Modern Pathology (2016) 29, 131–142

Silent subtype 3 pituitary adenomas are not always silent and represent poorly differentiated monomorphous plurihormonal Pit-1 lineage adenomas

Ozgur Mete[1], Karen Gomez-Hernandez[2], Walter Kucharczyk[3], Rowena Ridout[2], Gelareh Zadeh[4], Fred Gentili[4], Shereen Ezzat[2] and Sylvia L Asa[1]

[1]Department of Pathology, University Health Network, and Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada; [2]Department of Medicine, University Health Network, University of Toronto, Toronto, Ontario, Canada; [3]Department of Medical Imaging, University Health Network, University of Toronto, Toronto, Ontario, Canada and [4]Department of Neurosurgery, University Health Network, University of Toronto, Toronto, Ontario, Canada

Originally classified as a variant of silent corticotroph adenoma, silent subtype 3 adenomas are a distinct histologic variant of pituitary adenoma of unknown cytogenesis. We reviewed the clinical, biochemical, radiological, immunohistochemical and ultrastructural features of 31 silent subtype 3 adenomas to clarify their cellular origin. Among 25 with clinical and/or radiological data, all were macroadenomas; there was cavernous sinus invasion in 30% of cases and involvement of the clivus in 17% of cases. Almost 90% of patients were symptomatic; 67% had mass effect symptoms, 37% were hypogonadal and 8% had secondary adrenal insufficiency. Significant hormonal excess in 29% of cases included hyperthyroidism in 17%, acromegaly in 8% and hyperprolactinemia above 150 μg/l in 4%. Two individuals with hyperprolactinemia who were younger than 30 years had multiple endocrine neoplasia type 1. Immunohistochemically, all 31 tumors were diffusely positive for the pituitary lineage-specific transcription factor Pit-1. Although three only expressed Pit-1, others revealed variable positivity for one or more hormones of Pit-1 cell lineage (growth hormone, prolactin, thyroid-stimulating hormone), as well as alpha-subunit and estrogen receptor. Most tumors exhibited perinuclear reactivity for keratins with the CAM5.2 antibody; scattered fibrous bodies were noted in five (16%) tumors. The mean MIB-1 labeling index was 4% (range, 1–9%). Fourteen cases examined by electron microscopy were composed of a monomorphous population of large polygonal or elongated cells with nuclear spheridia. Sixty-five percent of patients had residual disease after surgery; after a mean follow-up of 48.4 months (median 41.5; range = 2–171) disease progression was documented in 53% of those cases. These data identify silent subtype 3 adenomas as aggressive monomorphous plurihormonal adenomas of Pit-1 lineage that may be associated with hyperthyroidism, acromegaly or galactorrhea and amenorrhea. Our findings argue against the use of the nomenclature 'silent' for these tumors. To better reflect the characteristics of these tumors, we propose that they be classified as 'poorly differentiated Pit-1 lineage adenomas'.
Modern Pathology (2016) **29,** 131–142; doi:10.1038/modpathol.2015.151; published online 8 January 2016

The last three decades have seen tremendous progress in our understanding of the molecular cytodifferentiation of adenohypophysial cells.[1,2] This has impacted significantly on the recognition and distinction of various clinically relevant histologic subtypes of pituitary adenomas.[1–5] Clinically nonfunctioning pituitary adenomas represent a significant fraction of these tumors, and are detected as incidentalomas during radiologic examination or at autopsy, or when the tumor causes symptoms associated with pituitary apoplexy (acute hemorrhagic necrosis), compression of adjacent structures or increased intracranial pressure.[3–6] There is now abundant evidence that nonfunctioning pituitary adenomas represent a heterogenous group of

Correspondence: Dr O Mete, MD or Dr SL Asa, MD, PhD, Department of Pathology, University Health Network, 200 Elizabeth Street, 11th Floor, Toronto, Ontario M5G 2C4, Canada.
E-mail: ozgur.mete2@uhn.ca or sylvia.asa@uhn.ca
Received 6 October 2015; revised 16 November 2015; accepted 17 November 2015; published online 8 January 2016

clinically relevant histologic subtypes with differing biologic aggressiveness.[3–19]

The application of pituitary cell lineage-specific transcription factors and other biomarkers has shown that most of these neoplasms are of gonadotroph origin.[3–5,8,19] In addition, a distinct subset of aggressive nonfunctioning pituitary adenomas includes silent corticotroph adenomas (Type 1 silent corticotroph adenoma: nonfunctioning densely granulated corticotroph adenoma; Type 2 silent corticotroph adenoma: nonfunctioning sparsely granulated corticotroph adenomas), which are characterized by a propensity to develop apoplexy, have a higher recurrence rate, and exhibit greater invasion.[8,10,14–17]

Originally classified as the third variant of silent corticotroph adenoma, silent subtype 3 adenomas are considered to be a distinct and aggressive histologic variant of pituitary adenoma.[11–13] Although these tumors have been traditionally termed 'silent', the initial retrospective review of 20 silent subtype 3 adenomas by Horvath *et al*, followed by 29 cases from St Michael's Hospital and 27 from the Mayo Clinic clearly showed that these tumors may be associated with clinical and biochemical evidence of hormonal excess.[11–13] None of these studies investigated the role of pituitary cell lineage transcription factors, and they concluded that the cytogenesis of silent subtype 3 adenomas remained unsettled.[11–13] We therefore undertook a systemic review of the clinical, biochemical, radiological, immunohistochemical and ultrastructural features of 31 silent subtype 3 adenomas in order to refine their classification by clarifying the cellular origin of these tumors.

Materials and methods

A retrospective review of institutional pathology records at the University Health Network from July 2001 to August 2015 revealed 31 silent subtype 3 adenomas from a total of 954 pituitary adenomas (3.2%). These tumors were diagnosed based on the following features: spindle cell morphology with or without associated epithelioid morphology; lack of development of features of other known pituitary adenoma subtypes; an unusual pattern of plurihormonality; and the presence of spheridia either seen by electron microscopy or on H&E-stained sections. The available medical records were reviewed to obtain relevant clinical information following institutional ethics review. Patient age and gender, clinical, biochemical, and radiological features, as well as immunohistochemical and ultrastructural characteristics of these tumors were recorded. In cases in which primary treatment or follow-up was not provided by physicians within the University Health Network, clinical information was incomplete or unavailable.

One neuroradiology expert retrospectively reviewed the available magnetic resonance imaging studies of all patients treated or followed at the University Health Network. Radiologic cavernous sinus invasion was defined as tumor extension lateral to the lateral tangent of the intra- and supracavernous internal carotid artery or total encasement of the intracavernous carotid artery (Knosp Grades 3 and 4).[20] Tumors that measured >1 cm in their greatest dimension were classified as macroadenomas, whereas those that measured >4 cm were classified as giant macroadenomas. Follow-up time was defined as the number of months between the histologic diagnosis and the last postoperative magnetic resonance imaging. In cases in which a postoperative magnetic resonance imaging was not performed or not available, the follow-up time was defined as the number of months between histologic diagnosis and last clinical assessment. The age at diagnosis was that in which the histologic diagnosis was made.

All surgical pathology specimens were reviewed by two endocrine pathologists. All tumors were subjected to a consistent panel of histochemical and immunohistochemical stains including the periodic acid–Schiff and Gordon-Sweet reticulin stains, and immunolocalization of pituitary cell lineage transcription factors (pituitary-specific transcription factor-1 (Pit-1), steroidogenic factor-1 (SF-1) and estrogen receptor (ER) for all tumors, and T-box transcription factor (Tpit) for hormone-negative tumors that also are negative for SF-1, ER and Pit-1), adenohypophysial hormones (adrenocorticotrophic hormone (ACTH), growth hormone (GH), prolactin (PRL), beta-thyroid-stimulating hormone (beta-TSH), beta-luteinizing hormone (beta-LH), beta-follicle-stimulating hormone (beta-FSH) and alpha-subunit), low molecular weight keratin (CAM5.2) and MIB-1. The MIB-1 labeling index was reported by counting 1000 tumor cells in hot spots of nuclear labeling. Fourteen specimens had tissue submitted for ultrastructural examination at the time of diagnostic assessment.

Results

The cohort comprises 16 female patients (52%) and 15 male patients (48%) with a mean age of 44.3 years (s.d. = 16.8) at the time of surgical pathology diagnosis. Relevant complete or partial clinical and radiological information was available for 25 patients (Table 1) of which information regarding the clinical presentation was unknown in one (case 10), baseline pituitary function tests were missing in one (case 16), magnetic resonance imaging at the time of initial presentation was not available in two (cases 2 and 16) and a magnetic resonance imaging after surgery was not available in two (cases 2 and 12). For the six cases that are not included in Table 1 only a complete pathology examination was available (Table 2 cases 26 to 31).

Table 1 Clinicopathological features of 25 patients with silent subtype 3 adenomas

Case	*Patient age (years) and gender*	*Clinical presentation*	*Main biochemical findings (limit of normal reference interval)*	*Radiological features*	*Treatment*	*Surgical outcome and other therapy*	*Follow-up*[a] *(months)*
1	66, F	Headaches and right third cranial nerve palsy	PRL = 28 μg/l (≤29.9), hypogonadism	Macroadenoma with suprasellar and downward growth as well as cavernous sinus invasion	TSS	Progressive residual tumor within the cavernous sinus	9
2	17, F	Hyperthyroidism	TSH = 1.14 mIU/l (< 5), FT4 = 40 pmol/l (< 23)	Not available	Surgery-surgical approach unknown	Resolution of hyperthyroidism	70*
3	53, F	Incidentaloma	PRL = 91.1 μg/l (≤29.9), hypogonadism	Macroadenoma with suprasellar and downward growth	TSS	Complete gross resection of the tumor without evidence of recurrence	73
4	68, M	Headaches and nasal obstruction	GH = 0.8 μg/l (≤9) IGF-1 = 264 μg/l (≤243)	Macroadenoma with predominant downward growth into the sphenoid sinus, erosion of the clivus, and cavernous sinus invasion	TSS	Residual tumor in the cavernous sinuses and throughout the clivus that progressed	58
5	42, F	Visual field deficit	PRL = 13.3 μg/l (≤29.9)	Giant adenoma with marked downward and suprasellar growth as well as cavernous sinus invasion	TCS	Residual sellar and infrasellar tumor that showed slow progression; hypopituitarism	58
6	30, F	Secondary amenorrhea, galactorrhea, headaches and visual field deficit	PRL = 67.4 μg/l (≤29.9), hypogonadism	Macroadenoma with predominant suprasellar extension, and downward growth	TSS	Complete gross resection with recovery of gonadal function and normalization of prolactin	41
7	47, F	Incidentaloma	PRL = 87.3 μg/l (≤29.8), GH < 0.3 μg/l (≤9), IGF-1 = 96 μg/l (178–295)	Macroadenoma with predominant downward growth and suprasellar extension	TSS	Residual tumor	5
8	13, M	Progressive bilateral temporal visual field loss	PRL = 10.6 μg/l	Macroadenoma with predominant suprasellar growth, symmetric downward remodeling of the sella, and cavernous sinus invasion	TCS followed by TSS	Suprasellar component of residual tumor was removed at second surgery; hypopituitarism with cavernous sinus disease	44
9	28, F	Irregular menses; PHPT and *MEN-1* mutation discovered during course of investigations	PRL = 45.2 μg/l (< 24)	Macroadenoma with suprasellar growth	TSS	Complete gross resection and no evidence of recurrence; resolution of menstrual irregularities and hyperprolactinemia	46
10	19, F	Unknown	PRL = 13 μg/l (< 24), normal thyroid function tests	Non-invasive macroadenoma without parasellar extension	TSS	Complete gross resection and no evidence of recurrence	39
11	68, M	Headaches	PRL 13.1 μg/l (≤18.1)	Macroadenoma with predominant suprasellar growth and cavernous sinus invasion	TSS	Residual tumor within the sella and cavernous sinus with mild progression of the intrasellar component	14
12	69, F	Hyperthyroidism			TSS		1*

Table 1 (Continued)

Case	Patient age (years) and gender	Clinical presentation	Main biochemical findings (limit of normal reference interval)	Radiological features	Treatment	Surgical outcome and other therapy	Follow-up[a] (months)
			TSH = 4.67 mIU/l (≤5), FT4 = 27 pmol/l (≤23), PRL = 6.1 μg/l (≤24)	Macroadenoma with mild suprasellar growth		Resolution of hyperthyroidism	
13	44, M	Chronic headaches and visual field defects followed by pituitary apoplexy; left cranial sixth nerve palsy	PRL = 18.2 μg/l (≤17.7), hypogonadism, adrenal insufficiency	Macroadenoma with marked downward and suprasellar growth	TSS	Lack of recovery of pituitary function; residual tumor progressed slightly inferiorly and invaded the cavernous sinus	103
14	43, M	Fatigue associated with central hypogonadism	PRL = 5.1 μg/l (≤13.1) LH described as 'inappropriately low' Free testosterone = 10.2 pmol/l (≤15.8)	Macroadenoma with predominant suprasellar growth, and invasion into the clivus	TSS	Residual tumor that progressed	55
15	75, F	Incidentaloma	PRL = 11 μg/l (≤24)	Macroadenoma with suprasellar growth	TSS	Resection of suprasellar component with stable residual disease within the sella	42
16	48, F	Visual field deficit	Unavailable—basal pituitary function tests were described as 'normal'	Postoperative MRI showed a macroadenoma with suprasellar and downward growth as well as cavernous sinus invasion	TSS and radiotherapy	Residual disease stabilized after radiation; visual field defects disappeared after surgery but she developed hypopituitarism	171
17	38, M	Chronic headaches, decreased libido and weight loss followed by visual field deficit	PRL = 48.4 μg/l (≤13.1), central hypogonadism and adrenal insufficiency	Macroadenoma with predominant suprasellar extension	TSSx2	Lack of recovery of pituitary function and residual progressive disease that led to a second surgery in which tumor resection was also incomplete	19
18	24, M	Visual field deficits	TSH = 1.44, FT4 = 34 pmol/l (≤22), FT3 6.1 pmol/l ≤ 6.8), PRL 26 μg/l (≤20)	Macroadenoma with suprasellar extension	TSSx2	Residual disease with hyperthyroidism persisted after first surgery; biochemical improvement after second surgery	12*
19	40, M	Visual field deficit and hypogonadism	LH = 2 IU/l (≤9), total testosterone = 6.2 nmol/l (≥7.6), PRL = 14.3 μg/l (≤31.1)	Macroadenoma with predominant suprasellar extension	TSS	Lack of recovery of pituitary function; complete gross resection of the tumor with recovery of peripheral vision	7
20	53, F	Headaches and fatigue with acromegalic features on physical examination	GH = 9.2 μg/l (≤9), IGF-1 = 703 μg/l (≤295), nonsuppressible GH during OGTT, PRL = 15.2 μg/l (≤29.9),	Macroadenoma with predominant downward growth with bone erosion and complete infiltration of the clivus; also suprasellar extension and invasion of the cavernous sinus	TSS	Residual tumor and lack of improvement of growth hormone excess	7*
21	34, F	Longstanding amenorrhea,	PRL = 332 μg/l (≤26.7) IGF-1 100 μg/l (182–	Giant adenoma with suprasellar	Dopamine agonist for		4

Table 1 (Continued)

Case	Patient age (years) and gender	Clinical presentation	Main biochemical findings (limit of normal reference interval)	Radiological features	Treatment	Surgical outcome and other therapy	Follow-up[a] (months)
		galactorrhea, headaches and visual field defects; MEN-1 diagnosis rendered during course of investigations because of finding of PHPT and pancreatic tumors	481), GH < 0.3 μg/l (≤9.9), hypogonadism	extension and downward invasion into the sphenoid sinus; cavernous sinus invasion	several years followed by TSS and radiotherapy	Residual tumor that was treated with radiotherapy	
22	55, M	Headaches, increase in ring and shoe size, joint pain, visual field deficits	GH = 3 μg/l (≤0.9), IGF-1 = 884 μg/l (≤295), PRL = 7.9 μg/l (≤18.1), hypogonadism	Macroadenoma with predominant downward growth and remodeling of the clivus	TSS	Complete gross resection with gradual decrease in IGF-1 levels and GH suppression on OGTT	10
23	31, F	Headaches, visual field deficits	PRL = 28 μg/l (≤24)	Macroadenoma with predominant suprasellar growth and mild downward growth	TSSx2	Complete gross resection after the second surgery; hypopituitarism	139
24	52, M	Headaches and hyperthyroidism	TSH = 6.9 mIU/l (≤5), FT4 = 29 pmol/l (≤25), FT3 = 3.6 nmol/l (≤2.7) PRL = 3 μg/l (≤13)	Macroadenoma with predominant suprasellar growth and downward growth with marked remodeling	TSS	Stable residual tumor with resolution of central hyperthyroidism and development of central hypothyroidism	119
25	35, F	Incidentaloma	PRL = 4.5 μg/l (≤24)	Macroadenoma with predominant suprasellar growth and mild downward remodeling	TSS	Complete gross resection	7

Abbreviations: FT3, free triiodothyronine; FT4, free thyroxine; GH, growth hormone; IGF-1, insulin-like growth factor-1; LH, Luteinizing hormone; OGTT, oral glucose tolerance test; PHPT, primary hyperparathyroidism; PRL, prolactin; TCS, transcranial surgery; TSH, thyrotropin; TSS, transsphenoidal surgery.
[a]Follow-up refers to the time from the initial surgery until the last postoperative magnetic resonance image except for the cases with an * in which it refers to the time from initial surgery until the last clinical assessment.

Clinical and Biochemical Findings

Twenty-one of 24 patients with complete history (88%) were symptomatic at the time of initial presentation. Manifestations of mass effect were present in 16 cases (67%). Headache and visual field deficits were the most frequent mass effect symptoms (11 patients; 46%), followed by cranial nerve palsy with or without pituitary apoplexy (2 patients; 8%).

Of the 24 patients with complete or partial basal pituitary function tests, 9 (37%) had central hypogonadism and 2 (8%) had central adrenal insufficiency. Significant hormonal excess occurred in 7 (29%) patients. Four individuals presented with central hyperthyroidism (17%) and two patients with acromegaly (8%). Hyperprolactinemia (mean = 89.7 μg/l; s.d. = 101.3) occurred in eight individuals (33%); only one of them (4%) had a prolactin level above 150 μg/l.

Two patients who presented before the age of 30 years were diagnosed with multiple endocrine neoplasia type 1 syndrome. In one case (case 21), the patient had presented in her early twenties with visual field deficits, amenorrhea and galactorrhea and was treated with dopamine agonists for most of the 9-year interval before the histologic diagnosis of silent subtype 3 adenoma. Her prolactin was elevated at 332 μg/l. Screening for multiple endocrine neoplasia type 1 was carried when she was referred to our institution and she was found to have primary hyperparathyroidism, as well as multiple pancreatic lesions with imaging features suggestive of neuroendocrine tumors along with significantly elevated fasting pancreatic polypeptide levels. The other patient (case 9) also had hyperprolactinemia that was mild but symptomatic. She was found to have primary hyperparathyroidism at the time of

Table 2 Immunohistochemical and ultrastructural features of 31 silent subtype 3 adenomas

Case no.[a]	*Transcription factors and hormones*	*MIB-1*	*CAM5.2*	*Spheridia (ultrastructure)*
1	Pit-1 (diffuse), PRL (patchy), GH (patchy), TSH (focal), ASU (focal)	NA	Perinuclear	NA
2	Pit-1 (diffuse), PRL (focal), TSH (focal)	2%	Perinuclear	Spheridia (+)
3	Pit-1 (diffuse), PRL (few cells), GH (focal), ASU (focal)	3%	Perinuclear	Spheridia (+)
4	Pit-1 (diffuse), ER (focal), GH (focal), ASU (focal)	4%	Negative	NA
5	Pit-1 (diffuse)	5%	Perinuclear	Spheridia (+)
6	Pit-1 (diffuse)	8%	Perinuclear	Spheridia (+)
7	Pit-1 (diffuse), ER (focal), GH (few cells), PRL (few cells)	4%	Diffuse	Spheridia (+)
8	Pit-1 (diffuse), ER (focal), PRL (few cells), TSH (focal), ASU (variable)	3%	Diffuse with scattered fibrous bodies	Spheridia (+)
9	Pit-1 (diffuse), ER (focal), PRL (variable), ASU (focal)	5%	Membranous	Spheridia (+)
10	Pit-1 (diffuse), ER (very focal), GH (variable), PRL (very focal), TSH (variable), ASU (variable)	3%	Diffuse	Spheridia (+)
11	Pit-1 (diffuse), ER (focal), ASU (focal), GH (focal), PRL (focal), TSH (focal)	4%	Perinuclear	Spheridia (+)
12	Pit-1 (diffuse), ER (few tumor cells), GH (variable), PRL (few tumor cells), TSH (variable)	2%	Perinuclear	NA
13	Pit-1 (diffuse), ASU (variable)	5%	Perinuclear and scattered fibrous bodies	NA
14	Pit-1 (diffuse)	3%	Diffuse	NA
15	Pit-1 (diffuse), ASU (focal), GH (few cells)	5%	Diffuse, but variable	NA
16	Pit-1 (diffuse), ASU (patchy)	1%	Diffuse	NA
17	Pit-1 (diffuse), ASU (abundant), PRL (variable)	9%	Diffuse and scattered fibrous bodies	Spheridia (+)
18	Pit-1 (diffuse), ER (focal), GH (variable), PRL (few cells), TSH (variable), ASU (abundant; patchy)	7%	Perinuclear	NA
19	Pit-1 (diffuse), ER (variable), PRL (variable), TSH (variable), ASU (variable)	2%	Diffuse	NA
20	Pit-1 (diffuse), ER (patchy), GH (patchy), PRL (patchy), TSH (patchy), ASU (patchy)	5.5%	Perinuclear and scattered fibrous bodies	NA
21	Pit-1 (diffuse), PRL (focal), TSH (focal), ASU (patchy)	3%	Membranous	NA
22	Pit-1 (diffuse), ER (focal), GH (focal), PRL (focal), ASU (focal), TSH (few cells).	7%	Diffuse	NA
23	Pit-1 (diffuse), GH (focal), TSH (variable), ASU (focal)	3%	Perinuclear	NA
24	Pit-1 (diffuse), GH (variable), TSH (scattered cells), ASU (variable)	5%	Negative	NA
25	Pit-1 (diffuse), ER (patchy), GH (patchy), PRL (patchy), TSH (patchy), ASU (patchy)	2%	Perinuclear	Spheridia (+)
26	Pit-1 (diffuse), ER (focal), GH (focal), PRL (scattered)	4%	Perinuclear	Spheridia (+)
27	Pit-1 (diffuse), ER (variable), GH (variable), PRL (variable)	7%	Perinuclear	NA
28	Pit-1 (diffuse), GH (variable), PRL (variable), TSH (variable)	6%	Diffuse and scattered fibrous bodies	Spheridia (+)
29	Pit-1 (diffuse), ER (patchy), GH (patchy), PRL (patchy), TSH (patchy), ASU (patchy)	2%	Perinuclear	NA
30	Pit-1 (diffuse), ER (few cells), GH (variable), PRL (few), TSH (variable)	1%	Diffuse and perinuclear	NA
31	Pit-1 (diffuse), ER (focal), GH (focal), PRL (few cells), TSH (variable), ASU (patchy)	4%	Negative	Spheridia (+)

Abbreviations: ASU, α-subunit; ER, estrogen receptor; GH, growth hormone; Pit-1, pituitary-specific transcription factor-1; PRL, prolactin; TSH, thyroid-stimulating hormone.
[a]Cases 1–25 correspond to cases 1–25 from Table 1.

diagnosis of her pituitary tumor and was eventually proven to have a pathogenic mutation in *MEN-1*.

Preoperative Sellar Imaging Studies

Preoperative sellar magnetic resonance imaging studies revealed that all individuals presented with pituitary macroadenomas with a mean maximal diameter of 2.8 cm (s.d. = 1.2); two (9%) were giant macroadenomas. Radiological evidence of cavernous sinus invasion was present in 7 of 23 cases (30%) and there was evidence of involvement of the clivus (Figure 1) in 4 cases (17%). Thirteen of the 23 (56%) macroadenomas had both suprasellar and downward growth. Predominant suprasellar growth was observed in 12 of 23 cases (52%), whereas predominant downward growth was present in 4 of 23 cases (17%).

Histopathology

All tumors showed diffuse solid growth (Figure 2). The tumors consisted of epithelioid to spindle-shaped cells with chromophobic to variably eosinophilic cytoplasm. Stromal fibrosis, cytologic atypia with enlarged nuclei, nucleolar prominence, spheridia and intranuclear pseudoinclusions were variably noted. None of the tumors exhibited acute

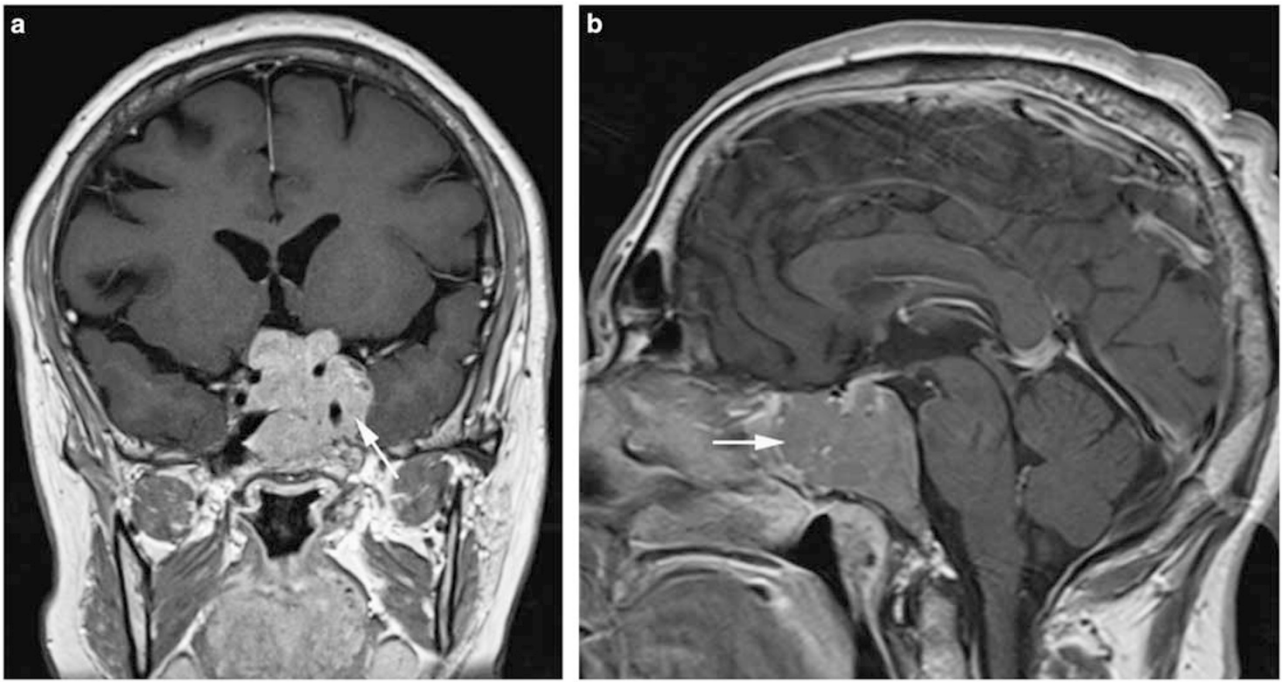


Figure 1 Magnetic resonance imaging of silent subtype 3 adenomas. Patients presented with macroadenomas with various degrees of parasellar extension. A macroadenoma exhibits both suprasellar and infrasellar growth and left cavernous sinus invasion (arrow) (**a**). A macroadenoma with marked downward growth fills the sphenoid sinus (arrow) and erodes the clivus (**b**).

hemorrhagic necrosis. The reticulin network was disrupted in all specimens. All tumors were negative with the periodic acid–Schiff stain. One tumor was associated with an adjacent gonadotroph adenoma, indicating the presence of a double adenoma.

Immunohistochemical Findings

Immunohistochemically, all tumors were diffusely positive for Pit-1 (Table 2 and Figure 3). Although three cases were only positive for Pit-1, others revealed focal or scattered positivity for one or more of the adenohypophysial hormones of Pit-1 cell lineage, GH, PRL, beta-TSH, as well as alpha-subunit and ER (Table 2 and Figure 3). All tumors were negative for SF-1, beta-FSH, beta-LH and ACTH. Twenty-eight (90%) tumors were positive for low molecular weight keratins using the CAM5.2 antibody, with predominant perinuclear or diffuse cytoplasmic staining patterns. Five tumors (16%) displayed scattered fibrous bodies in the background of diffuse or perinuclear cytoplasmic CAM5.2 reactivity (Figure 3). Two tumors displayed membranous CAM5.2 staining. The mean MIB-1 labeling index was 4% (range, 1–9%).

Ultrastructural Findings

Ultrastructural examination of 14 silent subtype 3 adenomas (Table 2) revealed that each neoplasm was monomorphous, composed of a single cell type of intermediate to large polygonal cells with prominent and interdigitating elongated cell processes (Figure 4). The cells contained abundant rough endoplasmic reticulum, a well-developed Golgi apparatus, and unevenly distributed clustered mitochondria. Two cases were rich in mitochondria, but they lacked abnormal mitochondrial dilatation or giant mitochondria. One case had focal mitochondrial calcification. Secretory granules were sparse and small with some heterogeneity in size and electron density; they tended to accumulate at the peripheral cell borders, as well as in cell processes. Cell membranes displayed plexiform interdigitations. In addition to a large prominent nucleolus, all tumors showed nuclear inclusions known as ‘spheridia’ (Figure 4). Three of the five tumors with scattered fibrous bodies identified on CAM5.2 immunohistochemistry were also subjected to ultrastructural examination; two of these tumors also had ultrastructural evidence of a few scattered juxtanuclear keratin aggresomes, diagnostic of fibrous bodies.

Treatment and Outcomes

The mean follow-up time was 48.8 months (median 41.5; range = 2–171) for the 25 patients included in Table 1. Two of 24 patients (8%) had initial pituitary tumor resection via a transcranial approach, whereas 22 of 24 patients (92%) had transsphenoidal surgery. In three (14%) of the patients who had

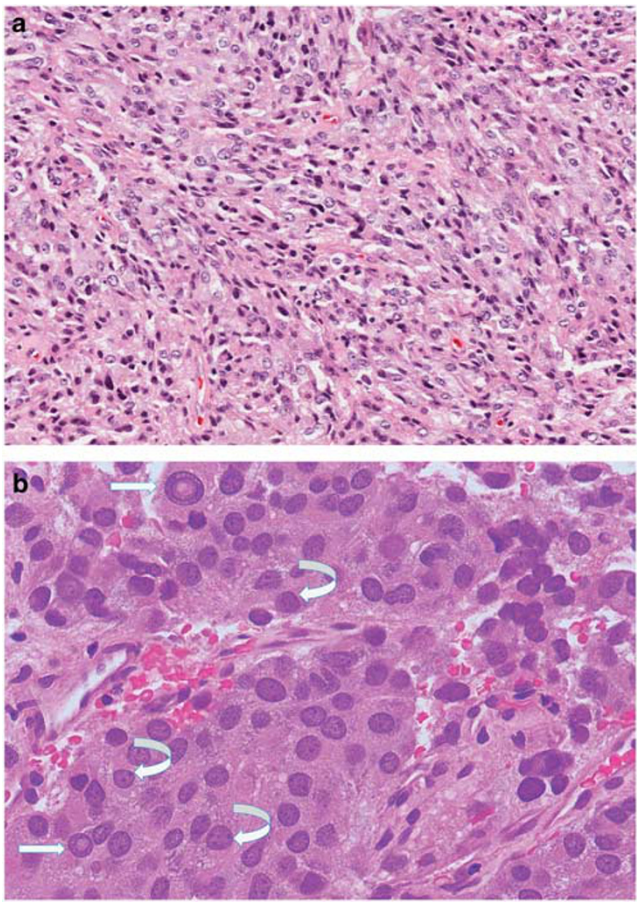


Figure 2 Histologic features of silent subtype 3 adenomas. The tumors showed diffuse solid growth (**a** and **b**) and consisted of spindle-shaped (**b**) and epithelioid (**b**) cells with chromophobic to variably eosinophilic cytoplasm (**a** and **b**). The tumor cell nuclei (**b**) show prominent structures that correspond to spheridia (curved arrows); occasional intranuclear pseudoinclusions (arrows) were also readily identified.

transsphenoidal surgery, a second operation was performed, whereas one patient in whom surgery was initially performed via a transcranial approach subsequently required a second surgery. Postoperative magnetic resonance imaging studies were available for 23 patients. Fifteen (65%) had residual tumor and in 8 (53%) progression was observed. Complete gross tumor resection was achieved in seven patients (30%) after one surgery. None of these patients had any radiological evidence of disease recurrence after a mean follow-up of 31.7 months (median = 39; range = 7–73). In one additional patient, complete gross resection was achieved after a second surgical intervention and she remained free of disease after 139 months of surveillance. None of the tumors that were grossly completely resected showed radiological evidence of invasion into bony structures or the cavernous sinuses.

Of the patients who presented with hyperprolactinemia, only one received dopamine agonist therapy for several years as she was initially thought to have a giant prolactinoma in spite of disproportion between prolactin levels and tumor size (case 21). Although medical therapy resulted in normalization of the prolactin, the tumor did not significantly change in size after years of treatment and for this reason she was referred for surgical management. One of the two patients with acromegaly was in remission 15 months after complete resection of his tumor, whereas the other continued to have elevated insulin-like growth factor-1 levels and an abnormal growth hormone response to a 75 g oral glucose tolerance test 7 months after surgery. All of four patients with central hyperthyroidism had resolution of their hormonal excess after surgery, although one required two operations.

Discussion

To our knowledge, the current series represent the largest institutional experience with a systematic review of clinical, biochemical, radiological, immunohistochemical and ultrastructural characteristics of silent subtype 3 adenomas. These tumors have been reported to occur in individuals who are younger than those with gonadotroph adenomas.[8] Furthermore, Yamaguchi-Okada *et al*[18] reported silent subtype 3 adenomas as the most frequent pituitary adenoma subtype among nonfunctioning adenomas occurring in patients younger than 25 years of age. In our cohort, the mean age was 44.3 years, ranging from 13 to 75 years.

Silent subtype 3 adenomas are well recognized for their aggressive behavior characterized by a propensity to invade and recur.[8,11–13,18] Although the frequency was approximately 1% of pituitary adenomas in the series of the Mayo Clinic;[13] our series reports a higher frequency of 3%. The explanation for this finding may be selection bias because of referrals of aggressive pituitary adenomas to our multidisciplinary endocrine oncology team.

Similar to other clinical series, most patients presented with macroadenomas with various degrees of parasellar extension. This series did not report magnetic resonance imaging evidence of sphenoid sinus invasion, as in some circumstances, it is difficult to make that radiological diagnosis with certainty. However, it is worth emphasizing that 13 of the 23 (56%) macroadenomas had both suprasellar and downward growth. Predominant suprasellar growth was observed in 12 of 23 cases (52%), whereas predominant downward growth, was present in 4 of 23 cases (17%). Cavernous sinus invasion was noted in 30% of the cases, which is similar to what was reported by Erickson *et al*[13] and about 50% of what Yamada *et al*[8] reported. Involvement of the clivus, which is not typically observed with the more common types of pituitary adenomas, occurred in 17% of cases. The

invasive behavior of a proportion of the tumors observed in the cohort partly explains the high percentage of individuals who were left with residual disease. However, the selected surgical approach and surgical expertise are also relevant and in this series when complete gross resection of the tumor was achieved (35%), long-term remission was also achieved (median 40 months; range 7–139).

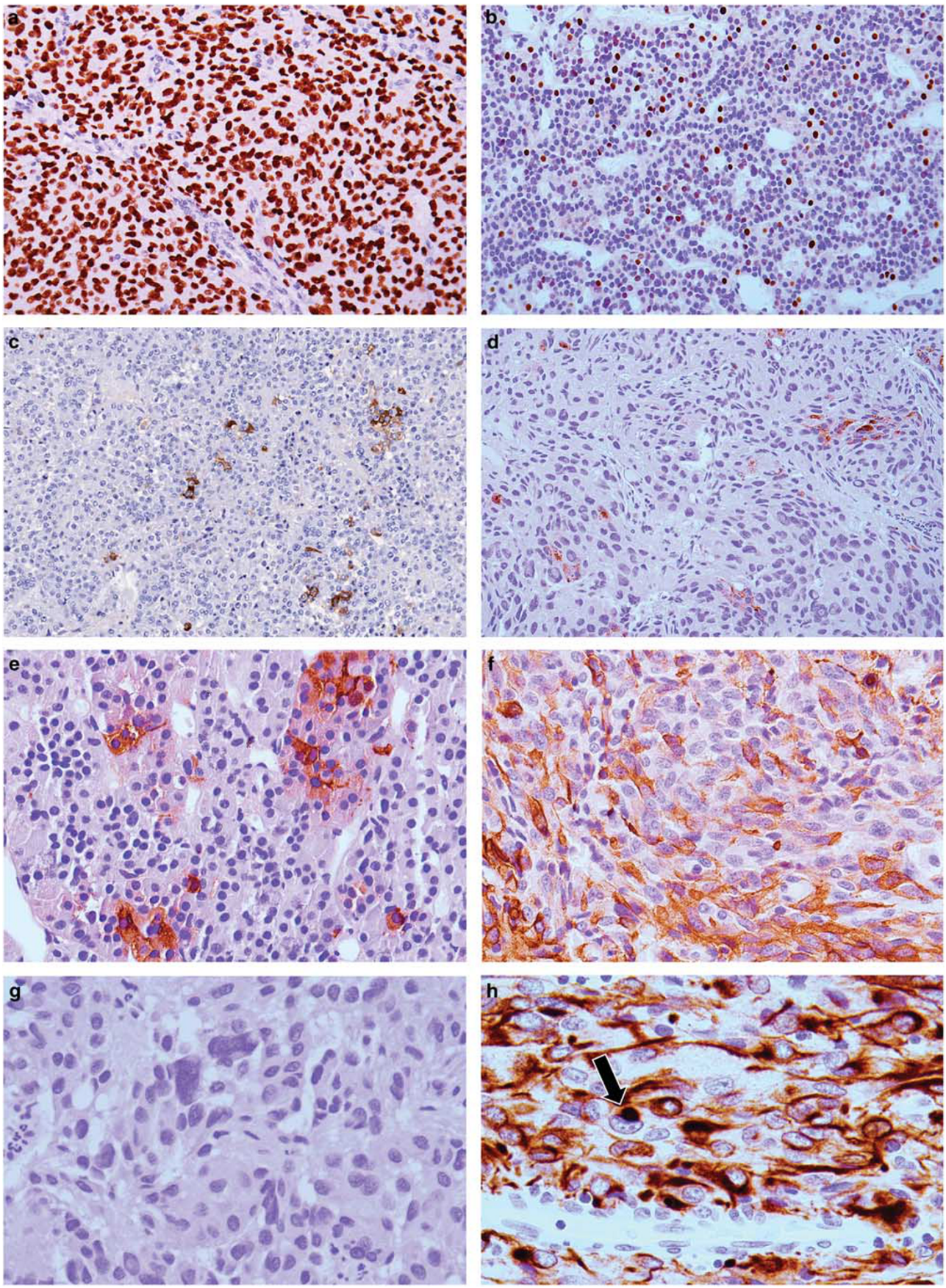
Based on our review, patients with silent subtype 3 adenomas were almost invariably symptomatic (88%) primarily due to headaches, and visual field deficits. The relatively low prevalence of hypopituitarism that we observed may be partly due to lack of complete baseline pituitary function testing in some cases. Certainly, given the size of most silent subtype 3 adenomas (mean: 2.8 cm), one would expect a higher degree of loss of pituitary function.

Although these tumors have been traditionally classified as 'silent', the initial series of 20 silent subtype 3 adenomas by Horvath *et al,*[11] followed by 29 cases from St Michael's Hospital[12] and 27 cases from the Mayo Clinic[13] showed that silent subtype 3 adenomas may be associated with clinical and biochemical evidence of hormonal excess leading to acromegaly,[11–13] amenorrhea and galactorrhea,[11–13] and even hyperthyroidism.[12,13] Consistent with findings of previous series,[11–13] the current cohort also shows that silent subtype 3 adenomas have the capacity to produce enough GH, PRL and TSH to cause clinical symptoms of hormone excess in approximately 30% of cases. In our series, hyperthyroidism was the most frequent (17%), followed by acromegaly (8%) and hyperprolactinemia above 150 μg/l (4%). It is important to emphasize that mild hyperprolactinemia (<150 μg/l) identified in previous reports,[11–13] as well as in seven cases in this study is likely due to 'stalk effect'; however, it is plausible that some of these tumors produce small amounts of prolactin (as reflected by focal PRL expression in these tumors) as a few patients have hyperprolactinemia that is higher than expected to result from stalk interruption. More strikingly, however, is the clinical presentation of hyperthyroidism. TSH-producing pituitary adenomas have traditionally been associated with clinically aggressive disease.[3–5] Based on our current findings, we propose that detailed histopathological examination of samples from patients with hyperthyroidism should allow prospective elucidation of the clinical, biochemical and radiological features that distinguish thyrotroph adenomas from silent subtype 3 adenomas and further clarification of whether they have differing outcomes and prognosis.

Interestingly and consistent with the studies by Horvath *et al*[11] and Erickson *et al,*[13] we also found a relatively high proportion of silent subtype 3 adenomas patients with multiple endocrine neoplasia type 1 syndrome. In this cohort, the two patients with multiple endocrine neoplasia type 1 syndrome had hyperprolactinemia, one exceeding 150 μg/l. These findings underscore the need to consider multiple endocrine neoplasia type 1 screening in patients younger than 30 years of age presenting with pituitary macroadenomas, particularly if hyperprolactinemia is present.[21]

The distinction of silent subtype 3 adenomas from other pituitary adenomas is of clinical significance given their well-known aggressive behavior. Although ultrastructural examination still has an important role in the workup of unusual pituitary adenomas, ultrastructural examination was the only reliable way to subtype and determine the cellular origin of pituitary adenomas in the past. Currently, the accurate classification of pituitary adenomas relies on the identification of many of the characteristic features of specific tumor types by immunohistochemistry. Since earlier cohorts[11–13] did not use antibodies against the pituitary transcription factors that can define cell lineages, they could not identify the cytogenesis of these neoplasms. In this cohort, diffuse nuclear positivity for Pit-1 in all silent subtype 3 adenomas is an important finding that indicates a cellular origin in the Pit-1 lineage. In addition to diffuse nuclear Pit-1 positivity, ER and alpha-subunit were also expressed variably in our cohort of silent subtype 3 adenomas. Although the combined expression of ER and Pit-1 are features of lactotrophs, alpha-subunit and Pit-1 expression are found in somatotrophs and thyrotrophs. Moreover, the ultrastructural features of silent subtype 3 adenomas resemble those of thyrotroph adenomas, whereas the identification of plurihormonality, and ER expression as well as the distinctive spheridia distinguish SS3As from thyrotroph adenomas. In animal studies, it has been suggested that there is a subgroup of Pit-1-independent thyrotrophs and previous investigators have questioned whether silent subtype 3 adenomas may be derived from these cells.[13] In humans, normal thyrotrophs and thyrotroph adenomas are well known to express Pit-1, and it follows that silent subtype 3 adenomas, which have some features of thyrotrophs, should express Pit-1.

Most Pit-1 lineage pituitary adenomas differentiate into somatotrophs, lactotrophs, mammosomatotrophs or thyrothyrophs.[3–4] As a consequence, differentiated adenomas usually exhibit diffuse adenohypophysial hormone positivity, with the exception of sparsely granulated somatotroph adenomas where positivity for GH can be focal and weak, however, the presence of fibrous bodies in $>75\%$ of tumor cells is identified.[3–5,22] In contrast to differentiated Pit-1 cell lineage adenomas, silent subtype 3 adenomas show variable, focal or scattered positivity for one or more adenohypophysial hormones including GH, PRL and beta-TSH. These features are consistent with a monomorphous plurihormonal adenoma of Pit-1 lineage in the vast


a
b
c
d
e
f
g
h

majority of our cases. However, as seen in three cases (10%) in this cohort, there were some silent subtype 3 adenomas lacking hormone expression and presenting with only Pit-1 expression. The ultrastructural examination available from two of these lesions identified spheridia and other ultrastructural features confirming the diagnosis of silent subtype 3 adenoma. However, such cases would have been mistaken for null cell adenomas without either electron microscopy or immunohistochemical staining for pituitary transcription factors.

Similar to a recent case that we reported but did not include in this cohort,[23] our results also highlight that silent subtype 3 adenomas should be added to the list of Pit-1 lineage adenomas that can present with scattered fibrous bodies, similar to acidophil stem cell adenomas,[3–5] which also express Pit-1. However, acidophil stem cell adenomas are distinguished from silent subtype 3 adenomas by their oncocytic nature and predominant diffuse cytoplasmic staining pattern for PRL with only focal GH expression to indicate plurihormonality in some cases.[3–5]

Conclusion

This study defines the Pit-1 cell lineage origin of silent subtype 3 adenomas. Our retrospective review identified these tumors as aggressive Pit-1 lineage adenomas, which may be associated with hyperthyroidism, acromegaly or clinical hyperprolactinemia. Although the distinction of silent subtype 3 adenomas from other pituitary adenomas is of clinical significance, our findings along with previously published series argue against the use of the nomenclature 'silent' for these neoplasms. Moreover, the identification of focal or scattered expression for Pit-1 lineage hormones in most cases and the absence of hormone expression in some cases suggest that silent subtype 3 adenomas are not differentiated Pit-1 lineage adenomas (Figure 5). In order to better reflect their clinical, biochemical and immunohistochemical characteristics, we propose the term 'poorly differentiated Pit-1 lineage adenoma' for these rare but aggressive pituitary neoplasms'.

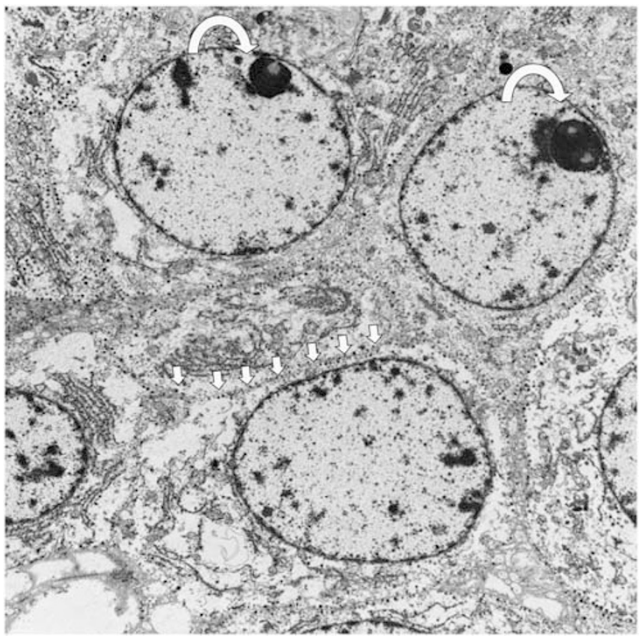


Figure 4 Ultrastructural features of silent subtype 3 adenomas. The tumors consisted of monomorphous intermediate to large polygonal cells. Secretory granules were sparse and small, and accumulated at the peripheral cell borders (short arrows). All tumors showed nuclear inclusions known as 'spheridia' (curved arrows).

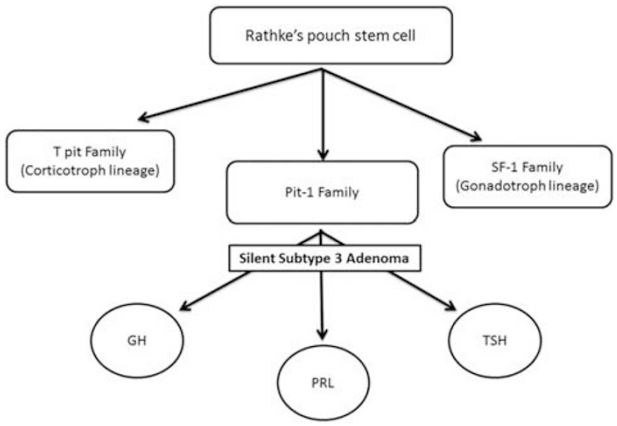


Figure 5 Cartoon illustrating silent subtype 3 adenomas in the molecular cytodifferentiation pathways of the adenohypophysis. Silent subtype 3 adenomas are true monomorphous plurihormonal poorly differentiated Pit-1 lineage adenomas, which can be associated with hyperprolactinemia, acromegaly or even hyperthyroidism. GH, growth hormone; Pit-1, pituitary-specific transcription factor-1; PRL, prolactin; SF-1, steroidogenic factor-1; Tpit, T-box transcription factor; TSH, thyroid-stimulating hormone.

Figure 3 Immunohistochemical features of silent subtype 3 adenomas. All tumors were diffusely positive for Pit-1 (pituitary-specific transcription factor-1) (**a**). Most tumors revealed focal positivity for estrogen receptor (ER) (**b**), and variable positivity for one or more of the adenohypophysial hormones of Pit-1 cell lineage: growth hormone (**c**), prolactin (**d**), beta-thyroid stimulating hormone (**e**), as well as alpha-subunit (**f**). Only three tumors lacked ER and adenohypophysial hormone expression (**g**), and were only positive for Pit-1. The CAM5.2 antibody revealed predominant perinuclear or diffuse cytoplasmic staining patterns for low molecular weight keratins (**h**), and five tumors displayed scattered fibrous bodies (**h**; arrow).

Acknowledgments

Ozgur Mete and Karen Gomez Hernandez contributed equally to this study and should be recognized as co-first authors.

Disclosure/conflict of interest

The authors declare no conflict of interest.

References

1 Asa SL, Ezzat S. Molecular determinants of pituitary cytodifferentiation. Pituitary 1999;1:159–168.
2 Asa SL, Ezzat S. The pathogenesis of pituitary tumors. Annu Rev Pathol 2009;4:97–126.
3 Asa SL. Pituitary adenomas. In: Asa SL (ed) Tumors of the Pituitary Gland. Fourth Series Fascicle 15 Armed Forces Institute of Pathology: Washington, DC, 2011, pp 55–172.
4 Mete O, Asa SL. Clinicopathological correlations in pituitary adenomas. Brain Pathol 2012;22:443–453.
5 Gomez-Hernandez K, Ezzat S, Asa SL *et al.* Clinical implications of accurate subtyping of pituitary adenomas: perspectives from the treating physician. Turk Patoloji Derg 2015;31(Suppl 1):4–17.
6 Dekkers OM, Pereira AM, Romijn JA. Treatment and follow-up of clinically nonfunctioning pituitary macroadenomas. J Clin Endocrinol Metab 2008;93: 3717–3726.
7 Mete O, Ezzat S, Asa SL. Biomarkers of aggressive pituitary adenomas. J Mol Endocrinol 2012;49: R69–R78.
8 Yamada S, Ohyama K, Taguchi M *et al.* A study of the correlation between morphological findings and biological activities in clinically nonfunctioning pituitary adenomas.Neurosurgery 2007; 61: 580–584.
9 Balogun JA, Monsalves E, Juraschka K *et al.* Null cell adenomas of the pituitary gland: an institutional review of their clinical imaging and behavioral characteristics. Endocr Pathol 2015;26:63–70.
10 Alahmadi H, Lee D, Wilson JR *et al.* Clinical features of silent corticotroph adenomas. Acta Neurochir (Wien) 2012;154:1493–1498.
11 Horvath E, Kovacs K, Smyth HS *et al.* A novel type of pituitary adenoma: morphological features and clinical correlations. J Clin Endocrinol Metab 1988;66:1111–1118.
12 Horvath E, Kovacs K, Smyth HS *et al.* Silent adenoma subtype 3 of the pituitary–immunohistochemical and ultrastructural classification: a review of 29 cases. Ultrastruct Pathol 2005;29:511–524.
13 Erickson D, Scheithauer B, Atkinson J *et al.* Silent subtype 3 pituitary adenoma: a clinicopathologic analysis of the Mayo Clinic experience. Clin Endocrinol (Oxf) 2009;71:92–99.
14 Scheithauer BW, Jaap AJ, Horvath E *et al.* Clinically silent corticotroph tumors of the pituitary gland. Neurosurgery 2000;47:723–729.
15 Lopez JA, Kleinschmidt-Demasters Bk Bk, Sze CI *et al.* Silent corticotroph adenomas: further clinical and pathological observations. Hum Pathol 2004;35: 1137–1147.
16 Bradley KJ, Wass JA, Turner HE. Non-functioning pituitary adenomas with positive immunoreactivity for ACTH behave more aggressively than ACTH immunonegative tumours but do not recur more frequently. Clin Endocrinol (Oxf) 2003;58:59–64.
17 Cho HY, Cho SW, Kim SW *et al.* Silent corticotroph adenomas have unique recurrence characteristics compared with other nonfunctioning pituitary adenomas. Clin Endocrinol (Oxf) 2010;72:648–653.
18 Yamaguchi-Okada M, Inoshita N, Nishioka H *et al.* Clinicopathological analysis of nonfunctioning pituitary adenomas in patients younger than 25 years of age. J Neurosurg Pediatr 2012;9:511–516.
19 Nishioka H, Inoshita N, Mete O *et al.* The complementary role of transcription factors in the accurate diagnosis of clinically nonfunctioning pituitary adenomas. Endocr Pathol 2015;26:349–355.
20 Knosp E, Steiner E, Kitz K *et al.* Pituitary adenomas with invasion of the cavernous sinus space: a magnetic resonance imaging classification compared with surgical findings. Neurosurgery 1993;33:610–627.
21 Cuny T, Pertuit M, Sahnoun-Fathallah M *et al.* Genetic analysis in young patients with sporadic pituitary macroadenomas: besides AIP don't forget MEN1 genetic analysis. Eur J Endocrinol 2013;168: 533–541.
22 Obari A, Sano T, Ohyama K *et al.* Clinicopathological features of growth hormone-producing pituitary adenomas: difference among various types defined by cytokeratin distribution pattern including a transitional form. Endocr Pathol 2008;19:82–91.
23 Pereira BD, Raimundo L, Mete O *et al.* Monomorphous plurihormonal pituitary adenoma of Pit-1 lineage in a giant adolescent with central hyperthyroidism. Endocr Pathol 2015; doi:10.1007/s12022-015-9395-2 [e-pub ahead of print].