Expression of somatostatin receptors on human pituitary adenomas *in vivo* and *ex vivo*

S. Nielsen*, S. Mellemkjær*, L.M. Rasmussen**, T. Ledet***, N. Olsen****, M. Bojsen-Møller*****, J. Astrup******, J. Weeke*, and J.O.L. Jørgensen*

*Medical Department M; **Laboratory for Molecular Pathology; ***Research Laboratory for Biochemical Pathology; ****Department of Nuclear Medicine; *****Department of Neuropathology; ******Department of Neurosurgery, Aarhus Kommunehospital, Aarhus University Hospital, Aarhus, Denmark

ABSTRACT. The distribution and biologic activity of somatostatin receptor subtypes (SSTR) in pituitary adenomas is not clarified, especially regarding clinically non-functioning adenomas (NFPA). We therefore characterized SSTR in human pituitary adenomas by combining molecular biology and *in vivo* scintigraphy. Co-expression of gonadotropin-releasing hormone receptor (GnRH-R) mRNA was also assessed to see whether this feature was associated with adenoma subtype and SSTR status. Pituitary tumor biopsies were obtained during transsphenoidal adenomectomy from 21 patients (11 NFPA, 7 acromegalics, 2 prolactinomas, 1 Cushing's disease). Expression of mRNA encoding the 5 known SSTR subtypes and the GnRH-R was determined by RT-PCR. Twelve patients also underwent a pre-operative somatostatin receptor scintigraphy. Most adenomas (no.=18) expressed mRNA for more than one SSTR. SSTR2 mRNA was expressed in 18 cases, whereas SSTR4 was absent in all but one. SSTR3 was frequently expressed in NFPAs. Somatostatin receptor scintigraphy was positive in most cases, and with a significantly higher uptake index in GH-producing adenomas all of which expressed SSTR2 mRNA. The uptake index appeared to be related to receptor density rather than tumor volume. Expression of GnRH-R mRNA was found in both NFPAs and GH-producing adenomas and was not significantly associated with a particular SSTR subtype population. In conclusion: 1) the distribution of SSTR is not significantly different between NFPA and GH-producing adenomas; and 2) somatostatin receptor scintigraphy reveals a higher uptake in GH-producing adenomas which is not significantly related to either SSTR distribution or tumor volume.

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INTRODUCTION

SS is widely distributed throughout the central nervous system and peripheral tissues. Hypothalamic SS inhibits the secretion of hormones from the anterior pituitary, in particular GH and TSH, and also exhibits anti-proliferative effects. Long-acting SS analogues are currently used to control hormonal hypersecretion and tumor growth in GH- and TSH-secreting pituitary adenomas. Moreover, SS receptor scintigraphy with radiolabeled octreotide demonstrates a high density of SS-binding sites in a large proportion of pituitary adenomas. It is, however, well known that both the clinical response to SS analogues as well as the outcome of *in vivo* receptor imaging display marked heterogeneity both between and within different pituitary tumor types (1-5). These differences are partly attributed to the existence of at least 5 distinct SS receptor subtypes (SSTR1-5), which exhibit differences regarding amino acid sequence, affinity for SS analogues, and post-receptor signaling pathways (6). Octreotide® and lanreotide® have high affinity for SSTR2 and SSTR5, however, octreotide predominantly visualizes SSTR2. On the contrary, *in vitro* data suggest that SSTR5 may be even more critical for the suppression of anterior pituitary hormone release (6). The distribution of SSTR expression in human pituitary adenomas has not yielded uniform results (7-13). It is, however, apparent that the majority of tumors expresses SSTR2 mRNA, whereas SSTR4 expression has only been reported in one study (10). The degree of expression of SSTR1, SSTR3, and

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Correspondence: Dr. Steen Nielsen, Medical Department M (Diabetes and Endocrinology), Aarhus Kommunehospital, DK-8000 Aarhus C, Denmark.

E-mail: stn@adfm.au.dk.

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SSTR5 appears to be more heterogeneous, especially in clinically non-functioning pituitary adenomas (NFPAs). Regarding NFPAs, the differences may reflect that this diagnostic entity comprises several subtypes (14-16). The suppression by SS of the secretion of gonadotropins and α-subunit is not seen in all NFPAs (1, 17). Moreover, functional SSTR protein has been documented by SS receptor scintigraphy in some NFPAs, but the outcomes of scintigraphy and SSTR expression have so far only been directly compared in a small study in which no association between the outcome of SS receptor scintigraphy and SSTR distribution could be documented (2). From a clinical point of view, more insight into the composition and function of SSTR in well-characterized pituitary adenomas may provide a basis for designing SS analogues with distinct receptor affinities for treatment of not only GH-secreting tumors, but also other pituitary diseases.

In this prospective study of acromegalic and NFPA patients undergoing transsphenoidal adenomectomy we investigated mRNA expression of SSTR1-5 in all tumors *ex vivo*, together with pre-operative *in vivo* SS receptor scintigraphy. In addition, we assessed mRNA expression of GnRH receptors in an attempt to further characterize NFPA and evaluate whether this feature was associated with a specific SSTR composition.

SUBJECTS AND METHODS

Patients (Table 1)

Twenty-one patients with pituitary adenomas were included in the study. All patients were characterized by a pre-operative routine endocrinological examination. Tissue samples from the 21 patients were obtained for reverse transcriptase polymerase chain reaction (RT-PCR) analysis, whereas immunohistochemical analysis was performed on tissue samples from 18 patients. Twelve patients underwent a pre-operative SS receptor scintigraphy, and a TRH-test with determination of TRH stimulated α-subunit levels were performed in 14 individuals. The diagnosis of a NFPA was based on the pres-

Table 1 - *Characterization of subjects.*

Pt. nr.	Sex	Age	Diagnosis	Treatment (prior to surgery)
3	F	60	NFPA	Thyroxine
5	F	27	NFPA	Insulin (T1DM)
7	F	67	NFPA	Estradiol, norethistrone
9	F	35	NFPA	Hydrocortisone
12	M	35	NFPA	Hydrocortisone, testosterone
13	M	61	NFPA	Hydrocortisone, testosterone, thyroxine
14	F	51	NFPA	-
15	M	61	NFPA	Hydrocortisone, testosterone
16	M	49	NFPA	-
17	M	51	NFPA	-
18	F	36	NFPA	Hydrocortisone
1	F	26	Acromegaly	Hydrocortisone, thyroxine, desmopressin
2	M	40	Acromegaly	-
4	M	65	Acromegaly	-
8	M	63	Acromegaly	Octreotide, lisinopril (hypertension)
10	F	49	Acromegaly	Antihypertensive drugs
11	M	47	Acromegaly	-
21	M	30	Acromegaly	Hydrocortisone
6	F	36	Prolactinoma	-
20	F	27	Prolactinoma	Bromocriptine
19	F	35	Cushing's disease	-

F: female; M: male; NFPA: non-functioning pituitary adenomas; T1DM: type 1 diabetes mellitus.

ence of a pituitary macroadenoma as assessed by either magnetic resonance imaging (MRI) or computed tomography (CT) in the absence of clinical and biochemical signs of hormonal hypersecretion. Prolactin levels were either normal or only moderately elevated (<50 µg/l). Normal to low levels of FSH and LH were found in all patients.

The diagnosis of acromegaly was based on clinical symptoms and signs together with significantly increased serum IGF-I levels (mean±SE: 980±150 µg/l, range 402-1662 µg/l) and sustained elevations in basal GH concentrations obtained from frequent serum sampling over a 5-h period with the patient in a supine position (mean±SE: 6.20±2.15 µg/l, range: 1.67-18.23 µg/l).

The 2 patients with prolactinomas had prolactin serum concentration of 221 and 880 µg/l (normal range: 1-18 µg/l), respectively, and both patients initially presented with amenorrhea. In the single case of Cushing's syndrome the diagnosis was based on clinical symptoms and markedly elevated excretion of cortisol: 2668, 2995 and 3024 nmol/24 h on 3 separate days. Furthermore, a significant ACTH response to CRH was demonstrated during a CRH test.

RT-PCR

The tissue samples were collected at the time of transsphenoidal adenomectomy, immediately frozen in liquid nitrogen and kept at -80 C until analyzed.

Total RNA was extracted from the tissue samples with Trizol® (GIBCO BRL). The amount and the quality of the RNA was assessed by measurement of the optical density at 260 and 280 nm.

From each sample 1 µg of RNA was used for reverse transcription in 30 µl of buffer with final concentrations of 50 mM Tris, pH 8.3, 75 mM KCl, 3 mM $MgCl_2$, 0.5 mM dATP, 0.5 mM dTTP, 0.5 mM dCTP, 0.5 mMdGTP, 30 pmol random hexamers/30 µl and 200 U MMLV reverse transcriptase. The samples were incubated for 75 min at 37 C followed by incubation for 15 min at 95 C. A similar procedure was performed in a duplicate sample but without the addition of the MMLV-RT. This non-reverse transcribed material served as a negative control for the PCR procedures. As a positive control expression of mRNA encoding the house-keeping enzyme GAPDH was determined by a similar RT-PCR procedure.

For the polymerase chain reaction 2 µl of the reverse transcription product was used in 20 µl of solution which contained final concentrations of 20 mM Tris, pH 8.4, 50 mM KCl, 1.5 mM MgCl2, 0.5 mM dATP, 0.5 mM dTTP, 0.5 mM dCTP, 0.5 mM dGTP, 2.5 U Taq DNA polymerase/20 µl and 500 pmol of each specific primer. The following primers were used: SSTR1: 5'-GGAACTCTATGGTCATCTAC-3' and 5'-GCTGAGCACAGTCAGACAGT-3' (3), SSTR2: 5'-TGACAGTCATGAGCATCGAC-3' and 5'-GCAAAGACAGATCATGGTGA-3' (3), SSTR3: 5'-TCATCTGCCTCTGCTACCTG-3' and 5'-GAGCCCAAAGAAGGCAGGCT-3' (3), SSTR4: 5'-ATCTTCGCAGACACCA GACC-3' and 5'-ATCAAGGCTGGTCACGACGA-3' (3), SSTR5: 5'-CGTCTTCATCATCTACACGG-3' and 5'-GGCCAGGTTGACGATGTTGA-3' (3), GnRH-R: 5'-CAGCAAAGTCGGACAGTC and 5'-AGTTGTAGTTCGTGGGGG (16) and finally GAPDH: 5'-GCCAAAAGGGTCATCATCTC-3' and 5'-GTAGAGGCAGGGATGATGTTC-3'. Amplification of DNA was performed with two initial cycles at 95 C for 60 sec followed by 60 sec at temperature of annealing which was specific for each primer and 60 sec at 73 C; this was followed by a number of cycles at 94 C for 45 sec, followed by 45 sec at the temperature of annealing and 45 sec at 73 C, and in the final cycle the samples were incubated for 2 min at 73 C. The temperature of annealing was 60 C in the case of SSTR1-4, 65 C in the case of SSTR5, and finally 50 C in the case of GAPDH and the GnRH-R. The amplification of DNA was carried out in 30 cycles in the case of GAPDH, 30 cycles for GnRH receptor and finally 38 cycles for SSTR1-5. The PCR product was separated by gel electrophoresis in a 2% agarose gel containing ethidium bromide. To ensure that an appropriate sensitivity level was used, the number of PCR cycles was chosen after optimization experiments using a pool of cDNA containing reverse transcribed RNA from several tumor types. This cDNA pool was subjected to different number of cycles between 30 and 40. The number of cycles which gave rise to a weak band was chosen for evaluation of all samples. The PCR products were visualized in ultraviolet light using equipment from BioRad®. As an arbitrary cut-off level a clearly visible band was considered as a sign of gene transcription. The results of the gel electrophoresis were determined by an independent investigator who was unaware of the clinical characteristics of the patients.

All PCR-reactions gave rise to DNA fragments with the expected size.

Somatostatin receptor scintigraphy

111 MBq of ^{111}In-pentetreotide (Octreoscan, Mallincrodt) was given as an intravenous injection. The profile of the cranium was imaged after 21 h using a Siemens Orbiter gamma camera equipped with a medium-energy, parallel-hole collimator and interfaced to a Picker Odyssey VP Computer. The pulse height analyzer windows were centered over

171 keV and 245 keV with window widths of 20% and 15%. The acquisition was performed with a hardware zoom of x 1.5, a matrix of 128 x 128 and total counts of 300.000 (18, 19). On the profile view, circular regions of interest (ROIs) were placed on the pituitary area and on the hemispheric brain. The pituitary ROI was small (9 pixels) and centered around the pixel with highest activity. The hemispheric ROI (212 pixels) was placed over the posterior part of the cerebrum. The uptake index was calculated as the ratio of the pituitary mean activity to the hemispheric mean activity (19, 20). An uptake index≥2.5 was considered positive.

Immunohistochemistry and α-subunit measurements

Adenoma tissue from all patients was fixed in phosphate buffered formalin for 24 h at room temperature, paraffin embedded and cut in 5 µm thick slices. All sections were boiled for 10 min in a microwave oven. Endogenic peroxidase was blocked by incubation for 30 min with methanol containing 0.9% H_2O_2. The antigens were visualized using a classical immuhistochemical technique in which all the primary polyclonal and the only monoclonal (α-subunit) antibodies were applied in a concentration between 1/400 and 1/1000. The secondary anti-

Table 2 - *Expression of mRNA encoding all 5 somatostatin receptor subtypes (SSTR) and the GnRH receptor (GnRH-R).*

Non-functioning pituitary adenomas						
Patient no.	SSTR1	SSTR2	SSTR3	SSTR4	SSTR5	GnRH-R
3	+	+	+			
5	+	+			+	+
7		+	+			+
9		+	+			+
12		+	+			+
13			+			
14		+	+		+	+
15		+	+			
16		+	+			
17		+	+		+	+
18	+	+				
Acromegaly						
Patient no.	SSTR1	SSTR2	SSTR3	SSTR4	SSTR5	GnRH-R
1		+	+			
2		+				
4	+	+	+		+	+
8	+	+	+			+
10	+	+			+	
11		+			+	
21		+	+			
Prolactinomas						
Patient no.	SSTR1	SSTR2	SSTR3	SSTR4	SSTR5	GnRH-R
6	+	+	+		+	+
20	+					
Cushing's disease						
Patient no.	SSTR1	SSTR2	SSTR3	SSTR4	SSTR5	GnRH-R
19			+	+		

body containing horseradish-peroxidase was used in a dilution of 1/100. The color was developed by diaminobenzidine and the nuclei were stained with hematoxylin. Antibodies against the following components were used: ACTH, GH, prolactin, FSH, LH, TSH, α-subunit and SS.

Serum levels of α-subunit were assessed by a monoclonal antibody radioimmunoassay (Biomerica®). The patients were admitted to the endocrine research laboratory at 09:00 h and a cannula was inserted in an antecubital vein. Blood samples for assessment of serum levels of α-subunit were drawn at -90, -75, -60, -45, -30, -15, 0, 15, 30, 45, 60, 75 and 90 min, and 400 μg TRH was given intravenously at 0 min. The peak value of α-subunit after administration of TRH was compared to the average α-subunit concentration in the samples taken prior to TRH administration.

Statistics

Non-parametric data were compared by Wilcoxon's signed rank test for paired samples while non-paired data were compared by Mann-Whitney's U test. Proportions were compared by the Chi-square test. A *p*-value less than 0.05 was considered statistically significant.

RESULTS

Immunohistochemistry and α-subunit measurements (Table 1)

All preparations showed adenoma tissue only. Staining for prolactin was found in both prolactinomas and 2 of 4 somatotroph adenomas, as well as one NFPA. Immunostaining for LH was more frequently positive among NFPAs than in the rest of the tumors in this series ($p<0.05$), whereas the frequency of the positive immunostaining for FSH, TSH and α-subunit did not differ significantly between the groups. In NFPAs staining for TSH was positive in 6 of 9 cases and staining for α-subunit was positive in 5 of 9 cases. Furthermore, α-subunit was found in 4 of 5 somatotroph adenomas, and in one corticotroph adenoma. The peak value of α-subunit increased significantly after injection of TRH (basal: median: 116.25 mIU/ml; TRH-stimulated peak: median 464 mIU/ml, $p=0.002$).

Somatostatin receptor and gonadotropin releasing hormone receptor mRNA expression (Table 2)

In most tumors (no.=18) expression of mRNA encoding multiple SSTR was demonstrated. SSTR2 was the most widely distributed (no.=18), whereas SSTR1 mRNA, SSTR3 mRNA and SSTR5 mRNA were expressed in 8, 15 and 7 tumors, respectively. SSTR4 mRNA was only detected in one tumor, which was the only ACTH-secreting tumor in the present study.

mRNA encoding the GnRH receptor was detected in 9 of 21 adenomas. More than half of the NFPAs (6 out of 11) expressed GnRH-R mRNA, but there was no statistical difference in the frequency of GnRH-R mRNA expression between the NFPAs and the rest of the tumors ($p=0.26$). No statistically significant association was detected between expression of GnRH-R mRNA and SSTR mRNA subtypes.

Somatostatin receptor scintigraphy (Table 3)

A pathological uptake index was found in 9 of 12 patients of whom 5 harbored a GH-producing pituitary adenomas, and 4 harbored NFPAs. In 2 patients with an NFPA and the patient with the ACTH-producing adenoma a normal uptake index was found. The mean uptake index among patients with GH producing adenomas was significantly higher compared to NFPAs [mean±SE: 4.58±0.63 (range: 3.60-7.00) *vs* 3.37±0.71 (range: 2.32-6.87), respectively ($p<0.05$)].

No distinct association was apparent between the pattern of SSTR mRNA expression and the outcome of SS receptor scintigraphy. Nevertheless, SSTR2 mRNA was demonstrated in 8 out of 9 with a pathological uptake index.

MRI showed a suprasellar tumor extension in 8 of 12 cases. Neither the volume nor the width of the tumor was significantly associated with the uptake index (volume: $r=0.469$, $p=0.124$; width: $r=0.381$, $p=0.222$) as assessed by linear regression.

DISCUSSION

The present study was performed to further our understanding of SS receptors in human pituitary adenomas by combining molecular biology and *in vivo* scintigraphy. Our main finding was that SSTR2 mRNA is expressed in the vast majority of somatotroph pituitary adenomas and NFPAs, whereas SSTR4 mRNA is absent in most cases. SS receptor scintigraphy was positive in most cases, and with a significantly higher uptake index in GH-producing adenomas all of which expressed SSTR 2 mRNA. Expression of GnRH-R mRNA was present in both NFPAs and some GH-secreting adenomas and was not associated with a specific pattern of SSTR.

Relatively few studies have evaluated the expression of SS receptor subtypes in human pituitary adenomas (7-10) and discrepancies remain regarding the distribution. In agreement with 2 of the studies (7, 9) we observed SSTR2 mRNA to be expressed in the

Table 3 - *Somatostatin receptor scintigraphy and somatostatin receptor subtypes (SSTR) mRNA expression.*

Patient no.	Diagnosis	SSTR mRNA					Uptake index	MRI		
		1	2	3	4	5		H	D	W
5	NFPA	+	+			+	2.79	6	5	5
9	NFPA		+	+			2.68	20	12	18
12	NFPA		+	+			2.32	31	22	34
13	NFPA			+			3.12	28	28	28
17	NFPA		+	+		+	2.40	31	21	23
18	NFPA	+	+				6.87	33	29	34
4	Acromegaly	+	+	+		+	4.64	12	17	14
8	Acromegaly	+	+	+			3.81	20	20	25
10	Acromegaly	+	+			+	7.00	42	18	24
11	Acromegaly		+			+	3.86	20	20	18
21	Acromegaly		+	+			3.60	11	11	11
19	Cushing			+	+		1.92	3	3	3

H, D and W: height, depth and width (mm) of the adenomas as measured by magnetic resonance imaging (MRI). Uptake index≥2.5 is considered positive.

vast majority of NFPAs and somatotroph pituitary adenomas. Moreover, we have confirmed that SSTR4 mRNA is absent in NFPAs as well as somatotroph pituitary adenomas. Especially regarding the SSTR3 the previous studies disagree, since Greenman *et al.* (8) demonstrated the expression of SSTR3 mRNA in all NFPAs and most somatotroph adenomas, whereas Miller *et al.* reported this receptor subtype to be absent in all NFPAs and most somatotroph adenomas. We found the expression of SSTR3 to be a common feature in both of these tumor types. Additionally, our study indicates that SSTR1 mRNA and SSTR5 mRNA are expressed in some but not all NFPAs and somatotroph adenomas. By contrast, Greenman *et al.* (8) found SSTR5 mRNA to be expressed in most somatotroph adenomas and Jaquet *et al.* (13) demonstrated the expression of SSTR5 mRNA in all 15 GH-secreting tumors included. However, in the latter study SSTR5 mRNA expression was subject to considerable variation, and in that context our data using an arbitrary cut-off level in a qualitative assessment of mRNA expression support different levels of SSTR5 mRNA expression in this particular adenoma type.

In this context it should be remembered that RT-PCR analysis only reveals the presence of mRNA and not the intact and functioning receptor itself. However, the presence of SS receptors in pituitary adenomas has previously been demonstrated by means of autoradiographic methods (21, 22). Consequently, it seems likely that SS receptors are expressed in most pituitary adenomas and the RT-PCR analysis reflects the distribution of SSTR in these tumors.

It is generally believed that SS receptor imaging mainly visualizes SSTR2 receptors (23), but our study is the first to provide a direct comparison between the outcome of octreotide scintigraphy and the distribution of pituitary SSTR in the same patient. As predicted SSTR2 mRNA was found in 8 of 9 tumors in which a pathological uptake index was found. SSTR2 mRNA was, however, also expressed in 2 NFPAs with a normal uptake index, which could be explained by detection of a very low level of SSTR2 receptors by the sensitive PCR method. Surprisingly, a pathological uptake index was found in a patient only expressing SSTR3 mRNA despite the relatively low affinity of octreotide for SSTR3. The accumulation of radioactivity was estimated by a scintigraphic index. No correlation between either tumor-volume or tumor-width, and uptake index could be demonstrated. These observations suggest that the uptake index primarily depends on the type of the tumor and the density of functioning receptors with a high affinity for the scintigraphic tracer (pentetreotide). The high uptake in acromegaly may therefore also suggest a higher SS receptor density in somatotroph adenomas compared to NFPAs which has also been proposed in a previous study (2).

The clinical value of a scintigraphic uptake index is still a matter of debate (2, 24, 25). A so-called den-

sity index (DI), based on the ratio between the uptake index of the whole pituitary area and the volume of the tumor obtained by MRI, has been suggest to provide a better predictive measure of the efficacy of SS analogue treatment of pituitary adenomas (25). In our study, however, a DI calculated as the ratio between the uptake index and the width of the adenoma did not give a better separation between NFPA and somatotroph adenomas than that of our uptake index (data not shown).

Expression of GnRH-R mRNA has mainly been documented in a small number of NFPAs (16, 26, 27). Our finding of GnRH-R mRNA in 6 out of 11 NFPA extends and supports the previous reports. Several lines of evidence suggesting that both hypothalamic- and pituitary-derived GnRH may play a role in the regulation of pituitary tumor cell growth (27), which makes it potentially relevant to study the association between mRNA expression of GnRH-R mRNA and SSTR in individual pituitary adenomas. We did not, however, find evidence of a unique pattern of co-expression of these receptors apart from the observation that 8 out of 9 GnRH-R positive adenomas also expressed SSTR 2 mRNA. Future experimental studies are needed to evaluate whether modulates the action of GnRH on pituitary tumor cell biology.

In summary, our study confirms that SSTR2 mRNA is expressed in most pituitary adenomas in contrast to SSTR4, which was absent in all but one. Moreover SSTR3 mRNA is frequently expressed in NFPAs and in a substantial proportion of other pituitary adenomas. SS receptor scintigraphy *in vivo* revealed a pathological or significant uptake index in 75% of the tumors, which appeared to be related to receptor density rather than tumor volume. Future studies with specific antagonists will undoubtedly teach us more about the biology of pituitary adenomas.

REFERENCES

1. Katznelson L., Oppenheim D.S., Coughlin J.F., Kliman B., Schoenfeld D.A., Klibanski A.
   Chronic somatostatin analog administration in patients with alpha-subunit-secreting pituitary tumors.
   J. Clin. Endocrinol. Metab. 1992, *75:* 1318-1325.
2. Broson Chazot F., Houzard C., Ajzenberg C., Nocaudie M., Duet M., Mundler O., Marchandise X., Epelbaum J., Gomez De Alzaga M., Schafer J., Meyerhof W., Sassolas G., Warnet A.
   Somatostatin receptor imaging in somatotroph and non-functioning pituitary adenomas: correlation with hormonal and visual responses to octreotide.
   Clin. Endocrinol. Oxf. 1997, *47:* 589-598.
3. Kubota A., Yamada Y., Kagimoto S., Shimatsu A., Imamura M., Tsuda K., Imura H., Seino S., Seino Y.
   Identification of somatostatin receptor subtypes and an implication for the efficacy of somatostatin analogue SMS 201-995 in treatment of human endocrine tumors.
   J. Clin. Invest. 1994, *93:* 1321-1325.
4. Plockinger U., Reichel M., Fett U., Saeger W., Quabbe H.J.
   Preoperative octreotide treatment of growth hormone-secreting and clinically nonfunctioning pituitary macroadenomas: effect on tumor volume and lack of correlation with immunohistochemistry and somatostatin receptor scintigraphy.
   J. Clin. Endocrinol. Metab. 1994, *79:* 1416-1423.
5. Rieger A., Rainov N.G., Elfrich C., Klaua M., Meyer H., Lautenschlager C., Burkert W., Mende T.
   Somatostatin receptor scintigraphy in patients with pituitary adenoma Somatostatin receptor scintigraphy in patients with pituitary adenoma.
   Neurosurg. Rev. 1997, *20:* 7-12.
6. Shimon I., Taylor J.E., Dong J.Z., Bitonte R.A., Kim S., Morgan B., Coy D.H., Culler M.D., Melmed S.
   Somatostatin receptor subtype specificity in human fetal pituitary cultures. Differential role of SSTR2 and SSTR5 for growth hormone, thyroid-stimulating hormone, and prolactin regulation.
   J. Clin. Invest. 1997, *99:* 789-798.
7. Greenman Y., Melmed S.
   Heterogeneous expression of two somatostatin receptor subtypes in pituitary tumors.
   J. Clin. Endocrinol. Metab. 1994, *78:* 398-403.
8. Greenman Y., Melmed S.
   Expression of three somatostatin receptor subtypes in pituitary adenomas: evidence for preferential SSTR5 expression in the mammosomatotroph lineage.
   J. Clin. Endocrinol. Metab. 1994, *79:* 724-729.
9. Miller G.M., Alexander J.M., Bikkal H.A., Katznelson L., Zervas N.T., Klibanski A.
   Somatostatin receptor subtype gene expression in pituitary adenomas.
   J. Clin. Endocrinol. Metab. 1995, *80:* 1386-1392.
10. Panetta R., Patel Y.C.
    Expression of mRNA for all five human somatostatin receptors (hSSTR1-5) in pituitary tumors.
    Life Sci. 1995, *56:* 333-342.
11. Florio T., Thellung S., Arena S., Corsaro A., Spaziante R., Gussoni G., Acuto G., Giusti M., Giordano G., Schettini G.
    Somatostatin and its analog lanreotide inhibit the proliferation of dispersed human non-functioning pituitary adenoma cells in vitro.
    Eur. J. Endocrinol. 1999, *141:* 396-408.
12. Jaquet P., Ouafik L., Saveanu A., Gunz G., Fina F., Dufour H., Culler M.D., Moreau J.P., Enjalbert A.
    Quantitative and functional expression of somatostatin receptor subtypes in human prolactinomas.
    J. Clin. Endocrinol. Metab. 1999, *84:* 3268-3276.
13. Jaquet P., Saveanu A., Gunz G., Fina F., Zamora A.J., Grino M., Culler M.D., Moreau J.P., Enjalbert A., Ouafik L.H.

Human somatostatin receptor subtypes in acromegaly: distinct patterns of messenger ribonucleic acid expression and hormone suppression identify different tumoral phenotypes.
J. Clin. Endocrinol. Metab. 2000, *85:* 781-792.

14. Kovacs K., Scheithauer B.W., Horvath E., Lloyd R.V.
The World Health Organization classification of adenohypophysial neoplasms. A proposed five-tier scheme.
Cancer 1996, *78:* 502-510.
15. Snyder P.J.
Extensive personal experience: gonadotroph adenomas.
J. Clin. Endocrinol. Metab. 1995, *80:* 1059-1061.
16. Alexander J.M., Klibanski A.
Gonadotropin-releasing hormone receptor mRNA expression by human pituitary tumors in vitro.
J. Clin. Invest. 1994, *93:* 2332-2339.
17. de Bruin T.W., Kwekkeboom D.J., Van't Verlaat J.W., Reubi J.C., Krenning E.P., Lamberts S.W., Croughs R.J.
Clinically nonfunctioning pituitary adenoma and octreotide response to long term high dose treatment, and studies in vitro.
J. Clin. Endocrinol. Metab. 1992, *75:* 1310-1317.
18. Krenning E.P., Kwekkeboom D.J., Bakker W.H., Breeman W.A., Kooij P.P., Oei H.Y., van Hagen M., Postema P.T., de Jong M., Reubi J.C., Lamberts S.W.
Somatostatin receptor scintigraphy with [111In-DTPA-D-Phe1]- and [123I-Tyr3]-octreotide: the Rotterdam experience with more than 1000 patients.
Eur. J. Nucl. Med. 1993, *20:* 716-731.
19. Duet M., Mundler O., Ajzenberg C., Berolatti B., Chedin P., Duranteau L., Warnet A.
Somatostatin receptor imaging in non-functioning pituitary adenomas: value of an uptake index.
Eur. J. Nucl. Med. 1994, *21:* 647-650.
20. Maini C.L., Sciuto R., Tofani A., Ferraironi A., Carapella C.M., Occhipinti E., Mottolese M., Crecco M.
Somatostatin receptor imaging in CNS tumours using 111In-octreotide.
Nucl. Med. Commun. 1995, *16:* 756-766.
21. Heitz P.U., Landolt A.M., Zenklusen H.R., Kasper M., Reubi J.C., Oberholzer M., Roth J.
Immunocytochemistry of pituitary tumors.
J. Histochem. Cytochem. 1987, *35:* 1005-1011.
22. Reubi J.C., Heitz P.U., Landolt A.M.
Visualization of somatostatin receptors and correlation with immunoreactive growth hormone and prolactin in human pituitary adenomas: evidence for different tumor subclasses.
J. Clin. Endocrinol. Metab. 1987, *65:* 65-73.
23. Hofland L.J., Lamberts S.W.
Somatostatin receptors and disease: role of receptor subtypes.
Baillieres Clin. Endocrinol. Metab. 1996, *10:* 163-176.
24. Plockinger U., Bader M., Hopfenmuller W., Saeger W., Quabbe H.J.
Results of somatostatin receptor scintigraphy do not predict pituitary tumor volume- and hormone-response to octreotide therapy and do not correlate with tumor histology.
Eur. J. Endocrinol. 1997, *136:* 369-376.
25. Duet M., Ajzenberg C., Benelhadj S., Lajeunie E., Lormeau B., Guillausseau P.J., Rohmer V., Vilain D., Mundler O., Warnet A.
Somatostatin receptor scintigraphy in pituitary adenomas: a somatostatin receptor density index can predict hormonal and tumoral efficacy of octreotide in vivo.
J. Nucl. Med. 1999, *40:* 1252-1256.
26. Miller G.M., Alexander J.M., Klibanski A.
Gonadotropin-releasing hormone messenger RNA expression in gonadotroph tumors and normal human pituitary.
J. Clin. Endocrinol. Metab. 1996, *81:* 80-83.
27. Sanno N., Jin L., Qian X., Osamura R.Y., Scheithauer B.W., Kovacs K., Lloyd R.V.
Gonadotropin-releasing hormone and gonadotropin-releasing hormone receptor messenger ribonucleic acids expression in nontumorous and neoplastic pituitaries.
J. Clin. Endocrinol. Metab. 1997, *82:* 1974-1982.