

RESEARCH

SST5 expression and USP8 mutation in functioning and silent corticotroph pituitary tumors

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Abstract

Objective: Somatostatin receptor type 5 (SST5) is inconsistently expressed by corticotroph tumors, with higher expression found in corticotropinomas having ubiquitin-specific protease 8 (*USP8*) mutations. Aims were to study the correlation between characteristics of corticotropinomas and SST5 expression/*USP8* mutation status and to describe the response to pasireotide in five patients.

Design: Retrospective cohort study.

Methods: Clinico-biochemical, radiological and pathological data of 62 patients, operated for a functioning or silent corticotropinoma between 2013 and 2017, were collected. SST5 expression was measured by immunohistochemistry (clone UMB-4, Abcam, IRS > 1 being considered positive), and Sanger sequencing was performed on 50 tumors to screen for *USP8* mutations.

Results: SST5 expression was positive in 26/62 pituitary tumors. A moderate or strong IRS was found in 15/58 corticotropinomas and in 13/35 functioning corticotropinomas. Among functioning tumors, those expressing SST5 were more frequent in women (22/24 vs 9/15, $P = 0.04$) and had a lower grade ($P = 0.04$) compared to others. *USP8* mutations were identified in 13/50 pituitary tumors and were more frequent in functioning compared to silent tumors (11/30 vs 2/20, $P = 0.05$). SST5 expression was more frequent in *USP8*mut vs *USP8*wt tumors (10/11 vs 7/19, $P = 0.007$). Among treated patients, normal urinary free cortisol levels were obtained in three patients (IRS 0, 2 and 6), while a four-fold decrease was observed in one patient (IRS 4).

Conclusion: SST5 expression appears to be associated with functioning, *USP8*mut and lower grade corticotropinomas. A correlation between SST5 expression or *USP8*mut and response to pasireotide remains to be confirmed.

Key Words

- ▶ SST5
- ▶ corticotroph pituitary tumors
- ▶ USP8
- ▶ pasireotide

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Introduction

Cushing's disease is a rare disorder defined as chronic hypercortisolism due to a corticotropin-secreting pituitary tumor (corticotroph tumor) (1). Chronic cortisol excess is responsible for multisystem morbidity, contributing to increased mortality and altered quality of life (2, 3). In some cases, corticotroph tumors are discovered during examination or analyses in patients showing no clinical signs of Cushing's disease and these are termed silent corticotroph tumors. Diagnosis of such tumors arises because of symptoms related to tumor mass, symptoms of hyperprolactinemia or pituitary deficiency, and they are often classified as non-functioning pituitary tumors (4, 5). Treatment of Cushing's disease is obligatory in order to control cortisol excess and to prevent morbi-mortality. Transsphenoidal surgical resection is the first-line treatment with a remission rate of approximately 75% when the surgery is performed by an expert pituitary surgeon (6, 7, 8). However, recurrence occurs in 15 to 66% of patients and some patients either refuse or do not benefit from the surgery (7). Alternative treatments include additional pituitary surgery, radiation therapy or medical treatment (9, 10). Medical treatments can be divided into the use of drugs that block cortisol production (anticortisol drugs) or block cortisol action (glucocorticoid receptor blockers), which do not control pituitary tumor growth and secretion, and those treatments that target the corticotroph tumor (cabergoline or pasireotide). Pasireotide, a second-generation somatostatin analog, acting on somatostatin receptor (SST) subtype 1,2,3 and 5, is the only approved pituitary-targeting drug for the treatment of Cushing's disease (11, 12, 13, 14, 15). Its efficacy is variable. In phase III studies, 24-h urinary free cortisol (24-h UFC) was reported to be normalized in 20 to 40% of patients after 6 months of treatment; however, it was associated with numerous side-effects including hyperglycemia and diabetes mellitus (13, 14). Predictive markers of response to pasireotide are, however, lacking as has been already been reported to be the case in somatotroph tumors, though an association between the expression of somatostatin receptor subtypes, in particular SST5, and response to treatment has been suggested (16). Most published studies have quantified SST5 mRNA expression in corticotroph tumors using quantitative RT-PCR and reported its expression in 80 to 100% of tumors (12, 17, 18, 19). However, using immunohistochemistry (IHC), SST5 expression was only found in 20 to 55% of corticotroph tumors (20, 21, 22). Somatic driver mutations in the ubiquitin-specific protease 8

(*USP8*) gene, identified in 35% to 60% of corticotroph tumors (21, 23, 24, 25, 26, 27, 28), lead to an increase in the deubiquitinase activity of USP8 and to an increase of ACTH secretion by the tumor (28). SST5 expression has been found to be higher in *USP8*mut corticotroph tumors (21). The increased SST5 expression in *USP8* mutated tumors suggests a positive response to pasireotide in those tumors. The prognosis associated with those mutations is controversial, as they seem to be associated with a greater likelihood of surgical remission (23) as well as a higher risk of recurrence of Cushing's disease (24).

The goal of the present study was to characterize SST5 IHC expression and *USP8* mutations in a large cohort of functioning and silent corticotroph pituitary tumors and to correlate its expression with clinical and biochemical characteristics of patients at diagnosis. Additionally, SST5 expression and response to pasireotide was studied in five patients.

Materials and methods

Patients and assessments

Between January 2013 and December 2017, 76 patients underwent surgery for a corticotroph tumor at the neurosurgical service of University Lyon Hospitals (Hospices Civils de Lyon) carried out by one expert neurosurgeon (E J). Six patients operated for recurrence, without access to initial pathology, were excluded from the study, and eight patients were excluded because of insufficient material being obtained to perform SST5 IHC. Initial clinical presentation data, including sex and age at surgery, preoperative hormonal assessment and pituitary MRI, were collected. Five patients were pre-treated with anticortisol drugs before their surgery, either by ketoconazole ($n=4$) or mitotane ($n=1$). Corticotroph tumors were reported as silent when no clinical or biochemical signs of Cushing's disease were present prior to surgery. Preoperative MRI were reviewed by a pituitary specialist radiologist (V L), and the tumor size, defined as the largest diameter observed on MRI, and cavernous sinus invasion (according to Knosp's classification (29)) were both noted.

The response to pasireotide was studied retrospectively in five treated patients operated for a corticotroph tumor at Lyon Hospital between 2010 and 2018. Patients with combined medical treatment of hypercortisolism were excluded. Initial 24-h-UFC levels were compared with those during and at the end of the treatment, and clinical improvement as well as any side-effects were reported.

The study was approved by the ethics committee of Hospices Civils de Lyon. Consent was obtained from each patient or subject after full explanation of the purpose and nature of all procedures used. Patient information was recorded in a local database (PITUI CARE-Lyon, registered with the French data protection agency CNIL, 16-021, and clinicaltrials.org, NCT 02854228).

Biochemistry

Cortisol and ACTH measurements were performed using automated immunoassays (Roche Diagnostics). For cortisol assays, inter-assay CVs were 1.9% at the 109 nmol/L level, 2.8% at the 333 nmol/L level and 2.8% at the 741 nmol/L level. For ACTH, inter-assay CVs were 9.2 % at the 20 ng/L level, 2.1% at the 52 ng/L level and 2.3% at the 925 ng/L level. 24-h UFC was measured with a direct competitive immunoassay (Abbott Diagnostics). Inter-assay CVs were 3.6%, 3.9%, 2.2% and 3.2% at concentrations of 124.0 nmol/L, 273.2 nmol/L, 465.6 nmol/L and 1035.1 nmol/L, respectively. Data for 24-h UFC were missing in 19 cases, including 15 silent corticotroph pituitary tumors. In accordance with the French Society of Endocrinology guidelines, biochemical exploration of the corticotroph axis was performed in clinically non-functioning pituitary tumors to screen for corticotroph deficiency and to exclude potential silent corticotroph tumors (30).

Pathology

All tumors ($n=62$) were stained for ACTH, SST5, Ki67 and p53 using IHC and were assessed by a single experienced pathologist (A V) who was blinded to the clinical data. Immunohistochemical analysis was performed on 4- μ m paraffin sections using the BenchMark® ULTRA automated immunostainer (Ventana Medical Systems Inc, Tucson, AZ, USA) and the following antibodies and dilutions: anti-ACTH (clone AH26, 1:300, Diagnostic Biosystems, Pleasanton, CA, USA), anti-SST5 (clone UMB-4, 1:250, Abcam), anti-Ki67 (clone MIB1, 1:100, Dako) and anti-p53 (clone D07, 1:100, Dako). SST5 immunohistochemistry required an additional amplification step (amplification kit, Ventana Medical Systems). To evaluate the expression of SST5, only membrane immunopositivity was considered (inter-endothelial junctional immunopositivity served as an internal positive control). SST5 immunopositivity was scored according to the IRS scoring system (Supplementary Table 1, see section on [supplementary materials](#) given at the end of this article). As defined by IRS classification, the staining was considered to be negative for IRS 0 and 1

and positive for IRS >1. Four tumors were considered SST5-positive despite the IRS being not evaluable, as the size of the removed tumor was insufficient to precisely assess the percentage of positive cells. The percentage of ACTH-positive cells and granular and strongly basophilic staining of the cytoplasm were analyzed as indicators of corticotroph tumor differentiation. Proliferation markers (mitotic activity, immuno-expression of p53 and Ki67) were also analyzed. A clinico-pathological classification was applied according to Trouillas *et al.* (29). This classification takes into account cavernous and/or sphenoid sinus invasion and the presence of proliferative markers (presence of at least two out of the three following features: more than two mitotic figures, positive expression of p53 or Ki67-positive cells superior or equal to 3%). In six patients, grading was not feasible because of the small size of the tumoral tissue.

Molecular analysis

USP8 analysis was performed in 50 tumors, while analysis could not be conducted in 12 tumors because of the lack of tumor tissue. Genomic DNA was extracted from formalin-fixed, paraffin-embedded tissue sections using the Maxwell RSC FFPE DNA Kit (AS1450). The DNA sequence was amplified by PCR with the CORE 10 (Mpbio) NH₄(SO₄)₂ Kit (MP Biomedicals, Irvine, CA, USA) using the forward primer 5'-CACCCCTCCAACACTCATAAAG-3' and reverse primer 5'-GTTCTAGGAGTTAAGATAAACATAC-3'. Sanger sequencing of PCR products was performed on the ABI 3500 Dx Analyzer (Applied Biosystems) after having employed the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Results were interpreted with Seqscape Version3 software. In seven complicated cases with results that were difficult to interpret, TOPO cloning was performed after PCR amplification using the TOPO® TA Cloning® Kit (Invitrogen). PCR, sequencing and analyses were carried out following the same protocols.

Statistical analysis

Data are presented as median and ranges (minimum and maximum). The association between SST5 expression (negative or positive) or *USP8* mutations (wild type or mutant) and patient/tumor characteristics were tested using Fisher's exact test for qualitative data and Wilcoxon rank test for quantitative data. Statistical analyses were performed using R software version 3.3.0 (www.r-project.org/). *P*-values <0.05 were considered as significant.

Results

Cohort description

The study population consisted of 62 (44F/18M) patients who were evaluated retrospectively. Patient characteristics are described in Table 1. The median tumor size was 11.5 mm with three tumors that were not visible on MRI which were operated after inferior petrosal sinus sampling. Overall, 26/62 tumors were SST5-positive. Eight different *USP8* mutations were detected in 13 out of the 50 tumors, and one mutation (Ser719del) was found in 7 of the 13 patients (Supplementary Table 2). Of the 62 patients in our cohort, 23 with neither clinical nor biochemical hypercortisolism were diagnosed postoperatively as having silent corticotroph tumors (Table 1).

Patients with silent corticotroph tumors were older than those with functioning corticotroph tumors (P -value=0.03). Although 24-h UFC and 08:00h cortisol were lower in silent tumors (both P -values <0.001), 08:00h ACTH levels were similar at diagnosis (P -value=0.41). While 26/39 functioning corticotroph tumors were microadenomas, silent corticotroph pituitary tumors were mostly macroadenomas (only one was a microadenoma), with a larger diameter (both P -value <0.001), and were more often invasive and positive for p53 (both P -values=0.05). Silent corticotroph tumors were less differentiated, with a lower percentage of ACTH expression (P -value <0.001, Fig. 1). SST5 expression and *USP8* mutations were less frequent in silent tumors compared to functioning corticotroph tumors (P -value <0.001 and 0.05).

Table 1 Clinical, radiological and pathological features of 62 patients operated for corticotroph tumors, categorized by functional characteristics.

	All patients <i>n</i> = 62	Functioning <i>n</i> = 39	Silent <i>n</i> = 23	<i>P</i> -value ^a
Sex ratio F/M	44/18	31/8	13/10	0.08
Age at surgery (years), median (range)	44 (11–75)	40 (11–73)	50 (22–75)	0.03
Cortisol 08:00 h (nmol/L), median (range)	496 (227–1191)	531 (297–1191)	376 (227–808)	<0.001
	NA = 5	NA = 2	NA = 3	
ACTH 08:00 h (ng/L), median (range)	50 (15–204)	55 (15–204)	45 (16–126)	0.41
	NA = 10	NA = 3	NA = 7	
24-h UFC (nmol/24 h), median (range)	377 (49–8657)	620 (102–8657)	96 (49–207)	<0.001
	NA = 19	NA = 4	NA = 15	
Macro adenomas	35	13	22	<0.001
Size on MRI (mm), median (range)	11.5 (0–70)	7 (0–50)	23.8 (7–70)	<0.001
	NA = 1		NA = 1	
Invasive corticotroph tumor	20	9	11	0.05
SST5 positive	26	24	2	<0.001
IRS categories				
Negative	36	15	21	0.001
Mild	7	7	0	
Moderate	12	10	2	
Strongly positive	3	3	0	
	NA = 4	NA = 4		
ACTH expression (%), median (range)	100 (5–100)	100 (30–100)	93 (5–100)	<0.001
	NA = 1		NA = 1	
Ki67 ≥3%	12	10	2	0.1
	NA = 6	NA = 5	NA = 1	
Mitosis > 2	5	4	1	0.64
	NA = 4	NA = 4		
Positive P53 expression	12	4	8	0.05
	NA = 5	NA = 5		
Grade	1a = 34	1a = 23	1a = 11	0.18
	1b = 4	1b = 3	1b = 1	
	2a = 17	2a = 7	2a = 10	
	2b = 1	2b = 1	2b = 0	
	NA = 6	NA = 5	NA = 1	
<i>USP8</i> mutations	13	11	2	0.05
	NA = 12	NA = 9	NA = 3	

^aCalculated using Fisher exact test and Wilcoxon rank test. 24-h FC, 24-h urinary free cortisol; NA, data not available.

Silent corticotroph tumors

Out of the 23 silent tumors, only two were SST5 positive, both with a moderate IRS. *USP8* analyses were performed in 20 tumors and two patients had mutations, both of these being Ser719del (Fig. 2 and Supplementary Table 2). Only one patient simultaneously showed positive SST5 expression and a *USP8* mutation. Among these three patients, with either *USP8* mutation or a positive SST5 expression or both, all were women, aged 16 to 75 years old. All tumors were grade 1a macroadenomas, with sizes varying from 11.5 to 21 mm.

Due to the small number of patients that were SST5-positive or showed *USP8* mutations, no comparisons could be made based on SST5 expression or *USP8* mutations.

Functioning corticotroph tumors

SST5 expression

Markers associated with SST5 expression were studied in the 39 functioning corticotroph tumors (Table 2). Among these, 24 of the patients were SST5-positive and 15 were SST5-negative. Positive SST5 expression was more frequent in women (22/24 vs 9/15, P -value=0.04). Although the grade was found to be lower in SST5-positive tumors (P -value=0.04), invasion was not statistically different for all functioning tumors but a difference was found in the subgroup of macroadenomas. Indeed, SST5-positive macroadenomas were less invasive than SST5-negative macroadenomas (4/24 vs 5/15, P =0.03). *USP8* mutations were more frequent in SST5-positive tumors (10/17 vs 1/13, P -value=0.007). The only significant difference between SST5-positive and SST5-negative functioning microadenomas was patient age, as patients with SST5-positive tumors were significantly older

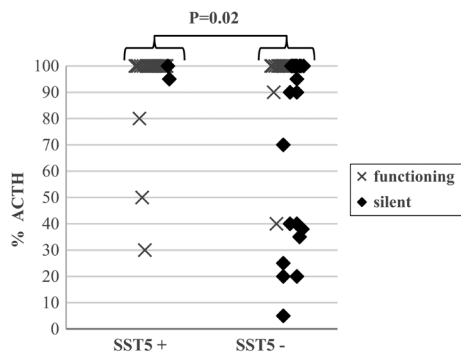


Figure 1 ACTH expression in SST5 positive and negative corticotroph tumors according to the functional status. P -value calculated using Wilcoxon rank test.

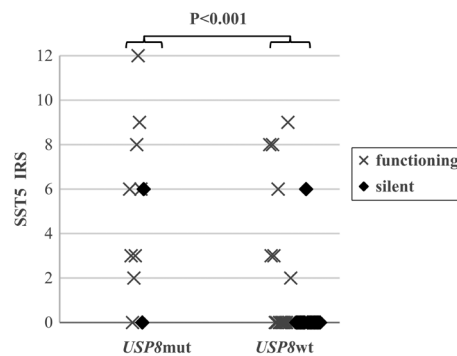


Figure 2 SST5 expression in *USP8*mut or *USP8*wt corticotroph tumors. mut, mutant; wt, wild-type. P -value calculated using Wilcoxon rank test.

(46 vs 29 years, P -value=0.01). Among patients pre-treated by anticortisolic drugs, only one patient pre-treated with ketoconazole had a significant SST5 expression (IRS 6).

USP8 mutations

Among the 20 functioning tumors in which *USP8* sequencing was performed, 11 had *USP8* mutations (Table 3). *USP8*mut corticotroph tumors were more often SST5 positive (10/11 vs 7/19, P -value=0.007) and had a higher IRS (P -value=0.04) compared to *USP8*wt, while no other significant differences were found for other characteristics.

Response to pasireotide treatment

The evolution of 24-h UFC of five patients treated by pasireotide, as well as their IHC results, are presented in Fig. 3. SST5 expression was negative in patient A (IRS 0), while it was positive in four cases with an IRS of 2, 4, 6 and 8 respectively (in patients B, C, D and E). Patients B, D and E were treated due to recurrence of Cushing's disease, occurring between 2 and 6 years after surgery, while patients A and C were treated prior to their pituitary surgery. In all patients, treatment was introduced at a dose of 0.6 mg twice a day. In one patient, the dosage was increased but then rapidly reduced because of digestive side-effects (diarrhea). At the end of treatment, three (A, B and D) out of five patients had a normal 24-h UFC, while a significant change (four-fold decrease) in 24-h UFC was observed in patient C. Clinical improvement of Cushing's symptoms during pasireotide treatment was observed in all cases. Side-effects were frequent with all patients suffering from digestive disorders, while disturbed blood glucose in patients with pre-existing diabetes was observed in four patients and diabetes was newly diagnosed in one patient.

Table 2 Clinical, radiological and pathological features in functioning corticotroph tumors based on SST5 expression ($n = 39$).

	SST5 + $n = 24$	SST5 - $n = 15$	P-value ^a
Sex ratio F/M	22/2	9/6	0.04
Age (years), median (range)	43 (22–73)	33 (11–67)	0.09
Cortisol 08:00 h (nmol/L), median (range)	559 (319–1191) NA = 1	513 (297–978) NA = 1	0.25
ACTH 08:00 h (ng/L), median (range)	59 (16–136) NA = 2	46 (15–204) NA = 1	0.49
24-h UFC (nmol/24 h), median (range)	620 (102–8657) NA = 3	479 (145–2300) NA = 1	0.78
Macroadenomas	8	5	1
Size on MRI (mm), median (range)	7 (0–22)	7 (0–50)	0.65
Invasive corticotroph tumor	4	5	0.27
ACTH expression (%), median (range)	100 (30–100)	100 (40–100)	1
Ki67 $\geq 3\%$	6 NA = 2	4 NA = 3	0.71
Mitosis >2	3 NA = 1	1 NA = 3	1
Positive P53 expression	1 NA = 2	3 NA = 3	0.12
Grade	1a = 18 1b = 1 2a = 2 2b = 1 NA = 2	1a = 5 1b = 2 2a = 5 2b = 0 NA = 3	0.04
USP8 mutations	10 NA = 7	1 NA = 2	0.007

^aCalculated using Fisher exact test and Wilcoxon rank test.
24-h UFC, 24-h urinary free cortisol; NA, data not available.

The reasons for the interruption of treatment were: digestive side-effects and no decrease in tumor volume in patient A, poor observance and difficulty of monitoring for patient B, pituitary surgery in patient C, hepatic cytolysis (five-fold increase in transaminases) and the appearance of gallstones in patient D and bilateral adrenalectomy in patient E.

Discussion

In our cohort of 62 patients, more than one-third of corticotroph pituitary tumors were silent tumors, clinically classified as nonfunctioning pituitary tumors but reclassified after pathology analysis as corticotroph tumors. Overall, USP8 mutations were found in 13/50 tumors and SST5 was expressed in 26/62 tumors, results which are in accordance with previous findings (20, 21, 22, 23, 24, 25, 26, 27, 28). Compared to silent tumors, functioning tumors more frequently showed USP8 mutations and positive SST5 expression, with about one-third expressing SST5 receptor with an IRS either moderate or strong. Our results demonstrated an

association between the presence of USP8 mutation and SST5 expression among functioning tumors. Moreover, functioning corticotroph tumors expressing SST5 were more frequent in women and had a significantly lower grade on clinicopathological classification, suggestive of a better prognosis.

The value of SST5 expression studied by IHC in predicting the response to pasireotide is unknown in corticotroph tumors. In somatotroph pituitary tumors, pasireotide has been shown to decrease GH secretion *in vivo* and *in vitro* and to reduce tumor volume (31, 32, 33), with an efficacy which seems to be correlated to SST5 expression (16). In lactotroph pituitary tumors, pasireotide was recently reported to be effective in a few cases of tumors that were resistant to dopamine agonists and expressed SST5, producing biochemical control and either stabilization or reduction in tumor volume (34, 35, 36). One limiting factor in the evaluation of the role of SST5 is the interpretation of its expression using IHC. The intensity of SST5 expression can be evaluated using different scoring systems, which sometimes take into account cytoplasmic-positivity (16, 22, 37). We chose to consider only membrane immunopositivity and,

Table 3 Clinical, radiological and pathological features in functioning corticotroph tumors based on the presence of *USP8* mutations ($n = 30$).

	<i>USP8</i> mut $n = 11$	<i>USP8</i> wt $n = 19$	P-value^a
Sex ratio F/M	10/1	14/5	0.37
Age (years), median (range)	41 (22–73)	37 (11–67)	0.64
Cortisol 08:00 h (nmol/L), median (range)	547 (366–922)	531 (297–1191)	0.76
ACTH 08:00 h (ng/L), median (range)	NA = 1 55 (16–119)	NA = 1 54 (15–204)	0.84
24-h UFC (nmol/24 h), median (range)	NA = 1 214 (10–8657)	NA = 2 465 (145–2300)	0.26
Macroadenomas	5	7	0.71
Size on MRI (mm), median (range)	8 (7–22)	7 (0–50)	0.18
Invasive corticotroph tumor	2	5	1
SST5 positive	10	7	0.007
IRS categories			
Negative	1	12	0.04
Mild	3	3	
Moderate	3	3	
Strongly positive	2	1	
	NA = 2		
ACTH expression (%), median (range)	100 (30–100)	100 (40–100)	0.54
Ki67 $\geq 3\%$	3	5	1
		NA = 2	
Mitosis >2	3	1	0.27
		NA = 2	
Positive P53 expression	2	1	0.54
		NA = 2	
Grade	1a = 7 1b = 2 2a = 1 2b = 1	1a = 12 1b = 0 2a = 5 2b = 0 NA = 2	0.09

^aCalculated using Fisher exact test and Wilcoxon rank test.

24-h UFC, 24-h urinary free cortisol; NA, data not available; mut, mutant; wt, wild-type.

after comparison of SST5-positive and -negative tumors, to use the IRS scoring system in order to more precisely evaluate SST5 expression. This methodology has been employed in previous studies to assess SST expression in pituitary tumors (21, 38, 39), which allowed a more reliable comparison between studies.

In functioning corticotroph tumors, pasireotide is indicated in order to decrease hypercortisolism. *In vitro* studies on corticotroph tumor cells have found a decrease in ACTH release following use of pasireotide, which seems to be mediated by SST5 (12, 17, 40, 41, 42). Indeed, SST5 is the most highly expressed somatostatin receptor in corticotroph tumors (12, 20, 42), and it has been shown in previous studies that octreotide, which targets SST2, is less effective than pasireotide in decreasing cortisol levels and that SST2 is downregulated in the context of exposure to high glucocorticoid levels (41, 42). The response rate to pasireotide treatment in Cushing's disease obtained

in previously published clinical trials was between 20 to 40% (13, 14), which is in agreement with the number of patients with functioning corticotroph tumors expressing SST5 with an IRS score of moderate or strong in our cohort, suggesting that only one-third of functioning corticotroph pituitary tumors are likely to be sensitive to pasireotide treatment. In our study, functioning tumors that were SST5-positive had a lower grade and probably a lower risk of recurrence, suggesting that these tumors are less likely to require pasireotide treatment. However, it has been previously reported that *USP8*mut tumors had a higher risk of recurrence (24). The correlation between the expression of SST5 in corticotroph tumors and the efficacy of pasireotide is lacking. Unfortunately, only five patients operated for a corticotroph tumor in Lyon between 2010 and 2018 were treated with pasireotide monotherapy, without combined medical treatment of their hypercortisolism. Four patients expressed SST5,

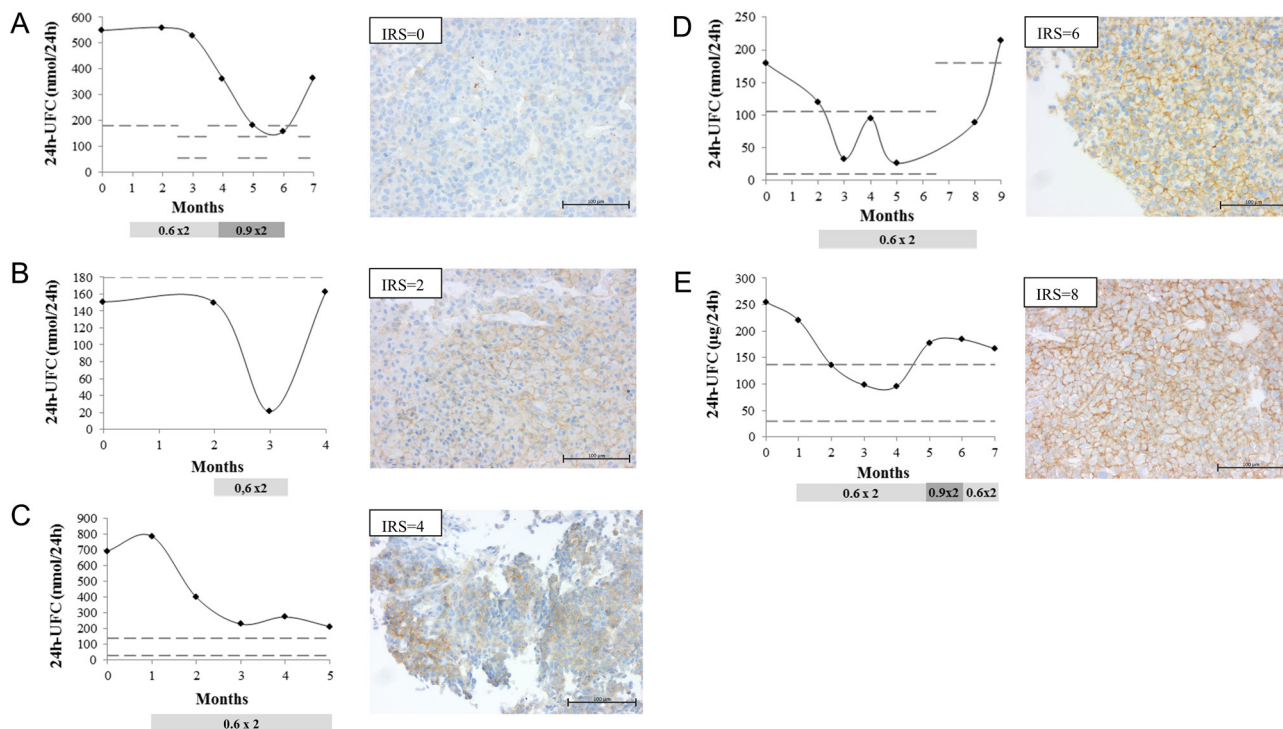


Figure 3

Evolution of 24-h UFC after introduction of pasireotide in patients A to E (left) and SST5 immunohistochemistry (original magnification $\times 200$) (right). 24-h UFC, 24-h urinary free cortisol; IRS, immunoreactive score. Dotted lines on graphs represent 24-h-UFC norm, and daily dosage of pasireotide is represented below each graph, expressed in mg. Photographs were taken in the most immunoreactive area and are not representative of the whole tumor.

one patient showed mild expression and three patients had moderate expression. Though an improvement of hypercortisolism and of Cushing's symptoms was observed in most of the patients, including those that were SST5-negative, the small number of patients and the difference in duration of treatment do not allow us to reach firm conclusions. Pasireotide was used in preoperative treatment, showing a good response in two patients, with an IRS of 0 and 4. It has been reported that preoperative octreotide treatment can result in decreased SST2 expression. SST trafficking is modulated by pasireotide in a different manner than its modulation by octreotide (40, 43). However, as the effect of preoperative treatments on SST5 is not yet well understood, it is not possible to exclude a decrease in SST5 expression following pasireotide treatment.

In previous *in vitro* and *in vivo* studies, pasireotide has also been associated with an anti-tumoral effect (12, 44, 45, 46). A recent publication reporting the use of long-acting pasireotide demonstrated that median tumor volume decreased by 17.8% and 16.3%, respectively, after 12 months of treatment with pasireotide at 10 or 30 mg/month in patients with Cushing's disease (14).

However, the role of SST5 in this anti-tumoral effect is controversial (12, 44). If the anti-tumoral effect of pasireotide is partly mediated via SST5, it suggests that silent corticotroph pituitary tumors may not be good candidates for pasireotide treatment, since only 2 out of the 22 patients in our cohort with silent tumors expressed SST5. These results are in agreement with the lower level of SST5 mRNA found in silent corticotroph tumors in comparison to functioning corticotroph tumors that was reported in a previous study (19). On the contrary, SST5 expression was present in 8 of the 13 patients with a functioning macro-corticotroph tumor in our cohort, suggesting that those tumors are more likely to respond to pasireotide. This result is interesting, since macroadenomas associated with Cushing's disease present both a lower rate of remission as well as a higher rate of recurrence (47, 48).

In published studies consisting of 11 and 20 patients with silent corticotroph tumors, somatic mutations of *USP8* were found in 0% to 20% of those tumors (25, 49). In contrast, *USP8* mutations were found in 24 to 62% of functioning corticotroph tumors (21, 23, 24, 25, 26, 27, 28), with a higher level of SST5 expression in

*USP8*mut tumors (21), which is consistent with the results of our study. However, unlike previous findings, mutations in our cohort were not statistically associated with female gender (21, 23, 25, 50) and no differences were found for the age at diagnosis or the tumor size (21, 24, 25). The *USP8* gene encodes a protein with a deubiquitinase activity, which inhibits the degradation of the EGF receptor (EGFR). The activity of *USP8* is regulated by the 14-3-3 protein, and all known mutations are found in exon 14, within or close to the 14-3-3 protein binding motif (715–720). Among the mutations found in our cohort, five were previously known mutations (Ser719del, Pro720Arg, Ser718Cys, Ser718Pro and Pro720-Gln724del) (21, 25, 51, 52, 53). Novel mutations were found in two patients, one adjacent to the binding motif (Asp721Glu) in one patient and two mutations in the other patient (Ser719_Gln724delinsLeu and Asn741Asp). The presence of double *USP8* mutations in the same patient has already been reported (28, 54). In our case, apart from the mutation affecting the 14-3-3 protein binding motif (Ser719_Gln724delinsLeu), the functional impact of the other mutation is difficult to assess. Previous studies have found a greater likelihood of surgical remission (23) as well as a higher risk of recurrence of Cushing's disease (24) in *USP8*mut tumors, while in our study no difference was found between functioning tumors with or without mutations in terms of tumor grade. IHC for *SSTR5* is not routinely performed during histopathological analysis of pituitary tumors. The presence of *USP8* mutations, frequently associated with positive *SSTR5* expression, could be used as a marker to predict *SSTR5* expression and then response to pasireotide treatment. However, such molecular analyses are more costly to perform than immunohistochemistry.

This study has several limits, such as its retrospective nature and the presence of missing data. The number of patients studied, which may seem small, can be explained by the rarity of corticotroph pituitary tumor. Response to pasireotide could be studied only on a small number of patients.

In conclusion, *SSTR5* expression and *USP8* mutations are more frequently found in functioning corticotroph tumors. We did not identify clinical predictive marker of *SSTR5* expression, except for the association with functioning tumors and patient gender. The value of *SSTR5* expression as a theranostic marker of response to pasireotide by corticotroph tumors should be the subject of future studies on a larger prospective cohort and may represent a new avenue for personalized therapy.

Supplementary materials

This is linked to the online version of the paper at <https://doi.org/10.1530/EC-20-0035>.

Declaration of interest

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