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ORIGINAL ARTICLE

The silent variants of pituitary tumors: demographic, radiological and molecular characteristics

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Abstract

Introduction Tumors of the anterior pituitary gland (PTs) are mostly benign tumors with a low prevalence, which has nevertheless increased with advances in brain radiology techniques. Nearly half of PTs are not associated with a clinical endocrine syndrome. These tumors have been indistinctly named non-functioning pituitary adenomas (NFPAs) or silent pituitary tumors (SPTs) and the mechanisms of silencing are not fully known.

Aim To study the frequency and characterize the silent variant of PTs in a large local series, and to assess their pituitary adenohypophyseal gene expression.

Methods This observational, cross-sectional study was performed in a Pituitary Tumor Center of Excellence and involved 268 PTs. After identifying the different subtypes according to the immunohistochemical (IHC) expression of adenohypophyseal hormones, we studied their gene expression by RT-qPCR.

Results We found that silent tumors were larger and more invasive, but not more proliferative than their functional counterparts. The RT-qPCR complements the IHC typification of PTs, reducing the proportion of null-cell subtype. Finally, some silent PT subtype variants showed lower specific adenohypophyseal hormone gene expression than their functional counterparts, which may contribute to the absence of endocrine manifestations.

Conclusions This paper highlights the importance of identifying the silent variant of the PTs subtypes. As expected, silent tumors were larger and more invasive than their functioning counterparts. However, there was no difference in the proliferation activity between them. Finally, the lower specific gene expression in the silent than in the functioning counterparts of some PTs subtypes gives insights into the silencing mechanisms of PTs.

Keywords Pituitary tumors · Non-functioning pituitary adenomas (NFPA) · Silent pituitary tumors (SPTs) · Adenohypophyseal hormone gene expression

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Introduction

The prevalence of pituitary tumors (PTs) has increased with the use of highly sensitive brain imaging techniques. Recent studies have estimated prevalence to be around 1 per 1000 population. Historically, most PTs were diagnosed as prolactin-producing tumors. However, important advances in brain imaging techniques have changed this profile, and now non-functioning PTs have emerged as the most prevalent ones, both in surgical and non-surgical series [1]. The term non-functioning pituitary adenoma (NFPA) has been widely used in the literature. NFPAs are benign adenohypophyseal tumors not associated with a clinical endocrine syndrome. The acronym has usually been related with gonadotroph and null-cell (NC) tumors. But most series include other tumor subtypes, such as silent corticotropinomas and other silent subtypes, under the NFPA umbrella [2]. Altogether, different series estimate that NFPAs represent 20–50% of pituitary tumors [2–5], of which 5–30% have typically been considered null cell tumors (NCTs) [6].

The identification of the specific pituitary-cell lineage of the NFPAs is very important because the behavior of silent corticotroph tumors (CTs) can be different than that of silent gonadotroph tumors (GTs) [7–9]. In this way it is expected that the incorporation of the analysis of pituitary-cell lineage transcription factors to the IHC study of PTs, according to the recommendations laid out in the World Health Organization's (WHO) Classification of Pituitary Tumors [10], will allow a better typification of NFPAs. Furthermore, previous results of our research group demonstrated that the molecular study of the gene expression of the adenohypophyseal hormones complements the IHC ones, allowing a more accurate identification of NFPAs [11, 12].

The silencing mechanisms of PTs are not fully understood. While there are plenty of theories on silent corticotroph tumors (S-CTs) [13–19], the information for other PTs subtypes is scarce. To date, no study has explored the hypothesis that low gene expression of pituitary hormones could be related to the absence of hormonal hypersecretion in PTs. Therefore, the aims of the present study were to calculate the prevalence of the PTs subtypes in a large sample of 268 pituitary tumors, according to the protein (IHC) or gene expression (RT-qPCR) of adenohypophyseal hormones. In addition, to analyze the demographic, clinical, radiological and molecular differences between the functioning and silent counterparts PTs subtypes.

Materials and methods

The study was conducted in a Pituitary Tumor Center of Excellence and in the Research Laboratory of the Institute for Health and Biomedical Research of the Alicante General University Hospital-University Miguel Hernández. This study complies with the Declaration of Helsinki and was approved by the local ethics committee (Ref. CEIm:PI2018/127 Ref.ISABIAL: 180361). All patients signed their informed consent.

Samples

We selected 268 PTs with clinical, biochemical, radiological, IHC, and molecular information from the pituitary tumor collection of the Alicante General University Hospital biobank (Supplemental Table 1). PTs in this collection come from four university hospitals within the Spanish Molecular Registry of Pituitary Adenomas (REMAH) network: Alicante General University Hospital, University Hospital La Ribera, Polytechnic University Hospital La Fe, and the University of Albacete Hospital Complex.

PTs subtypes were identified based on the IHC expression of pituitary-specific hormones, following the recommendations of the 2017 WHO classification of tumors, but without taking into account the pituitary-lineage transcription factors [20].

Demographic, clinical, hormonal, and radiological variables

Demographic, clinical, hormonal and radiological information of the patients were anonymously collected from the REMAH 2.0 and local databases. Variables were categorized depending on whether or not PTs were functioning or silent.

Serum prolactin (PRL), cortisol, thyroid-stimulating hormone (TSH), and free thyroxine (FT4) were quantified by electrochemiluminescence immunoassay using a Cobas 801 automated autoanalyzer (Roche Diagnostics, Mannheim, Germany). Adrenocorticotropic hormone (ACTH) was measured in plasma on the same analyzer. Growth hormone (GH) was measured using the Immulite analyzer (Siemens Diagnostics; Marburg, Germany; Siemens Medical Solutions Diagnostics Limited, Glyn Rhonwy, Llanberis, UK) and IGF-1 on the Liaison analyzer (DiaSorin, Inc, Stillwater, MN, USA), both by chemiluminescent immunoassay.

Tumor size was defined by the maximum tumor diameter observed on magnetic resonance imaging [21]. Invasiveness was graded according to the Knosp grades (invasive tumors: grades III–IV; non-invasive tumors: grades I–II) [22].

Immunohistochemical studies

IHC studies were carried out in the pathology departments of the four participating reference hospitals. Formalin-fixed paraffin-embedded tissue was used with standard automated techniques in the Autostainer Link48 (Dako-Agilent) with the Envision (Dako) high-sensitivity visualization system.

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Samples of 2 mm paraffin cylinders were taken from each tumor to build a block using a matrix tissue device (Beecher instruments). Each block included 20 cases plus 2 controls.

Immunostaining was performed against the following pituitary hormones: GH, PRL, ACTH, follicle-stimulating hormone (FSH), luteinizing hormone (LH), TSH and alpha subunit. Immunostaining was detected using the Envision kit in an automatic immunostainer. PTs were scored based on the percentage of immunoreactivity of positive cells ($3 \geq 67\%$; $2 = 34–66\%$; $1 = 5–33\%$; $0 = 0–4\%$) or semi-quantitatively ($3 = +++$, $2 = ++$, $1 = +$, $0 = 0$).

Cell proliferation was estimated by IHC of the MIB1-LI/Ki-67 staining index. Ki-67 quantification was carried out on hot spots in complete sections. At least 500 cells were quantified at each point, considering any intensity of nuclear staining positive. The result was expressed as a percentage, with Ki67 of more than 3% defined as high proliferation [23].

Molecular studies

Molecular studies were centralized in the Alicante General University Hospital-Institute for Health and Biomedical Research (ISABIAL) Research Laboratory. All 268 samples were preserved immediately after surgery in RNAlater solution at 4 °C for 24 h and then stored at − 20 °C. The biological samples were disintegrated in the TissueLyser (Qiagen, Hilden, Germany). We used the AllPrep DNA-RNA-Protein kit (Qiagen) for manual RNA extraction and measured their concentration and purity in the Nanodrop spectrophotometer (Thermo 465 Scientific, Waltham, MA, USA). For each retrotranscription reaction, we used 2 µg of RNA in a total volume of 20 µL, employing the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems).

We performed RT-qPCR following the manufacturer's instructions in the 7500 Fast Real-Time PCR System (Life Technologies, CA, USA) using: SYBR Green primers in 133 tumors [12], and TaqMan Fast Advanced PCR Master Mix and assays based on hydrolysis probes (TaqMan Gene Expression Assays, Life Technologies) in the remaining 135 tumors [11]. The use of two methods did not affect the study since they have both shown good correlation with clinical diagnosis [11]. Specifically, gene expression levels of all pituitary hormones, type 1 corticotrophin-releasing hormone receptor (*CRHR1*) and arginine vasopressin receptor 1b (*AVPR1B*) were measured.

A pool of RNA from nine normal pituitary samples obtained from autopsies served as a calibrator. All samples were analyzed in duplicate. The relative differences in gene expression were expressed as fold change (FC) and were obtained with the $2^{-\Delta\Delta C}$t method (SDS software, Applied Biosystems).

Statistical analysis

Qualitative variables, including PTs subtypes, were expressed as absolute and relative frequencies. Participant age was expressed as mean ± standard deviation (SD). The Shapiro–Wilk test was used to determine the normality of the distribution of the quantitative variables. Associations between demographic variables (age, sex) and clinical variables (functioning tumor vs. silent tumor) with molecular variables (FC) were analyzed by the Student's *t*, Mann–Whitney, Kruskal–Wallis, or Chi squared tests, as appropriate. *p* values of less than 0.05 were considered statistically significant. Statistical analysis was performed with the SPSS 15.0 software (IBM Software, Armonk, NY, USA).

Results

Demographic, clinical and radiological characteristics of the overall sample (*n* = 268)

Most patients (95.5%) were aged older than 25 years (mean 49.74 ± 15.37), 51.9% were women, and 89.6% were macroadenomas, of which 56.3% were invasive. One hundred sixty-one (60.1%) tumors were silent, including all GT and NCT.

Distribution of PTs subtypes in the whole series, by their IHC or pituitary-hormone gene expression

Table 1 shows the distribution of the 268 PTs of the series along with the functioning and silent variants of the different subtypes, according to IHC or molecular identification. The frequency of the different subtypes changed depending on the method of identification. The most important differences between IHC and molecular identification were observed in NCTs and GTs.

Demographic, clinical, and radiological differences between functioning and silent PTs subtypes according to IHC identification

Table 2 presents the prevalence of the different subtypes in the whole sample, the functioning and silent counterparts within them, and their demographic, biochemical, and radiological characteristics. Silent tumors, as a whole, were larger (26.65 ± 9.76 mm vs 17.2 ± 10.20 mm, $p < 0.001$) and more invasive (65.8% vs 40.2%, $p < 0.001$) than functioning ones. Moreover, most (61.7%) were

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Table 1 IHC and molecular characterization of PTs subtypes of the series, according to their clinical diagnosis

Clinical diagnosis	*n*	IHC subtype	Molecular subtype
Acromegaly	68	35 ST 28 ST MIXED 3 PH 2 NCT	42 ST 7 ST MIXED 13 PH 2 NCT 2 TT 1 GT 1 LT
Cushing	28	11 CT 2 NCT 14 PH 1 ST	23 CT 4 PH 1 NCT
Hyperprolactinemia	12	11 LT 1 ST MIXED	8 LT 1 CT 3 NCT
Hyperthyroidism	3	1 TT 1 ST 1 ST MIXED	3 TT
Non-functioning	157	56 NCT 40 GT 14 CT 9 LT 17 PH 8 ST 10 ST MIXED 3 TT	30 NCT 80 GT 20 CT 6 LT 10 PH 2 ST MIXED 9 TT
Total	268	268	268

PT pituitary tumor, *IHC* immunohistochemistry, *F* functioning, *S* silent, *ST* somatotroph tumor, *ST Mixed* somatotroph–lactotroph tumor, *CT* corticotroph tumor, *LT* lactotroph tumor, *TT* thyrotroph tumor, *GT* gonadotroph tumor, *PH* plurihormonal, *NCT* null cell tumor

found in men, and they were diagnosed later in life than their functioning counterparts (54.71 ± 14.84 years vs 42.25 ± 12.98 years; $p < 0.001$).

Adenohypophyseal hormone gene expression in silent versus functioning variants of PTs subtypes, as identified by IHC

Figure 1 shows the differences in the pituitary hormone gene expression between the different PTs subtypes. In short, pure and mixed STs and LTs expressed more *GH* and *PRL*, respectively, than their silent counterparts. In contrast, there were no significant differences in the expression of *POMC*, *CHRH* and *AVPR1B* between functioning and silent CTs. Functioning plurihormonal tumors (FPH), as a whole, showed higher expression of *PRL*, *POMC* and *AVPR1* and lower expression of *FSH* than their silent counterparts, regardless of whether they were PH-Pit 1 or PH-unusual.

Discussion

This study aimed to properly identify the silent counterparts of a large series of PTs subtypes and assess the differences with the functioning ones. The main strengths of the research are the large number of tumors studied and the molecular analyses performed. The most relevant limitation is that the tumors came from four different hospitals, and, unlike the RT-qPCR, the IHC procedures were not centralized.

Definition of non-functioning PTs

An important percentage of PTs do not present a recognizable endocrine syndrome and are only diagnosed due to their neuro-ophthalmological manifestations or incidentally during the performance of a brain imaging technique. In a recently published and very large series of 1055 pituitary tumors, 48.2% of the global series demonstrated this behavior [24], compared to 60.1% in ours. Traditionally, these tumors have been known as NFPAs, but specialists increasingly prefer the term silent pituitary tumor (SPT) [2] to define pituitary tumors that express some adenohypophyseal hormones or their transcription factors on IHC but do not secrete clinically relevant hormones [2, 25]. This overlapping terminology generates confusion and complicates comparisons between series. Therefore, we agree with these authors that the term NFPA should be substituted by the more specific SPT following surgery and once the immunohistochemical study is available.

Typification of PTs subtypes

The principal challenge in the identification of non-functioning PTs subtypes has been the limited accuracy of IHC in characterizing the expression of adenohypophyseal hormones, with the main surgical series reporting a high percentage of apparently NCTs [6, 26]. In the present study, the percentage of non-functioning PTs largely depended on the technique used to identify them. Strikingly, the percentage of NCTs dropped from 21 (56/268) to 11.2% (30/268) and the GTs increased from 14.9 (40/268) to 29.8% (80/268) by IHC and by RT-qPCR respectively, (Table 1).

Fortunately, the introduction of the measurement of PTF of pituitary cell lineage, according to the WHO 2017 recommendations [27], has allowed a better typification of non-functioning PTs. Indeed the number of NCTs has decreased and the number of GTs and S-CTs has increased in the main series published [24, 28]. Nishioka et al. reclassified 119 IHC NCTs (out of 516 consecutive NFPAs) as gonadotroph (SF-1 positive; 66.4%), corticotroph (Tpit positive; 26.9%) or Pit-1 positive (6.7%)

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Table 2 Clinical, pathological and radiological differences between functioning and silent PTs subtypes according to their IHC identification

Subtype	Total *n* (%)	Functioning *n* (%)	Silent *n* (%)	*p* value
ST [*n* (%)]	45 (16.79)	37 (82.22)	8 (17.78)*	
Age [(years), mean ± SD]	44.67 ± 12.76	44.14 ± 12.96	47.3 ± 12.28	0.63
Women [*n* (%)]	28 (62.22)	25 (89.3)	3 (10.7)	0.12
Macro [*n* (%)]	43 (95.55)	35 (94.6)	8 (100)	0.37
Tumor diameter [(mm), mean ± SD]	19.6 ± 9.65	18.81 ± 9.41	23.25 ± 10.54[a]	0.18
Invasive [*n* (%)]	21 (46.67)[b]	16 (43.2)	5 (62.5)	0.32
Proliferative [*n* (%)]	5 (11.1%)	5 (13.5%)	0 (0%)	na
GH [ng/mL, (*n*) mean ± SD]		(33) 20.78 ± 24.16	(5) 2.36 ± 2.73	0.009
IGF-1 [ng/mL, (*n*) mean ± SD]		(36) 864.29 ± 361.69	(6) 204.17 ± 135.19	0.001
Mixed ST [*n* (%)]	40 (14.92)	30 (75)	10 (25)	
Age [(years), mean ± SD]	45.80 ± 11.02	45.17 ± 10.39	47.7 ± 13.14	0.77
Women [*n* (%)]	20 (50)	16 (80)	4 (20)	0.46
Macro [*n* (%)]	33 (82.5)	24 (80.0)	9 (90.0)	0.45
Tumor diameter [(mm), mean ± SD]	17.79 ± 9.17	16.0 ± 8.41	22.8 ± 9.81[a]	0.045
Invasive [*n* (%)]	18 (45)[b]	12 (40.0)	6 (60.0)	0.27
Proliferative [*n* (%)]	4 (10)	3(10)	1(10)	na
GH [ng/mL, (*n*) mean ± SD]		(24) 22.56 ± 36.58	(8) 0.244 ± 0.13	< 0.001
IGF-1 [ng/mL, (*n*) mean ± SD]		(25) 879.52 ± 431.67	(8) 136.95 ± 60.036	< 0.001
PRL [ng/mL, (*n*) mean ± SD]		(23) 161.29 ± 463.52	(9) 35.88 ± 31.57	0.867
CT [*n* (%)]*	25 (9.32)	11 (44)	14 (56)**	
Age [(years), mean ± SD]	41.52 ± 13.25	40.27 ± 10.98	42.5 ± 15.13	0.76
Women [*n* (%)]	13 (52)	7 (53.8)	6 (46.2)	0.30
Macro [*n* (%)]	19 (76)	5 (45.4)	14 (100)	< 0.001
Tumor diameter [(mm), mean ± SD]	20.67 ± 13.25	13.55 ± 9.83	26.69 ± 13.57[c]	0.016
Invasive [*n* (%)]	10 (40)	4 (36.4)	6 (42.9)	0.49
Proliferative [*n* (%)]	3 (12)	1 (9%)	2 (14.2)	na
Serum cortisol [μg/dL, (*n*) mean ± SD]		(11) 23.62 ± 11.99	(10) 18.95 ± 12.32	0.39
ACTH [pg/mL, (*n*) mean ± SD]		(11) 98.49 ± 60.83	(7) 103.23 ± 83.76	0.751
LT [*n* (%)]	20 (7.46)	11 (55)	9 (45)	
Age [(years), mean ± SD]	48.25 ± 22.92	35 ± 20.61	64.44 ± 13.42	0.007
Women [*n* (%)]	8 (40)	3 (37.5)	5 (62.5)	0.20
Macro [*n* (%)]	19 (95)	10 (90.9)	9 (100)	0.27
Tumor diameter [(mm), mean ± SD]	27.95 ± 12.03	26.45 ± 14.90	29.78 ± 7.71	0.22
Invasive [*n* (%)]	13 (65)	7 (63.6)	6 (66.7)	0.89
Proliferative [*n* (%)]	3 (15)	2(18.2)	1 (11.1)	na
PRL [ng/mL (*n*) mean ± SD]		(10) 2379.34 ± 4965.71	(9) 49.90 ± 42.66	0.022
TT [*n* (%)]	4 (1.49)	1 (25)	3 (75)	
Age [(years), mean ± SD]	51.25 ± 16.03	40	55 ± 17.35	0.35
Women [*n* (%)]	0 (0)	0 (0)	0 (0)	na
Macro [*n* (%)]	4 (100)	1 (100)	3 (100)	na
Tumor diameter [(mm), mean ± SD]	26.75 ± 17.84	10 ± 0	32.33 ± 17.04	0.18
Invasive [*n* (%)]	1 (25)	0 (0)	1 (33.3)	0.41
Proliferative [*n* (%)]	1 (25)	0 (0)	1 (33.3)	na
TSH [mU/L (*n*) mean ± SD]		(1) 2.40	(2) 2.17 ± 2.651	na
FT4 [ng/dL (*n*) mean ± SD]		(1) 2.84	(2) 0.75 ± 0.353	na
PH [*n* (%)]	34 (12.68)	17 (50)	17 (50)	
Age [(years), mean ± SD]	48.00 ± 16.18	39.12 ± 11.33	56.88 ± 15.63	0.001
Women [*n* (%)]	26 (76.47)	15 (57.69)	11 (42.31)	0.10
Macro [*n* (%)]	24 (70.58)	8 (47.1)	16 (94.1)	0.001
Tumor diameter [(mm), mean ± SD]	18.75 ± 11.21	12.19 ± 7.06	25.31 ± 10.88	0.001

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Table 2 (continued)

Subtype	Total *n* (%)	Functioning *n* (%)	Silent *n* (%)	*p* value
Invasive [*n* (%)]	14 (41.17)	4 (28.57)	10 (71.43)	0.034
Proliferative [*n* (%)]	4 (11.7)	3 (17.6)	1 (5.8)	na
GT [*n* (%)]	40 (14.92)	0 (0)	40 (100)	
Age [(years), mean ± SD]	54.75 ± 14.51		54.75 ± 14.51	na
Women [*n* (%)]	17 (42.5)		17 (42.5)	na
Macro [*n* (%)]	40 (100)		40 (100)	na
Tumor diameter [(mm), mean ± SD]	26.92 ± 8.02		26.92 ± 8.02[c]	na
Invasive [*n* (%)]	29 (72.5)		29 (72.5)	na
Proliferative [*n* (%)]	3 (7.5)		3 (7.5)	na
NCT [*n* (%)]	60 (22.39)	0 (0)	60 (100)	
Age [(years), mean ± SD]	57.63 ± 13.66		57.63 ± 13.66	na
Women, [*n* (%)]	27 (45)		27 (45)	na
Macro [*n* (%)]	58 (96.7)		58 (96.7)	na
Tumor diameter [(mm), mean ± SD]	27.19 ± 9.49		27.19 ± 9.49	na
Invasive [*n* (%)]	44 (73.3)		44 (73.3)	na
Proliferative [*n* (%)]	5 (8.3)	0	5 (8.3)	
Total	268 (100%)	107 (39.92%)	161 (60.07%)	
Tumor diameter [(mm), mean ± SD]	22.86 ± 10.95	17.20 ± 10.20	26.65 ± 9.76	0.001
Invasive [*n* (%)]	149 (55.6)	43 (40.2)	106 (65.8)	< 0.001
Proliferative [*n* (%)]	28 (10.4)	14 (13.1)	14 (8.7)	0.25

ST somatotroph tumor, *ST Mixed* somatotroph–lactotroph tumor, *CT* corticotroph tumor, *LT* lactotroph tumor, *TT* thyrotroph tumor, *PH* plurihormonal, *GT* gonadotroph tumor, *NCT* null cell tumor, *SD* standard deviation, *na* not applicable

*Two acromegaly were diagnosed as silent because the IHC was NCT; two Cushing were diagnosed as silent because the IHC was NCT; these 4 NCT explain the differences in NCT between Tables 1 and 2

[a] $p = 0.894$

[b] $p = 0.878$

[c] $p = 0.335$

tumors and 5% of NCTs [28]. And in Mete's study [24] the percentage of NCTs were only the 4.5%, while GTs represented 42.5% of the whole series. Other authors have found similar results [29]. These figures matched better with our RT-q-PCR identification of non-functional PTs (50.9% of GTs and 11.1% of NCTs) than with the IHC one (25.5% of GTs and 35.6% of NCTs) (Table 1). In addition, the analysis of transcription factors in our series by RT-qPCR and by IHC in a subgroup of 56 tumors, reduced even more the frequency of NCTs of 3.2% (8/251) and 3.6% (2/56,) respectively [30]. The discrepancy between the IHC and RT-qPCR study of the adenohypophyseal hormones in the present study could be attributed to the performance of the IHC studies in four different pathology departments, in contrast to the molecular analyses, all conducted in our research laboratory; the limited sensitivity of adenohypophyseal hormone antibodies for detecting low protein expression, and the subjectivity among observers in interpreting such low values.

Characteristics of SPTs

Most silent pituitary tumors are GTs or NCTs, notwithstanding the important pool of silent tumors from other lineages. In Nishioka study, the prevalence of SPTs ranged from 9.2% of plurihormonal Pit-1 tumors to 99.3% of all gonadotropinomas and 100% of NCTs, within their respective subtypes [28]. In Mete et al.'s series, silent tumors accounted for 100% of the NCTs subtype and 99% of GTs [24]. In our series, the prevalence of SPTs ranged from 18% of STs to 100% of GTs and NCTs of the respective subtypes.

By definition, all operated silent tumors are macroadenomas. Compared with GTs, tumors from the other lineages are larger (Pit-1 lineage), more invasive (NC and CT), or present greater recurrence rates (Pit-1 and CT) [24]. Our study had a cross-sectional design, so we could not collect information about the recurrence rates of tumors during the follow-up. However, the silent tumors, as a whole, were significantly larger than the functioning ones ($p = 0.001$; Table 2). By subtypes, there were only significant differences in size between

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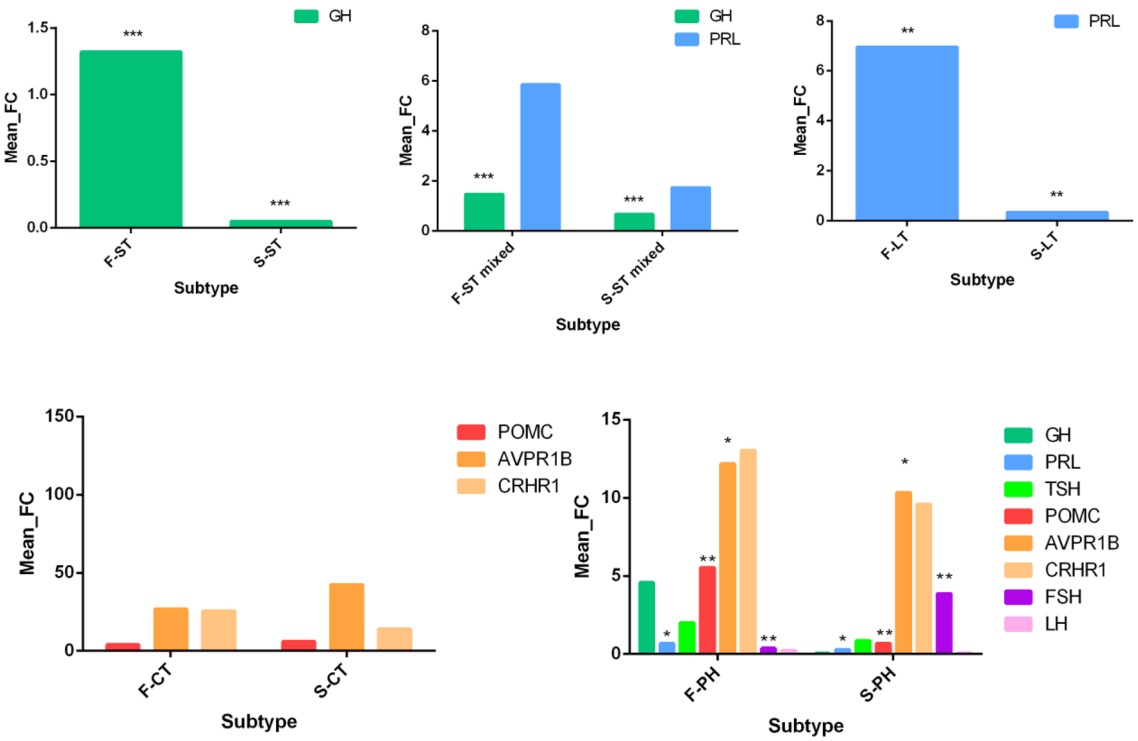

Fig. 1 Differences in adenohypophyseal hormone gene expression between functioning and silent variants, of PTs subtypes

counterparts in the corticotroph, mixed somatotroph, and plurihormonal subtypes. Consequently, silent tumors were also more invasive than functioning ones. Among subtypes, the percentage of invasive silent tumors ranged from 42.9% of CTs to 73.3% of NCTs. Contrary to previous reports [24, 31], we did not find differences in the size or invasiveness between NCTs and GTs, which is likely attributable to the fact that most NCTs in our series were GTs, as we demonstrate with the molecular study. These findings reinforce the need to properly identify the different subtypes of PTs before searching for clinical differences between them.

As a whole, PTs are low proliferative tumors. However, the WHO 2017 classification still recommends determining the Ki-67 labelling index and other proliferative indices, such as p53 or number of mitoses, depending on the former. As tumors of our series came from four different pathology departments, we only took into consideration only the Ki-67 labelling index. In contrast to Mete et al.'s results, where 60% of the series exceeded this threshold, only just 10.4% of the tumors in our series did, with no difference between functioning and silent tumors. These notable differences between the two series, one coming from a single center and the second from four centers, could be attributed to the technique's heterogeneity and the subjective interpretation of Ki-67 IHC results among observers. Therefore, we could not properly compare the differences in the proliferative behavior between functioning and silent counterparts of PTs nor among subtypes. However, the tumor subtype and its radiological extent have been considered better predictors of aggressive behavior of PTs than the Ki67 index [32, 33]. The differences also highlight the importance of centralizing the analysis and treatment of pituitary pathologies in Pituitary Tumor Centers of Excellence [34].

The silencing mechanisms of PTs are not well known. The first subtype described as having a different clinico pathological reality compared with its functioning counterpart was the silent corticotroph tumor (S-CT) [14]. Since then, multiple cases have been published (Table 3). CTs are tumors of Tpit pituitary lineage, and they are immunopositives for ACTH and TPIT. Globally, 20–35% are not associated with a Cushing syndrome [35, 36], and together they constitute approximately 5% of all PTs [37], although with important variability among series (Table 3). Nishioka et al. [28] and Mete et al. [24] reported the percentage of SPTs within their histological subtype. In Nishioka et al.'s study, the prevalence of silent CTs comprised 32.9% of all corticotropinomas. In Mete et al.'s series, silent tumors accounted for 33% of CTs. The discrepancies in the prevalence of S-CTs within PTs and within tumors of corticotroph lineage have been attributed to differences in the techniques used in their identification. In our study, S-CTs constituted 56% of tumors of corticotroph lineage and 5.2% of all PTs when the subtype was identified by IHC. Contrarily to our results, S-CTs have been reported

Table 3 Characteristics of previous case series of S-CT and other non-functioning pituitary adenomas (NFPAs)

Sample [*n* (%)]	Gender (M/W)	*p* value[a]	Age [years mean ± SD (range)]	*p* value[a]	Size (cm)	*p* value[a]	Invasiveness	*p* value[a]
Cho et al. [9] (162 NFPAs, all macroadenomas)					Mean ± SD		Hardy grade: *n* (%)	
S-CT, 28 (17%)	Ratio 1:2.1		44.5 (13–67)		2.8 ± 0.8		2:16 (57.1) 3:11 (39.3) 4:1 (3.6)	
Non S-CT, 134 (83%)	Ratio 1.3:1	0.042	50.2 (18–79)	0.026	3.0 ± 0.9	0.32	2:87 (64.9) 3:47 (35.1) 4:0 (0)	0.077
Yamada et al. [38] (213 NFPAs)							*n* (%)	
S-CT, 26 (12%)	4/22 (85% W)		52.1 ± 2.5 (35–72)				22 (85%)	
GT, 136 (64%)	95/41 (30% W)	< 0.001	54.2 ± 1.9 (35–72)				15 (11%)	$p < 0.001$
NC, 39 (18%)	17/22 (56% W)	0.017	51.9 ± 2.3 (32–75)				15 (38%)[b]	< 0.001
Subtype 3, 9 (4%)	6/3 (33% W)	0.007	28.3 ± 1.8 (19–34)				6 (67%)[c]	
Nishioka et al. [28] (516 NFPAs)					Giants (> 4 cm), *n* (%)		*n* (%)	
S-CT, 51 (10%)	12/39 (76% W)		51 ± 13 (23–81)		16 (31.4%)		23 (45.1%)	
GT, 376 (73%)	230/146 (39% W)	< 0.001	56 ± 13 (18–83)	na	31 (8.2%)	na	20 (5.3%)	< 0.001
Langlois et al. [39]				Mean ± SD			(%)	
S-CT, 37 (4.5%)	17/20 (54% W)		51.1 ± 16.1		2.53 ± 1.15		43	
S-ST, 17 (2.1%)	3/14 (82% W)		42.8 ± 16.3	< 0.001	1.52 ± 0.99 (~ 50% macro)[e]		25	0.40
GT, 70 (18.9%)	44/26 (37% W)[d]		60.0 ± 15.1	0.018	2.87 ± 1.97		41[f]	
Cooper et al. [13] (109 NFPAs)					Mean ± SD (*n* analyzed)		*n*/*N* analyzed (%)	
S-CT, 25 (23%)	13/12 (48% W)		53 ± 14.3		3.0 ± 1.7 ($n = 17$)		7/17 (41%)	
Others, 84 (77%)	55/29 (35% W)		54.9 ± 14.9		3.1 ± 1.3 ($n = 58$)	ns	22/58 (38%)	ns
Jahngiri et al. [40]					Mean ± SD		*n*/*N* analyzed (%)	
S-CT, 75 (3%)	38/37 (49% W)		50 (19–83)		2.2 ± 0.9 (3% micro)		16/54 (30%)	
F-CT, 134 (6%)	20/114 (85%)	< 0.001	39 (10–77)	< 0.001	0.9 ± 0.9			

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Table 3 (continued)

Sample [*n* (%)]	Gender (M/W)	*p* value[a]	Age [years mean ± SD (range)]	*p* value[a]	Size (cm)	*p* value[a]	Invasiveness	*p* value[a]
Others, 1726 (91%)	836/890 (52.% W)	0.70	49 (9–89)	0.90	2.0 ± 1 (13% micro)	0.20	297/1655 (18%)	0.03
Alahmadi et al. [35]				Mean			%	
S-CT, 20	9/11 (56% W)		51.1 (24–78)		3.5 ± 2.19 (all macro)		31%	
NFPA (GT + NC), 30	14/16 (53% W)	0.85	51.5	0.93	5.95	0.074	23%	$p = 0.48$
Cohen-Inbar et al. [41] (357 NFPAs)					Mean		*n* (%)	
S-CT, 50 (14%)	19:29 (40:60%)		50.9 ± 13		3.3		38 (77.6%)	
Others, 307 (86%)	137:129 (52:48%)	0.17	55.2 (13.7–87)	0.06	3.1	0.36	210 (70.9%)	0.43
Ioachemiscu et al. [42] (159 NFPAs, all macroadenomas)					Mean ± SD		*n* (%)	
S-CT, 33 (21%)	19/14 (42.4% W)		49.6 ± 14.1		2.83 ± 0.97		45.5% (15)	
Others, 126 (79%)	72/54 (42.9% W)	0.96	55.6 ± 12.8	0.02	2.84 ± 0.99	0.98	30.2% (38)	0.09
Torregrosa et al. (161 NFPAs)					Macro, *n* (%)		*n* (%)	
S-CT, 14 (5.2%)	8/6 (46.2% W)		42.5 ± 15.13		14 (73.7%)		6 (63.6%)	
F-CT, 11 (4.1%)	4/7 (53. 8% W)	0.30	40.27 ± 10.98	0.76	5 (26.3%)	< 0.001	4 (36.4%)	0.49
GT, 40 (14.9%)	23/17 (42. 5% W)		54.75 ± 14.51		40 (100%)		29 (72.5%)	

NFPA non-functioning pituitary adenoma, *S-CT* silent corticotroph tumor, *F-CT* functioning corticotroph tumor, *GT* gonadotroph tumor, *NC* null cell tumor, *S-ST* silent somatotroph tumor, *M* men, *W* women, *Macro* macroadenoma (≥ 1 cm), *Micro* microadenoma (< 1 cm), *na* not available, *ns* not significant

[a]Column *p* values in comparison to S-CTs

[b]NC vs GT $p < 0.001$

[c]Subtype 3 vs GT $p < 0.001$

[d]S-ST vs GT $p = 0.002$

[e]S-ST vs GT $p = 0.009$

[f]S-ST vs GT, $p > 0.05$

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as more frequent in women, while the age of diagnosis is lower than that of other silent subtypes (Table 3).

Several theories have been proposed to explain why some CTs do not cause Cushing syndrome, the most attractive of which is based on the processing of POMC and ACTH in corticotroph cells [15, 16]. Indeed, by studying 24 silent and 23 functioning CTs, we recently demonstrated that the lack of secretory activity of S-CT was related to an impaired processing of POMC and a high degradation of ACTH [19]. According to this hypothesis, we did not find significant differences in the expression of *POMC*, *CHRH*, and *AVPR1B* between functioning and silent corticotroph tumors.

Silent somatotroph tumors (S-STs) present positive immunostaining for GH but are not associated with acromegaly. Their estimated prevalence is nearly 2% of all PTs [39, 43]. In Mete et al.'s series, silent tumors accounted for 9.2% of Pit-1 lineage. In the present study, S-STs constituted 3% of all PTs and 17.7% of the IHC-diagnosed STs. The latter results were recently confirmed through the study of *PTF* gene expression [30]. As most S-STs are plurihormonal [44], the discrepancy between IHC and RT-qPCR identification of S-STs suggests an important cross-reaction between GH with α-subunit or prolactin antibodies. S-STs have been reported as more prevalent in women and are diagnosed earlier than other silent subtypes [37, 44]. Accordingly, in the present series, the age at diagnosis was also lower than that of other subtypes, with the exception of S-CTs. However, we found a higher prevalence in men. Similarly to our results, it has been reported that S-STs secrete less GH than their functioning counterparts [44]. However, the silencing mechanisms of STs have yet to be clarified [25]. Several hypotheses have been raised: a low secretory activity of GH [44], a defective post-transcriptional processing of GH [45, 46], or low biological activity of the secreted GH [46, 47]. In this study, we found significantly lower *GH*-gene expression in silent compared to functioning STs (Fig. 1), what it is not surprising because most of silent ST are sparsely granulated tumors.

Although LTs are considered one of the most prevalent PTs subtypes, silent LTs (S-LT) have rarely been reported. Previous to surgery, they behave as NFPAs with normal or slightly elevated PRL levels, and it is difficult to differentiate the hyperprolactinemia to that related with the compression of dopaminergic pathways. Therefore, diagnosis is established only after surgery. The prevalence of LTs among silent pituitary tumors has been estimated at 0.6–1.65% [48, 49]. In the present series, S-LTs represented 5.5% of all silent pituitary tumors and 45% of LTs. They were more prevalent in women and were diagnosed late in life (64.4 ± 13.4 years). The silencing mechanisms have not been studied. Similarly, to S-STs, pure S-LT expressed less *PRL* than F-LTs; this finding may contribute to a lower secretion of PRL. Indeed, the circulating PRL levels of S-LTs were significantly lower than those of F-LTs (Table 2).

A positive PRL stain in silent tumors is frequently reported with GH-positive immunostaining, and these tumors are considered silent mixed somatotroph–lactotroph tumors. In our series, the silent mixed somatotroph–lactotroph tumors represented 6.2% of all silent PTs (Table 2) and were clearly differentiated from pure S-LTs. Silent mixed somatotroph–lactotroph tumors were more prevalent in men and were diagnosed earlier than pure LTs (47.7 ± 13.14 years). The expression of *GH* was lower than in their functioning counterparts, and again, this fact may contribute to the absence of endocrine symptoms in these tumors (Fig. 1).

Finally, silent TTs have also been scarcely reported. Their prevalence within tumors of thyrotroph lineage ranged from 31 to 75% in two independent series [50, 51]. In our series, they comprised 1.8% of the total silent PTs and 75% of the tumors of TSH cell lineage. Because of their low number, we were unable to compare the silent and functioning variants, but the three silent TTs were macroadenomas, although only one of them was invasive.

Conclusion

This paper highlights the importance of identifying the silent variant of the PTs subtypes, because the behaviour of a non-functioning PTs is different depending on the lineage it derives from. Moreover, and as expected, silent tumors were larger and more invasive than their functioning counterparts. However, there was no difference in the proliferation activity between them. The molecular study of the expression of the adenohypophyseal hormone genes complements the IHC identification of the PTs subtypes, especially the NCTs ones. Moreover, the lower specific gene expression in the silent than in the functioning counterparts of some PTs subtypes, gives insights to the silencing mechanisms of PTs.

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Compliance with ethical standards

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Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

Consent for publication Samples were donated by patients to the Institutional BioBank for research purposes with the corresponding consent for publication.

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