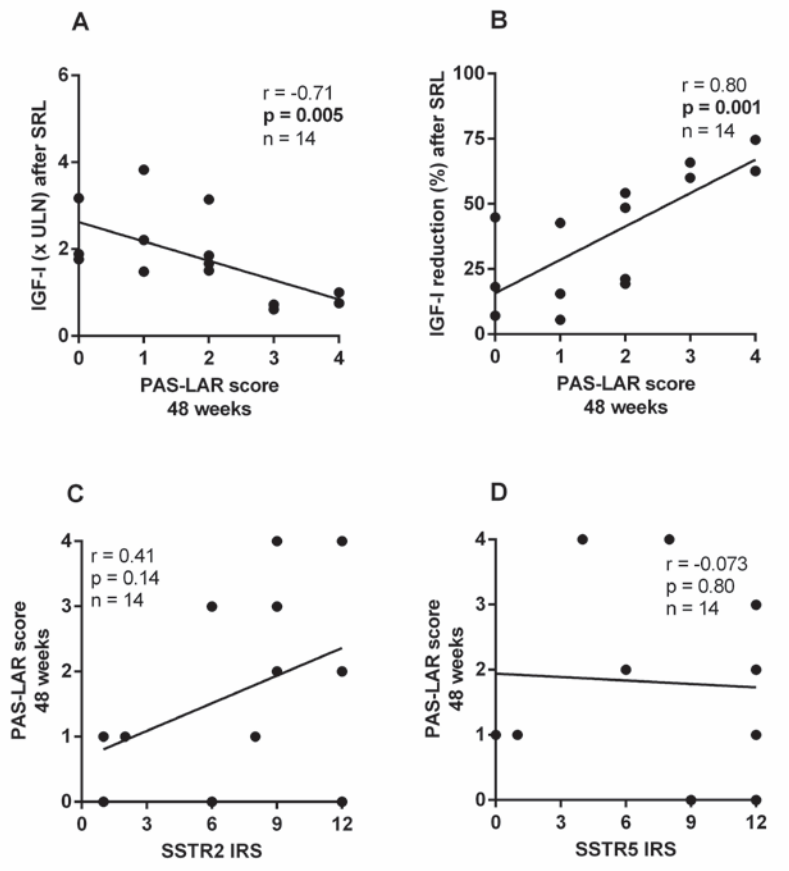

Figure 5. Relation between response to SRL treatment and the response to PAS-LAR treatment at 48 weeks in the SSTR subcohort. The PAS-LAR score at 48 weeks was inversely correlated to IGF-I (x ULN) levels after SRL treatment (5A), and positively correlated to the percentage IGF-I reduction after SRL treatment (5B). The PAS-LAR score showed a trend for a relation with SST_2 expression (5C), but SST_5 expression did not show any relation (5D).

DISCUSSION

Our results suggest that in acromegaly patients the responsiveness to PAS-LAR treatment is mainly correlated to SST_2 expression, and not to SST_5. This observation is further supported by the finding that after 48 weeks treatment the percentage IGF-I reduction after SRL treatment was correlated to the PAS-LAR treatment response score. Our study provides the novel finding that the *in vivo* response to PAS-LAR is directly correlated to both the clinical response to first-generation SRL treatment and SST_2 expression on adenoma tissue.

However, these results are not unexpected. Indeed, our data confirm previous *in vitro* studies, carried out in primary cultures of GH-secreting adenomas, showing that the efficacy of naïve somatostatin (SRIF-14), octreotide and pasireotide in the reduction of GH secretion was positively correlated with SST_2 mRNA expression, but not with SST_5 (13, 21, 22). According to these findings, our group and other authors have reported that the effect of octreotide and pasireotide on GH suppression is almost superimposable both *in vitro* and *in vivo* (12, 13, 23).

The observed positive relation between SST_2 protein expression and the percentage IGF-I reduction after SRL treatment, is in line with previous studies (5, 6). Furthermore, an inverse relation between the PEGV dose and SST_2 expression was recently reported, suggesting that the required PEGV dose to normalize IGF-I levels in patients with partial response to SRLs is a surrogate marker for the degree of SRL resistance (8). In our cohort, the SRL pretreated patients had a trend for a higher SST_2 expression than medically naïve patients. This finding is in contrast to previous studies (5, 6), and it is probably correlated to a lack of statistical power in our study (5 vs 9 adenoma samples).

Our results show that IGF-I levels after PAS-LAR treatment were directly correlated to SST_2 expression, and not to SST_5 expression. After exclusion of SRL pretreated patients, SST_2 IRS was also significantly correlated to the PAS-LAR score, a tool designed to uncover the impact of switching to PAS-LAR treatment in patients using SRLs and PEGV combination treatment. While there is no evidence that SST_5 expression is affected by SRL presurgical treatment, it has been widely demonstrated that patients receiving SRL treatment prior to neurosurgery show significantly lower SST_2 protein expression compared to medically naïve patients (4-6, 13). Although this is not evident in our cohort, a pooled analysis of SRL pretreated and medically naïve patients can introduce bias. Exclusion of the SRL pretreated patients from our analysis

(n = 5) resulted in a stronger relationship between SST_2 IRS and the response to PAS-LAR treatment.

A strength of our study lies in the relatively large number of patients in which the clinical efficacy of SRLs, SRL/PEGV combination treatment and PAS-LAR treatment were systematically investigated in combination with data on SSTR expression of somatotroph adenomas in a well characterized subgroup of patients. The main limitation of our study lies in the retrospective collection of data on IGF-I levels during SRL treatment and the use of different IGF-I assays during follow-up. The IGF-I levels measured after 24 weeks PAS-LAR treatment may be partly influenced by the carry-over effect of withdrawal of SRLs after 12 weeks. While the carry-over effect of SRLs may have influenced the short-term response to PAS-LAR after 24 weeks, the response to PAS-LAR treatment after 48 weeks is probably not affected. In our cohort, the PAS-LAR score at 48 weeks might therefore be the most informative marker for responsiveness to PAS-LAR treatment.

Two studies have previously investigated the relationship between the immunohistochemical expression of SST_2 and SST_5 in somatotroph adenomas and the clinical response to first-generation SRL and PAS-LAR treatment in acromegaly (24). *Iacovazzo* et al suggested that SST_5 expression drives the responsiveness to PAS-LAR treatment in patients resistant to first-generation SRLs. These authors investigated a cohort of 39 acromegaly patients requiring post-operative SRL treatment, of which 11 patients were resistant to SRL and were switched to PAS-LAR treatment. They observed that none of the patients lacking SST_5 expression was responsive to PAS-LAR, whereas 5 out of 7 patients with membranous expression of SST_5 were responsive to PAS-LAR. Furthermore, patients with a higher SST_5 score had a greater reduction in IGF-I levels. However, they found no difference in SST_2 expression between pasireotide responders and non-responders.

These results are in contrast with our findings, which suggest that SST_2 expression, and not SST_5 expression, is more important for the clinical response to PAS-LAR. The main difference between our study and the study from *Iacovazzo* et al. (24), is that we included mainly patients who were partially responsive to SRLs, while Iacovazzo et al included only SRL resistant patients. Secondly, the patients in our cohort all received pegvisomant treatment before switching to pasireotide LAR during the PAPE study, whereas the patients in the Iacovazzo study did not receive PEGV treatment, and were directly switched to pasireotide LAR (Table 2). In addition, these differences are unlikely to be explained by the use of a different SSTR expression scoring system. Although the other authors used a scoring method proposed by *Volante* et al

which takes into account both subcellular localization and extent of staining (25), the method we used from *Remmele* et al is a semiquantitative score which takes into account both intensity and percentage of positive cells (19). Interestingly, both scoring systems have been recently found to show high inter-laboratory and inter-observer agreement for SSTRs expression in neuroendocrine tumors (26).

Furthermore, in our cohort we cannot rule out a direct effect of PEGV treatment on SSTR expression (8). PEGV is known to increase serum GH levels (27), which could result in reduced hypothalamic GHRH secretion, which in turn may lead to a down-regulation of SSTR expression. Although the impact of PEGV on SSTR expression is plausible (28), there is no evidence that PEGV treatment plays a major role in the modulation of SSTR expression via the activation of GH-IGF-I axis.

While our study suggests that pasireotide acts mainly via SST_2 in somatotroph adenomas, in corticotroph adenomas SST_5 seems to be more important. Several *in vitro* studies have demonstrated that pasireotide is more effective than octreotide in reducing ACTH secretion and/or intracellular cAMP levels in AtT20 cells or corticotroph adenomas primary cultures (29-32). On the other hand, preclinical studies have indicated that pasireotide and octreotide are equally effective (in vitro) in lowering GH levels (21, 23, 33, 34). This suggests a predominant role of SST_2 in mediating the inhibitory effect of pasireotide on GH secretion in somatotroph adenomas.

In conclusion, our results suggest that SST_2 expression of somatotroph adenomas is more important than SST_5 in driving the responsiveness to PAS-LAR treatment in a peculiar subset of acromegaly patients (e.g. partial responders). It is plausible that the enhanced efficacy of PAS-LAR compared to first-generation SRLs is mediated by its stronger inhibition of insulin secretion, rendering the liver less sensitive to GH action (35). The enhanced efficacy of PAS-LAR could also be the consequence of a differential activation of SST_2 by the different compounds (e.g. reduced activation of SST_2 internalization and faster recycling on the cell membrane) (36, 37), rather than by the higher binding affinity of PAS-LAR for SST_5. Future studies should investigate whether this is indeed the case.

Acknowledgements
We are grateful for the assistance of Hans Stoop from the pathology department for the immunohistochemistry. We also acknowledge the study nurses who contributed to the study, and the patients for their participation in the PAPE study.

Author contributions

AM, LH, and SN conceived and designed the study. AM was responsible for data management and statistical analyses after discussion with all authors. All authors participated in data interpretation and in writing of the report.

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SUPPLEMENTAL DATA

Relationship between SSTR immunoreactivity and response to SRL treatment

The percentage IGF-I reduction after SRL treatment was positively related to SST_2 IRS (r = 0.54, *P* = 0.046, n = 14), but no correlation was found with SST_5 IRS (r = 0.090, *P* = 0.76, n = 14).

IGF-I (x ULN) levels after SRL treatment showed an inverse trend with SST_2 IRS (r = −0.31. P = 0.28, n = 14), while no correlation was observed with SST_5 IRS (r = −0.12, *P* = 0.69, n = 14) (Figure 2A and 2B). Exclusion of SRL pretreated patients did not change the relation to SST_2 (r = −0.34, *P* = 0.37, n = 9) and SST_5 IRS (r = 0.10, *P* = 0.98, n = 9).

Relationship between baseline PEGV dose during combination treatment and SSTR immunoreactivity

We observed an inverse correlation between the PEGV dose at baseline and SST_2 IRS (r = −0.61, *P* = 0.020, n = 14), but SST_5 IRS was not correlated with the PEGV dose (r = 0.32, *P* = 0.27, n = 14).

SUPPLEMENTAL FIGURE 1