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CLINICAL STUDY

Quantitative analysis of somatostatin receptor subtype (SSTR1–5) gene expression levels in somatotropinomas and non-functioning pituitary adenomas

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Abstract

Objective: It is believed that the variable effectiveness of somatostatin analogs in post-surgical management of somatotropinomas and non-functioning pituitary adenomas (NFPA) may be due in part to variable expression of somatostatin receptor isoforms (SSTR1–5), within and between pituitary tumor types.

Design and methods: Quantitative real-time RT-PCR was used to compare absolute mRNA copy numbers for all five SSTR isoforms in 23 somatotropinomas and 19 NFPA.

Results: Somatostatin receptor subtype 5 mRNA was present at the highest level in somatotropinomas, followed by SSTR2 > SSTR3 ≫ SSTR1 ⋙ SSTR4. In contrast, SSTR3 mRNA was present at the highest level in NFPA, followed by SSTR2, while SSTR1, SSTR4, and SSTR5 transcripts were only detectable in select tumors. Among somatotropinomas, a positive correlation was found between SSTR2 mRNA levels and the percent decrease of GH (%GH) after 3 and 6 months of therapy with octreotide long acting repeatable (LAR) ($r=0.51$ and $r=0.66$; $P=0.05$ and $P=0.008$). Also the percent decrease of IGF-I (%IGF-I) after 3 months of octreotide LAR was negatively correlated with SSTR5 and %IGF-I after 6 months of octreotide LAR was positively correlated with SSTR2.

Conclusions: The present report is a large series examining SSTR mRNA levels in somatotropinomas and NFPA. These initial findings suggest that detailed knowledge of the SSTR mRNA expression profile in somatotropinomas can help to predict the hormonal response to therapy with LAR. Also, it appears that SSTR3 in NFPA may be a potential target for SSTR3 preferential or universal ligands such as pasireotide.

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Introduction

The most common approach of treating patients with somatotropinomas and non-functioning pituitary adenomas (NFPA) is surgical removal of the tumor, with the primary clinical objective being the reduction of mass effects and preservation of pituitary function (1). For somatotropinomas, an additional treatment goal is to reduce growth hormone (GH) hypersecretion, obtaining biochemical control, defined as random GH levels less than 2.5 μg/l, suppression of GH to below 1 μg/l during an oral glucose load and normalization of insulin-like growth factor-I (IGF-I) levels (2). However, surgical 'cure', in acromegaly, occurs in only 50% of the patients with non-invasive macroadenomas (3–5). Among NFPA, tumor regrowth has been reported in 25–75% following craniotomy (6) and 40–50% after transsphenoidal surgery (TSS) of non-irradiated patients (7, 8).

Since surgery alone is frequently not curative, adjuvant medical treatment is often necessary. In acromegaly, the long-acting somatostatin analogs (SA) octreotide and lanreotide are routinely used, and are reported to reduce GH levels to less than 2.5 μg/l in 56–72% of the patients, with normalization of IGF-I in 66–75% (9, 10). In addition, a review of studies (11) reporting the effectiveness of SA as a primary medical management of somatotropinomas showed that the response is variable among patient population, where 51% experience significant tumor shrinkage (6–92% of original volume). In contrast to somatotropinomas, the

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majority of NFPA do not respond to the currently available SA, with respect to reduction of tumor size and re-growth following surgery (12, 13).

The poor response rate of about one-third of the somatotropinomas and the majority of NFPA to the currently available SA therapy has been attributed to inappropriate expression and/or function of somatostatin receptors (SSTR) (14). Somatostatin receptors are G-protein-coupled receptors, which are encoded by five separate genes (SSTR1–5). All the five genes are expressed in normal adult human pituitaries, however, SSTR4 mRNA is found at extremely low levels (15, 16). Somatostatin receptors expression in pituitary adenomas is reported to be highly variable within and between tumor subtypes (17–33). In studies conducted with non-pituitary tissues, all SSTR subtypes have been shown to have anti-proliferative effects in some cell types and SSTR2 and SSTR3 were specifically shown to initiate apoptosis (34). However, the exact SSTR subtypes that are involved in SA-mediated pituitary tumor shrinkage remains to be elucidated. On the other hand, SSTR2 and SSTR5 are clearly established as the primary inhibitors of GH secretion (19, 35). More recently, SSTR1 selective agonists were shown to be effective in suppressing GH release from GH-secreting pituitary adenomas, *in vitro* (22, 36). The currently used long-acting SA, octreotide, and lanreotide, bind preferentially to SSTR2, with a greater than tenfold lower affinity for SSTR5 and minimal binding to the other SSTR subtypes (SSTR3 ≫ SSTR1 ≫ SSTR4) (34). Therefore, the SSTR selectivity of octreotide and lanreotide, coupled with the highly variable expression of SSTR subtypes by pituitary adenomas, may in part explain why only a subset of somatotropinomas and very few NFPA are responsive to the therapeutic actions of the currently available SA. To circumvent this problem, SA with high affinity for SSTR1, SSTR2, SSTR3, and SSTR5 (SOM-230-pasireotide) or with selective specificity for SSTR1 (BIM-23 296 and CH 275) and SSTR5 (BIM-23 206 and BIM-23 268) have been developed and are under investigation for clinical use (37, 38).

The majority of studies which have examined SSTR expression patterns in pituitary adenomas have utilized standard RT-PCR techniques, which can only accurately assess whether the receptor is expressed or not (17, 18, 22–24, 26–29, 31). More recently, quantitative real-time RT-PCR has become available and has been used to assess the absolute levels of SSTR subtypes in somatotropinomas (19, 20, 32, 36) and gonadotropinomas (33). Some of these studies also evaluated the relationship between SSTR1–5 mRNA levels in somatotropinomas and acute *in vivo* or *in vitro* response to octreotide (18–21). In the present study, we have developed and applied this same quantitative technique to compare and contrast the SSTR1–5 mRNA levels in somatotropinomas and NFPA. Preliminary studies were also conducted by examining the relationship between SSTR1–5 mRNA copy number in somatotropinomas and the hormonal response to 3- and 6-month post-surgical treatment with octreotide long-acting repeatable (LAR).

Subjects and methods

Subjects

Tumor tissue samples were obtained from 23 somatotropinomas and 19 NFPA during transsphenoidal surgery. A portion of the tumor was retained for pathological examination and the remaining fragments were snap frozen in liquid nitrogen until RNA extraction or placed in a nucleic acid stabilizing solution (RNA Later, Ambion, Austin, TX, USA) overnight at 4 °C, as specified in the manufacturer's protocol, and then stored in liquid nitrogen until RNA extraction.

Diagnosis of acromegaly was based on the presence of classic clinical features and the lack of GH suppression to <1 μg/l during an oral glucose tolerance test and/or positive GH staining of the tumor specimen by immunocytochemistry. One acromegalic patient (no. 3) exhibited prolactin co-secretion as noted by elevated prolactin levels and confirmed by positive immunostaining for prolactin of tumor specimen. Diagnosis of NFPA was based on the presence of a tumor in the sella turcica visualized by magnetic resonance imaging (MRI) or computed tomography (CT) in the absence of symptoms suggesting hormone hypersecretion and biochemical confirmation of normal or hypofunctioning pituitary. Immunocytochemistry revealing negative immunostaining for all anterior pituitary hormones or positive immunostaining for glycoprotein hormones and/or β-subunit confirmed the diagnosis.

In the acromegalic patients for whom surgery was not curative (defined by the lack of GH suppression to less than 1 μg/l during an oral glucose tolerance test and IGF-I above the upper limit of reference values (ULRV) for age and sex assessed at least 3 months after surgery), medical treatment with octreotide LAR was started with 20 mg at 28 days intervals. After 3 months of therapy, GH and IGF-I levels were assessed on the day of the fourth injection, just before it, and octreotide LAR dose was increased to 30 mg in the next injection if biochemical parameters of acromegaly were not achieved (GH <2.5 μg/l and normal IGF-I for age and sex). To date, we have complete data on 15 of these patients, including GH and IGF-I levels at diagnosis, after surgery and 3 and 6 months after beginning medication.

This study was approved by the Ethics Committee of the Hospital Universitário Clementino Fraga Filho and the Institutional Review Board of the UIC and JBVAMC, Chicago. Informed consent was obtained from each patient before the study.

Selection of primers. All primer sets were selected using genomic sequences obtained from Genbank (National Center for Biotechnology Information (NCBI)) and Primer 3 software (NCBI) with selection parameters set to (i) pick primers that differ by no more than 0.2 °C in annealing temperature, (ii) exclude primers that may form primer dimers, and (iii) amplify a product of 100–200 bp. Sequences of selected primers were used in BLAST (NCBI) searches to check for potential homology to sequences other than the designated target. The primers, the expected product sizes, annealing temperatures, and Genbank accession numbers are provided in Table 1.

Verification of primer specificity. To verify the primer specificity, each primer set was used in a standard PCR (MRI Fermentas PCR Master Mix–Fermentas, Hanover, MD, USA) to amplify cDNA generated by RT (RT; MRI Fermentas First-Strand Synthesis Kit-Fermentas) using random hexamer priming of total RNA isolated from normal pituitary tissue (obtained during surgery for the resection of a somatotropinoma-histological examination revealed normal tissue). Thermal cycling profile consisted of a pre-incubation step at 95 °C for 10 min, followed by 35 cycles of denaturation (95 °C, 1 min), annealing (61–64 °C, 1 min) and extension (72 °C, 1 min). Products were run on an agarose gel and stained with ethidium bromide to confirm that only one band was amplified and no primer dimers formed. An aliquot of the PCR products was then purified using the MinElute PCR Purification kit (Qiagen). Purified PCR products were then sequenced to confirm target specificity. Aliquots of purified PCR products were used to construct standard curves for real-time PCR (see below).

Confirmation of primer efficiency and construction of standard curves – initial screening of primer efficiency was performed using real-time PCR amplifying twofold dilutions of RT products where optimal efficiency was demonstrated by a difference of a CT between dilutions. For real-time PCR, we use the SYBR PCR Master Mix (Bio-Rad). The thermocycling and fluorescence detection were performed using a Stratagene Mx3000p Real-Time PCR machine (Stratagene, La Jolla, CA, USA). If preliminary efficiency tests were confirmed, the concentration of purified PCR products were determined using Picogreen DNA quantification kit (Molecular Probes, Eugene, OR, USA) and the PCR products were serial diluted to obtain standards containing 10^1, 10^2, 10^3, 10^4, 10^5, and 10^6 copies of the template per microliter. One microliter of each standard was amplified by real-time and standard curves generated by the Stratagene Mx3000p Software. The R^2 values for all standard curves generated ranged between 0.997 and 1.003. Primer sets that yielded efficiencies between 90 and 110% were accepted, where an efficiency of amplification of 100% indicates all templates in each cycle are copied. If all validation parameters were met, the selected primers and reaction conditions were used to amplify cDNA from tumor samples.

Quantitative real-time PCR of SSTR subtype cDNA from tumor samples – pituitary tumors were processed for a recovery of total RNA using the Trizol reagent (Life Technologies, Gaithersburg, MD, USA). Total RNA was then purified with the Absolutely RNA kit, with DNAase treatment (Stratagene). The amount of RNA recovered was determined by the Ribogreen RNA quantification kit (Molecular Probes). Total RNA (1 μg) was reverse transcribed (RT) in a 20 μl volume using the reagents supplied in the cDNA First-Strand Synthesis kit (Fermentas) and cDNA was treated with RNAase H. One microliter aliquots of the resulting cDNA were amplified by real-time PCR using the primers specified above. Thermal cycling profile consisted of a pre-incubation step at 95 °C for 10 min, followed by 40 cycles of denaturation (95 °C, 30 s), annealing (61–64 °C, 1 min), and extension (72 °C, 30 s). Total RNA samples that were not reverse-transcribed were run to control for genomic and/or technical DNA

Table 1 Primer sequences, product sizes, annealing temperatures, and GenBank Accession numbers used for quantitative assessment of SSTR1–SSTR5 and housekeeping genes (GAPDH, β-actin, cyclophillin, and HPRT) by real-time RT-PCR.

	Sense	Anti-sense	Product size (bp)	Annealing temperature (°C)	Genbank Accession no.
SSTR1	CACATTTCTCATGGGCTTCCT	ACAAACACCATCACCACCATC	165	61	BC035618
SSTR2[a]	GGCATGTTTGACTTTGTGGTG	GTCTCATTCAGCCGGGATTT	185	61	NM001050
SSTR3	TGCCTTCTTTGGGCTCTACTT	ATCCTCCTCCTCAGTCTTCTCC	190	61	NM001051
SSTR4	CGTGGTCGTCTTTGTGCTCT	AAGGATCGGCGGAAGTTGT	174	63	BC069063
SSTR5	CTGGTGTTTGCGGGATGTT	GAAGCTCTGGCGGAAGTTGT	183	61	NM001053
GAPDH	AATCCCATCACCATCTTCCA	AAATGAGCCCCAGCCTTC	122	64	NM002046
β-actin	ACTCTTCCAGCCTTCCTTCCT	CAGTGATCTCCTTCTGCATCCT	176	64	NM001101
HPRT	CTGAGGATTTGGAAAGGGTGT	TAATCCAGCAGGTCAGCAAAG	157	61	BT019350
Cyclophillin	TGGTCTTTGGGAAGGTGAAAG	TGTCCACAGTCGGAAATGGT	109	61	AF022115

GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HPRT, hypoxanthine ribosyltransferase.
[a]SSTR2 primers amplify both SSTR2A and SSTR2B isoforms.

contamination (background). It should be noted that a standard curve was run with each set of samples to estimate copy number. At the end of the amplification, the final product was subjected to graded temperature-dependent dissociation to verify that only one product was amplified. The detection limit of the method is ten copies.

Internal controls. To control for variations in the amount of sample, RNA used in the RT reaction and the efficiency of the RT reaction, the expression level (copy number) of four commonly used housekeeping genes (glyceraldehyde-3-phosphate dehydrogenase – GAPDH, β-actin, hypoxanthine ribosyltransferase – HPRT and cyclophillin A) was determined for each sample (see Table 1 for primers and annealing temperatures). To determine if these genes were appropriate to use as internal controls, the stability of expression was calculated using the GeNorm 3.3 Visual basic application for Microsoft Excel (http://medgen.ugent.be/~jvdesomp/genorm/) as previously developed and validated by Vandesompele *et al.* (39). This program calculates the average pairwise variation of a particular gene with all other control genes (*M*), allowing for the elimination of the worst scoring control genes and recalculation of new *M* values for the remaining genes. *M* values <1.5 are indicative of a 'stable gene'. The geometric means of copy numbers for the most stable genes are then used as a normalization factor (NF). In our study, calculation of the *M* values, for the four control genes assessed, revealed that the cyclophillin expression was the least stable ($M=1.7$). Therefore, *M* values were recalculated using only GAPDH ($M=0.751$), β-actin ($M=0.826$) and HPRT ($M=1.0420$) where all values were <1.5. Therefore, the geometric means of the copy numbers for these three genes within each sample were used as a NF. Results were then reported as median (minimum–maximum) of SSTR copy number-background/NF.

Statistical analysis

Comparison of the regression lines generated for each SSTR standard set revealed homogeneity of the regression coefficient, indicating that slopes for each standard curve did not differ (SSTR1, 3.5; SSTR2 3.59; SSTR3 3.56, SSTR4 3.44, and SSTR5 3.6), thus allowing for the direct comparison of the corrected mRNA copy number of the various SSTR subtypes within tumor type. Mann–Whitney's non-parametric test was used to compare numeric variables between groups. Correlations between numeric variables were studied using the Spearman's correlation test. *P* values less than 0.05 were considered significant.

Results

SSTR mRNA content in somatotropinomas

Fourteen patients were females (60%). Median age at diagnosis for the whole group was 40 years (16–62 years). A variable amount of mRNA for each of the five SSTR subtypes was found in somatotropinomas (Table 2), with no significant difference between age and sex. Examination of the relative amount of SSTR mRNA isoforms within individual tumors showed SSTR5 was the dominant SSTR isoform in 52% (12/23) of the tumors analyzed (nos 2, 4, 5, 7, 11, 12, 14, 18, 19, 20, 21, and 22), while SSTR2 mRNA was dominant in 39% (9/23; nos 1, 3, 8, 10, 13, 15, 16, 17, and 23; Table 2). In two patients (nos 6, and 9) the predominant mRNA was SSTR3 (Table 2). In sample no. 15, the SSTR mRNA for all SSTR subtypes was low. In this sample, the amount of GAPDH, β-actin, and HPRT mRNA were comparable with those of the other samples tested (data not shown), indicating that the low copy numbers of SSTR mRNA was not due to RNA degradation or poor RT efficiency.

SSTR mRNA content in NFPA

Nine patients were females (47%). Median age during diagnosis in the whole group was 51 years (18–84 years). A variable amount of the five SSTR subtypes mRNA was also found in NFPA (Table 3), with no significant difference between age and sex. Examination of the medians for normalized values for mRNA of each SSTR subtype revealed that SSTR3 mRNA exhibited the highest quantity in NFPA, followed by SSTR2, while SSTR1, SSTR4, and SSTR5 mRNA were only present in select tumors (Table 3). Examination of the SSTR mRNA amount within individual tumors (Table 3) showed SSTR3 was the dominant isoform in 58% (11/19) of the tumors (nos 1, 2, 3, 4, 7, 8, 9, 10, 12, 13, and 17). In the remaining patients, SSTR2 was dominant in three (nos 6, 18, 19), SSTR1 in three (nos 11, 14, and 16), while the level of SSTR5 mRNA copies was dominant in one (no. 5). SSTR4 mRNA was low or undetectable in the majority of NFPA samples with the exception of tumors no. 5 and 15 (Table 3).

Comparisons in SSTR mRNA content between somatotropinomas and NFPA

Overall, somatotropinomas showed higher levels of mRNA for SSTR1, SSTR2, and SSTR5 compared with NFPA, while the levels of SSTR3 did not differ between tumor types (Fig. 1). SSTR4 levels were not studied in comparisons since median expression among both tumor types was very low, except in select patients (acro no. 12 and NFPA no. 5 and 15; Tables 2 and 3).

Table 2 SSTR1-5 mRNA levels in individual somatotropinomas; estimated SSTR mRNA copy number corrected by a normalization factor (NF) derived from the expression of three housekeeping genes (HPRT, β-actin, and GAPDH).

No.	**Age** (year)	SSTR1/NF	SSTR2/NF	SSTR3/NF	SSTR4/NF	SSTR5/NF	NF
Females							
1	24	7	10 238	7	0	2344	2.29
2	26	10	337	66	7	1779	2.94
3	31	102	3010	12	37	1859	0.45
4	31	0	256	77	0	4170	0.54
5	40	35	173	433	5	2300	2.71
6	40	412	84	696	54	639	0.63
7	41	29	134	425	5	2240	1.46
8	42	85	2976	24	18	2729	0.64
9	42	16	90	1226	0	72	1.85
10	45	155	3873	628	57	3204	0.24
11	47	21	676	391	3	3069	0.89
12	48	0	370	217	254	1385	0.23
13	51	23	2981	680	0	2091	0.53
14	59	521	1060	49	4	2931	1.9
Median	41.5	26	523	304	5	2270	0.76
Males							
15	16	23	211	11	1	74	2.79
16	28	450	2418	6	1	1799	0.62
17	28	9	8985	9	1	1421	3.55
18	30	140	916	308	1	1763	1.25
19	34	329	1089	570	1	1310	2.22
20	38	37	419	892	5	2394	0.72
21	50	743	983	0	0	2769	0.39
22	58	184	1669	248	22	2460	0.36
23	62	14	2460	856	2	1168	3.07
Median	34	140	1089	248	1	1763	1.25
All							
Median	40	35	983	248	3	2091	0.89

NF, normalization factor calculated using GeNorm (Vandesompele 2002). GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HPRT, hypoxanthine ribosyltransferase.

Table 3 SSTR1-5 mRNA levels in individual non-functioning pituitary adenomas; estimated SSTR mRNA copy number corrected by a normalization factor (NF) derived from the expression of three housekeeping genes (GAPDH, β-actin, and HPRT).

No.	**Age** (year)	SSTR1/NF	SSTR2/NF	SSTR3/NF	SSTR4/NF	SSTR5/NF	NF
Females							
1	18	0	236	755	0	1	1.81
2	33	84	191	406	47	15	1.00
3	43	0	39	642	21	0	1.03
4	47	0	203	420	0	4	0.83
5	48	0	37	56	674	1417	0.66
6	49	0	382	69	0	7	0.75
7	57	0	74	306	0	7	0.62
8	68	0	12	1067	0	6	1.69
9	84	0	75	1218	0	5	0.57
Median	53	0	75	420	0	6	0.83
Males							
10	31	0	227	496	0	3	0.93
11	40	1360	34	0	0	112	1.72
12	53	0	30	719	49	5	0.46
13	56	0	208	2614	0	4	0.92
14	60	139	145	6	0	4	1.79
15	80	0	778	188	874	2	1.84
16	–	3567	0	0	0	2	0.47
17	–	79	109	463	9	29	1.83
18	–	0	591	31	0	3	1.58
19	–	0	310	26	0	4	1.18
Median	50.5	0	177	110	0	4	0.93
All							
Median	51	0	145	406	0	4	1.00

NF, normalization factor calculated using GeNorm (Vandesompele 2002). GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HPRT, hypoxanthine ribosyltransferase.

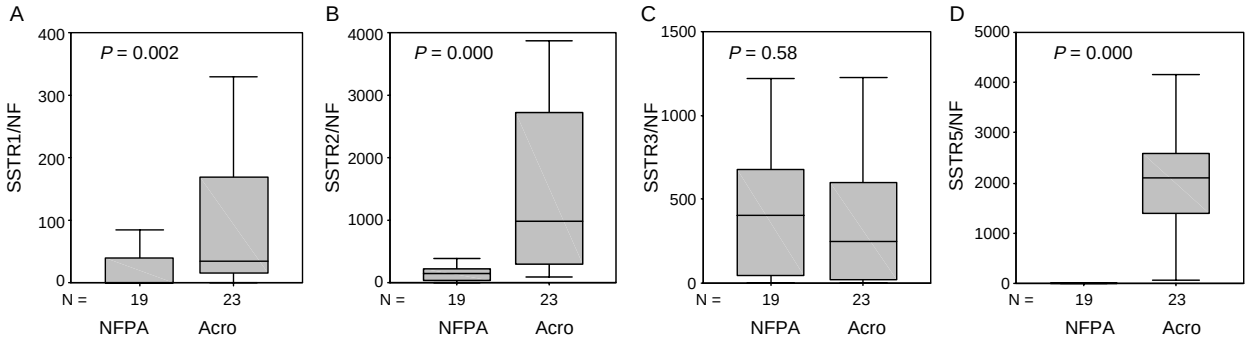

Figure 1 Comparison of SSTR1, 2, 3 and 5 mRNA amount (A to D respectively) between non-functioning pituitary adenomas (NFPA) and somatotropinomas (Acro) corrected for normalization factor (NF). Statistical significance was determined by Mann–Whitney's test. The lower and upper bars represent the first and third quartiles respectively. The line across the box represents median value. The lines above and below the box represent the highest and lowest values, excluding outliers.

Response to treatment with octreotide LAR and correlations with somatotropinoma SSTR1–5 mRNA copy number

Median GH at diagnosis was 32.2 μg/l (3.8–420; $n=20$) and median GH after surgery was 5.6 μg/l (2.3–139; $n=17$). Median IGF-I after surgery was 191.9% of the ULRV (103–616; $n=17$). Complete data on 15 of these patients, including GH and IGF-I levels at diagnosis, after surgery and 3 and 6 months after beginning octreotide LAR is presented in Table 4. Median GH at 3 and 6 months were 2.3 (0.1–12.9) and 2.1 (0.1–30.1) μg/l respectively. Median IGF-I at 3 and 6 months were 97.2 (36–475.2) and 92.0 (22.1–692.4) %ULRV respectively.

A positive correlation was observed between the SSTR2 mRNA levels and the percent decrease of GH (%GH) after 3 and 6 months of therapy with octreotide LAR ($r=0.51$ and $r=0.66$; $P=0.05$ and $P=0.008$ respectively; Table 5). Also a positive correlation was found between the SSTR2 mRNA levels and the percent decrease of IGF-I (%IGF-I) after 6 months of therapy with octreotide LAR ($r=0.56$; $P=0.03$) and a negative correlation was observed between the SSTR5 mRNA levels and the %IGF-I after 3 months of therapy with octreotide LAR ($r=-0.67$; $P=0.007$; Table 5). In contrast, there was no significant relationship observed between response to medical therapy and expression levels of SSTR1, SSTR3, and SSTR4 (Table 5).

The median SSTR2 mRNA levels were higher among patients with normalized IGF-I at 6 months of octreotide LAR therapy (2978×419; $P=0.01$). The median SSTR5 mRNA levels were lower among patients with normalized IGF-I levels at 3 months of octreotide LAR therapy (1779×2427; $P=0.03$; Fig. 2). No such differences among SSTR mRNA levels were found among patients with controlled GH using the criteria of GH <2.5 μg/l or the more strict criteria of 1 μg/l.

Table 4 Hormonal levels at diagnosis, after surgery and 3 and 6 month treatment with octreotide LAR in acromegalic patients.

			At diagnosis	After surgery		After 3 months of LAR				After 6 months of LAR			
No.	Age (year)	Sex	GH	GH	%ULRV IGF-I	GH	%GH decrease	%ULRV IGF-I	% IGF-I decrease	GH	%GH decrease	%ULRV IGF-I	% IGF-I decrease
1	24	F	86.3	139	151.4	4.5	96.8	147.2	2.8	6	95.7	99	34.6
2	26	F	9.2	5.7	110.6	2	64.9	93.4	15.6	2.9	49.5	59.3	46.4
3	31	F	25.7	3.2	103	0.1	95.6	38.9	62.2	0.1	96.9	22.1	78.5
5	40	F	35.2	6.8	221.9	5.4	20.6	160	27.9	3.5	48.5	208.9	5.9
8	42	F	51.3	10.3	171.2	3.3	68	49	71.4	2.7	73.8	64.7	62.2
10	45	F	14.1	4.2	144.8	4.2	0	144.8	0	0.5	88.1	73.2	49.4
11	47	F	35.2	5.4	136.6	4	26.5	475.2	−247.9	4.7	13.6	692.4	−406.9
12	48	F	8	2.3	197.3	2.5	−7.3	145.2	26.4	2.3	1.3	232.6	−17.9
13	51	F	77	2.5	276.3	0.7	72	75.6	72.6	0.2	92.8	52.3	81.1
16	28	M	26.5	5.7	190.9	0.6	88.8	53.4	72	1.7	70.2	95.5	50
19	34	M	92.9	21.3	209.5	0.8	96.1	58.3	72.2	1.9	70.5	97	53.7
20	38	M	120	112	137	12.9	88.5	293.3	−114.1	30.1	91.1	289.9	−111.6
21	50	M	–	7.3	277.5	3	58.9	266.7	3.9	2.5	73.1	221.3	20.3
22	58	M	6.8	2.8	193	0.2	91.8	101	47.7	1.8	65.8	91.8	54.2
23	62	M	127	36	300.4	1.4	96.1	52.9	82.4	0.9	97.5	72.4	75.9

GH in μg/l; ULRV, upper limit of the reference values; LAR, octreotide long acting repeatable.

Table 5 Correlations between the mRNA content for each SSTR subtypes and growth hormone (GH) levels at diagnosis and percent decrease in GH and insulin-like growth factor-I (IGF-I) levels after 3 and 6 months of therapy with octreotide LAR.

	After 3 months with octreotide LAR			After 6 months with octreotide LAR	
	GHat diagnosis (n=20)	% GH decrease (n=15)	% IGF-I decrease (n=15)	% GH decrease (n=15)	% IGF-I decrease (n=15)
SSTR1					
r	−0.76	0.02	0.02	0.11	0.23
P	0.75	0.94	0.9	0.7	0.41
SSTR2					
r	0.18	0.51	0.26	0.66	0.56
P	0.45	0.05*	0.35	0.008*	0.03*
SSTR3					
r	0.14	−0.05	0.11	0.14	0.05
P	0.57	0.87	0.69	0.61	0.87
SSTR4					
r	−0.37	−0.41	−0.17	−0.29	−0.02
P	0.12	0.12	0.55	0.29	0.95
SSTR5					
r	−0.36	−0.41	−0.67	−0.26	−0.31
P	0.12	0.13	0.007*	0.35	0.25

Statistical significance was determined by Spearman's correlation test. *Significant *P* Value.

Discussion

To date, this is the largest series examining SSTR1–5 mRNA levels in somatotropinomas and NFPA and it is the first study to compare the corrected mRNA copy numbers of mRNA for all SSTR subtypes between the tumor types. We report that SSTR5 is the dominant SSTR subtype in 52% of the somatotropinomas tested; while in 39%, SSTR2 mRNA levels were dominant. The current results support and extend the early findings of several groups, which used relative or quantitative real-time PCR and found SSTR5 to be dominant in the majority of the somatotropinomas tested (17–20, 32).

Given the affinity of the long-acting SA, octreotide and lanreotide is above tenfold higher for SSTR2 than for SSTR5 (40, 41), it is expected that patients with tumors expressing higher levels of SSTR2 would respond more favorably to the available SA therapies. In fact, a positive correlation was found between the SSTR2 levels and the percent decrease of GH after a 3- and 6-month and that of IGF-I decrease after a 6-month course of octreotide LAR. Consistent with these findings, several authors described that tumors removed from patients resistant to octreotide therapy tended to have lower levels of SSTR2 mRNA (18, 19, 21). On the other hand, in the current study, the percent decrease in IGF-I levels after a 3-month course of octreotide LAR negatively correlated to SSTR5 levels. This may be due to the fact that tumors expressing high levels of SSTR5 tended to express low levels of SSTR2 (although a negative correlation between these SSTR subtypes did not reach statistical significance – data not shown). On the other hand, the percent decrease in IGF-I levels after a 6-month course of octreotide LAR did not correlate with SSTR5 levels and this finding may be due to the size of the studied sample. Taken together, these reports

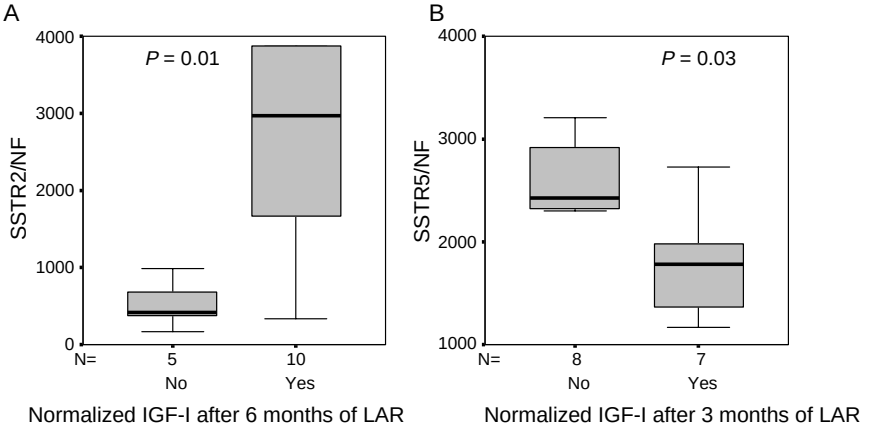

Figure 2 Comparison of SSTR2 and 5 mRNA copy numbers (A and B respectively) between patients with normalized IGF-I after a 3 and 6-month course of octreotide long acting repeatable (LAR) therapy corrected for normalization factor (NF). Statistical significance was determined by Mann–Whitney's test. The lower and upper bars represent the first and third quartiles respectively. The line across the box represents median value. The lines above and below the box represent the highest and lowest values, excluding outliers.

suggest the absolute levels of both SSTR2 and SSTR5 mRNA may be critical for the maximal GH and IGF-I suppressive effects of octreotide.

In the current report, we observed that NFPA, in contrast to somatotropinomas, expressed relatively low or undetectable levels of SSTR1, SSTR2 and SSTR5. Given the likely role of SSTR2 and SSTR5 in mediating the antiproliferative actions of octreotide and lanreotide (21, 22, 36, 42), the limited expression of these receptors in NFPA may explain the relative insensitivity of NFPA to the antiproliferative actions of the current SA therapies (24). However, it should be noted that in the current study, select NFPA did express SSTR1 (nos. 11 and 16), SSTR2 (nos. 15 and 18) and SSTR5 (no. 5) at levels higher than or within the range of somatotropinomas and therefore this small subset of tumors may be responsive to octreotide/lanreotide, as has been reported by a few authors (24, 43) or they may respond to SSTR1 and SSTR5 selective agonists.

Despite the low levels of SSTR1, SSTR2, and SSTR5 mRNA content, NFPA content of SSTR3 mRNA was comparable to that observed in somatotropinomas, where SSTR3 mRNA was dominant in 56% of the NFPA examined. These observations are consistent with the findings of some groups (17, 33) and in contrast to others (26, 27). These discrepancies may be related to the methodology used (real-time PCR vs relative RT-PCR) more than to the population studied.

Nonetheless, the predominance of SSTR3 mRNA in NFPA is intriguing and may be advantageous in targeting medical therapies to reduce mass effects. The importance of SSTR3 in mediating ligand-stimulated cell death was first elucidated by Sharma *et al.* (44), who reported that transfection of CHO cells with SSTR3 exclusively conferred somatostatin apoptotic activity. If ligand activation of SSTR3 mediates similar apoptotic pathways in pituitary cells, we might predict from our current observations that a large portion of NFPA (those that express functional levels of SSTR3) may regress in response to the selective SSTR3 agonists; this may also be the case for a subset of somatotropinomas. Therefore, the recent development of pasireotide, a SA that binds with high affinity to SSTR3, as well as SSTR1, SSTR2, and SSTR5 may prove to be an effective clinical tool in managing the growth of NFPA, as well as somatotropinomas.

The potential to select and predict the outcome of post-surgical medical management by prescreening tumors for functional SSTR has long been a clinical goal. Targeted selection of the most appropriate SA would not only improve clinical outcome (reduction in hormone secretion and tumor mass), but may also be useful in reducing side effects (16). However, *in vitro* data (21, 35) strongly suggest that the maximal effect of SA treatment likely requires activation of multiple receptors that signal through distinct pathways which can interact to evoke synergistic effects. Therefore, in the analysis of SSTR, one should consider not only the level of each SSTR but also the relationship between of the SSTR isoforms. The true predictive value of the present type of analysis will require a large sample population with detailed clinical follow-up to assess the effectiveness of surgical and medical management; however, the results of this preliminary work are encouraging. To date, such comparisons have only been made in small sample populations (19, 20, 32, 33, 36). Therefore to this end, our laboratory is continuing to collect outcome data on the available patients recruited for the present study and are also recruiting additional patients in order to confirm the findings that SSTR2 and SSTR5 mRNA content of somatotropinomas will be useful predictors of the subsequent response to SA therapy.

In summary, this report assesses the mRNA levels of all five SSTR subtypes in somatotropinomas and non-functioning pituitary adenomas by qRT-PCR, and it is the first series to compare the expression levels of each receptor between the tumor types. Our results also indicate that the hormonal response to octreotide in patients with somatotropinomas is positively correlated to SSTR2 and negatively correlated to SSTR5 mRNA levels, suggesting that the assessment of SSTR expression profiles by qRT-PCR may represent an effective screening tool to predict the outcome of post-surgical medical management of somatotropinomas. Also, it appears that SSTR3 in NFPA may be a potential target for SSTR3 preferential or universal ligands such as pasireotide.

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