Pituitary Adenomas

The European Neuroendocrine Association's Young Researcher Committee Overview

Gianluca Tamagno
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Preface

Pituitary is a fascinating gland for endocrinologists, being the main center of the regulation of the rest of endocrine glands, in synergy with the hypothalamus. The bulk of scientific knowledge regarding pituitary physiology and dysfunctions, or diseases, increases year after year due to the efforts implemented by numerous clinical, translational, and basic researchers all over the world. Among the conditions possibly impacting on pituitary function, pituitary adenomas represent the largest group and the most frequent clinical scenario faced in pituitary clinics.

Considering the continuous changes and novelties arising in our understanding of the function of this master gland and the diseases that may affect that, together with the progressive optimization of the clinical care of the patients on the basis of the evolving scientific evidence and with the support of the international guidelines, we decided to build up a comprehensive and exhaustive overview of pituitary adenomas. As members of the European Neuroendocrine Association Young Researchers Committee (EYRC), we envisioned the delivery of a book focusing on the most relevant aspects of pituitary adenomas for the young endocrinologists, especially those who are active in the field of neuroendocrinology either from a clinical or a research point of view. With the priceless commitment of the authors of the chapters and the expert reviewers and endorsers of the same, all of them very well-known physicians and researchers in the field of neuroendocrinology, we have generated a book aiming to support the education and the scientific development of the young colleagues and pointing toward to help them in the management of their first pituitary adenoma patients with a straightforward and exhaustive collection of evidence-based data and expert advices.

Young physicians and researchers from worldwide scientific groups renowned in the field of pituitary diseases have greatly contributed as authors to the creation of this book. In other words, this work has been mostly done by young physicians and researchers for the benefit of other young physicians and researchers. The basic and translational aspects have been integrated with the clinical aspects arising from everyday practice, with the goal of representing a useful companion for every early-stage endocrinologist. Illustrative clinical scenarios and a “questions & answers” section have been included in order to translate the theoretical knowledge into the practice and for giving the readers the possibility of reviewing their clinical and research approaches.

The combined enthusiasm and expertise of authors, reviewers, and editors, on behalf of EYRC, has finally shaped an original monothematic book on pituitary adenomas, which is at the same time solid and innovative, with an appropriate balance between the traditional knowledge and the most exciting innovations coming from recent or ongoing research.

Dublin, Ireland — Gianluca Tamagno
Cordoba, Spain — Manuel D. Gahete

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Anatomy of the Pituitary Gland

1

Nicolas Coronel-Restrepo, Luis V. Syro, Fabio Rotondo, and Kalman Kovacs

1.1 Anatomy

The pituitary gland or hypophysis is a small, bean-shaped gland located in the sella turcica or pituitary fossa, which is the largest bone depression in the superior surface of the sphenoid bone. The *tuberculum sellae*, a variable median elevation, forms the anterior boundary of the sella turcica; the posterior border is formed by the *dorsum sellae*, and its floor is the roof of the sphenoid sinus. Bone and dura mater protect the gland in its anterior, inferior, and posterior surfaces, whereas the lateral and the superior surfaces are only covered by dura (Fig. 1.1). The lateral walls of the sella have only one layer of dura, but a double-layered dura covers all other surfaces. Superiorly, the pituitary gland is covered by the *diaphragma sellae* (sellar diaphragm), a dural sheath with a medial opening which allows the passage of the pituitary stalk [1, 2]. The optic chiasm lies anterior to the pituitary stalk and, in approximately 70% of the cases, above the sellar diaphragm; it is *prefixed* when located more anteriorly, at the level of the tuberculum sellae, or *postfixed*, when it is

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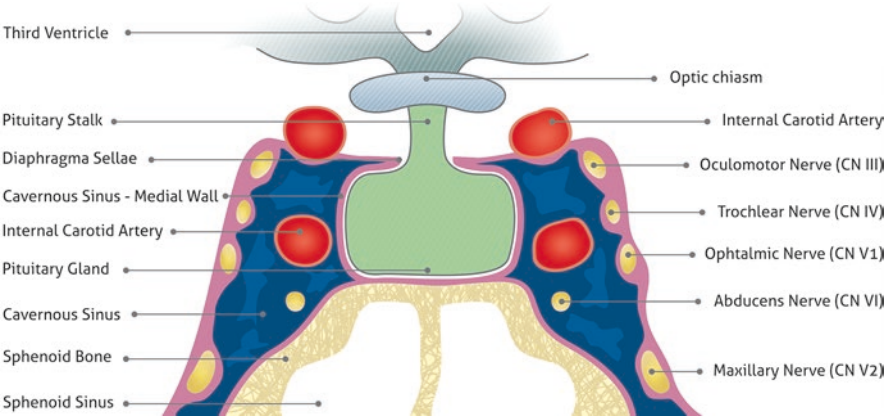

Fig. 1.1 Sellar and parasellar region

situated posteriorly, above the dorsum sellae. Depending on the position of the optic chiasm—prefixed, normal, or postfixed—any lesion that extends upwards may cause different visual field defects [3].

The cavernous sinuses, containing the internal carotid artery, the oculomotor, trochlear, and abducens nerves, are located bilaterally to the pituitary gland [4] (Fig. 1.1). Hence, the medial wall of the cavernous sinuses forms the lateral border of the sella. The cavernous sinus is the only anatomic site in the body where an artery traverses completely through a large venous channel. The cavernous sinuses are connected in the midline by the anterior and posterior intercavernous sinuses; they drain into the superior and inferior petrosal sinuses and finally into the internal jugular veins. In some cases, below the pituitary gland, there exists an inferior intercavernous sinus or venous plexus that needs to be underlined as a potential source of bleeding during any surgical procedure. The anatomic variability in the size of the diaphragm opening, and the fact that the medial wall of the cavernous sinus has only one single layer of dura, may explain the growth pattern of some pituitary tumors.

The normal weight of the pituitary gland, in adults, is approximately 0.6 g and measures 13 mm (transverse), 10 mm (anteroposterior), and 6 mm (vertical). The pituitary gland changes according to different physiological stages. At puberty, or during pregnancy and postpartum, hypertrophy of the pituitary gland can be seen [5]. Since the pituitary gland is close to several vital neurovascular structures, any benign or malignant lesion may present with a broad clinical spectrum and diverse outcomes [6–8].

The human pituitary gland consists of two distinct lobes: the anterior lobe or adenohypophysis, and the posterior lobe or neurohypophysis, each one of different embryologic origin [9] (Fig. 1.2). The adenohypophysis develops from Rathke's pouch, an invagination of the ectodermal primitive oral cavity, whereas the neurohypophysis develops from a downward extension of the neural ectoderm in the floor

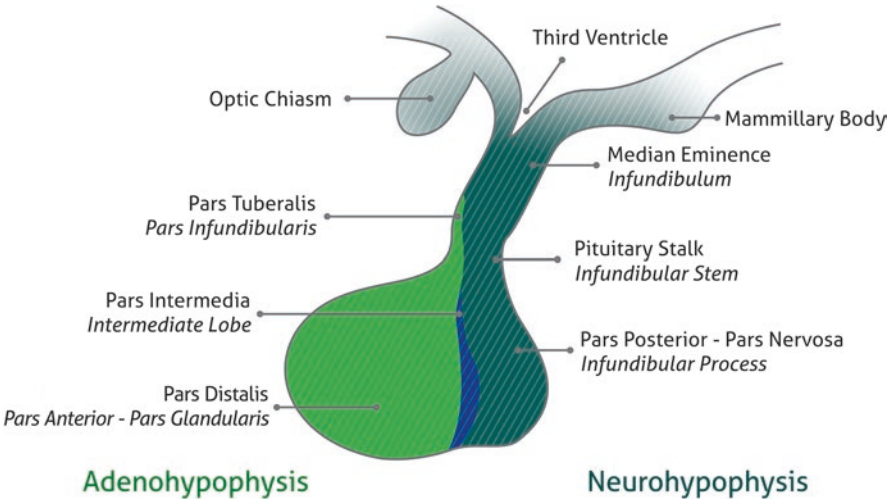

Fig. 1.2 Anatomy of the pituitary gland

of the diencephalon. The adenohypophysis consists of the *pars distalis*, the *pars intermedia*, and the *pars tuberalis*. The *pars distalis* (also known as pars anterior or pars glandularis) comprises 80% of the gland. The adult human pituitary gland has no anatomically distinct *pars intermedia*. It is made up of a small area of cells located at the junction of the two lobes, but it is morphologically rudimentary, and is incorporated into the *pars distalis*. The *pars tuberalis* is a minor upward extension of the adenohypophysis attached to the lower pituitary stalk. Histologically, the adenohypophysis comprises a central median wedge bordered by two lateral wings. The different hormone-producing cell types are distributed in an uneven, but specific manner. They are arranged within uniformly sized acini surrounded by a delicate reticulin fiber network, giving the pituitary its distinct architecture. The non-endocrine folliculo-stellate cells are localized in the center of the acini. The neurohypophysis is composed of nerve fibers that arise in the hypothalamic nuclei. It consists of three parts: the *medial eminence* or *infundibulum*, the *pituitary stalk* or *infundibular stem*, and the *pars nervosa* or *infundibular process* (Fig. 1.2).

The vascular supply and blood flow to the pituitary gland play an important role in the regulation of the adenohypophysial hormone secretion [9–11]. They carry the stimulatory and inhibitory hypothalamic hormones from the medial eminence to the adenohypophysis (Fig. 1.3). The blood irrigation of the gland is supplied by the superior and inferior hypophysial arteries. The *superior hypophysial arteries* – often three or four on both sides, joining around the proximal part of the pituitary stalk – originate from the supraclinoid segment of the internal carotid arteries, or the posterior communicating arteries. They reach and encircle the medial eminence generating an external and an internal capillary plexus. The external plexus is composed of small arteries that surround the upper part of the pituitary stalk. The

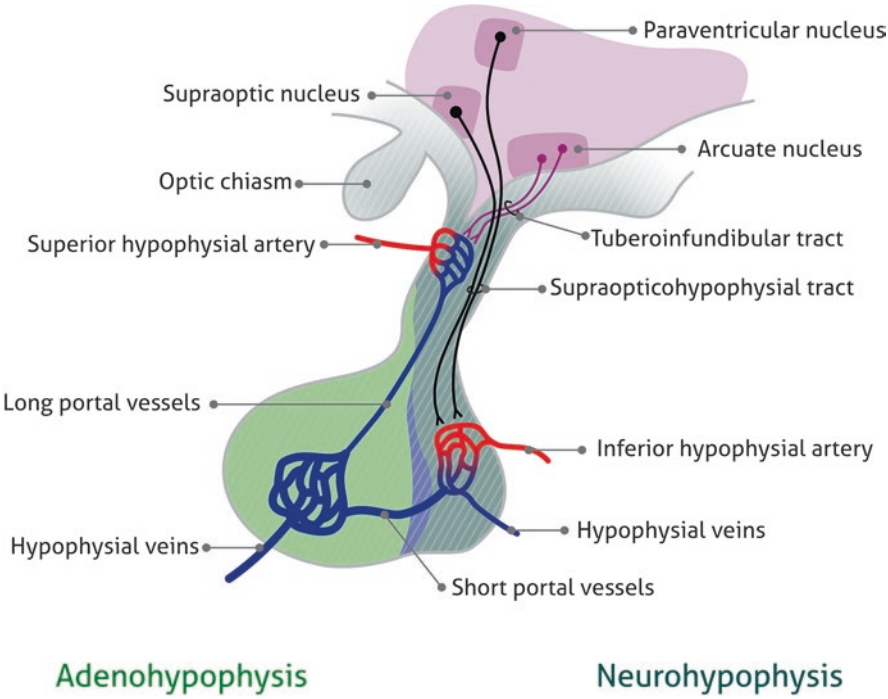

Fig. 1.3 Blood circulation to the pituitary gland

internal plexus is formed by a mesh of capillaries that contribute to the formation of the gomitoli, specialized vascular structures composed of a central muscular artery surrounded by a dense spiral network of capillaries [9, 11]. The function of the gomitoli is not clear, but it appears that they regulate the blood flow, influencing the release of the hypothalamic hormones in the pituitary paracrine network. The *inferior hypophysial arteries* originate from the meningohypophysial trunk within the cavernous sinus. They anastomose with each other to form an arterial circle around the posterior lobe, providing most of the blood supply to the posterior lobe, and to the lower portion of the stalk.

The hypophysial portal system connects the hypothalamus with the anterior pituitary; it stems from the internal capillary plexus of the median eminence and stalk [11] (Fig. 1.3). The *long portal vessels* originate from the median eminence and superior pituitary stalk; they descend on the anterior surface of the pituitary stalk and provide 90% of the blood that nourishes the anterolateral pars distalis. The *short portal vessels* originate in the lower part of the pituitary stalk; they go to the central portion of the anterior lobe and provide the remaining 10% of blood supply to the anterolateral pars distalis. Aside from a minor direct arterial supply from the inferior hypophysial arteries, most of the anterior lobe circulation is venous, coming from the portal vessels. The blood supply of the posterior lobe is arterial, in contrast to

that of the anterior lobe, which is venous, and is provided by the hypophysial portal system. This type of circulation has important clinical issues. If the pituitary stalk is damaged by a trauma that compromises the hypophysial portal system, the anterior pituitary can suffer a hemorrhagic infarct [12]. The fact that the anterior lobe receives minimal arterial supply can explain the infrequent metastases in that area [9].

The two most important pathways that link the hypothalamus to the pituitary are: the *tuberoinfundibular tract* and the *supraopticohypophysial tract* [13] (Fig. 1.3). The *tuberoinfundibular tract* arises from small neurons situated in the arcuate nucleus and the periventricular zone. The hypothalamic releasing hormones are carried by the axons down to the median eminence and to the infundibulum, where they are released into the hypophysial portal system. The *supraopticohypophysial tract* arises from large neurons located in the supraoptic and paraventricular nuclei. They synthesize oxytocin and vasopressin, which are transported and stored in specialized axon terminals (Herring bodies). When released, they enter the capillary plexus in the posterior pituitary and reach the general circulation via the hypophysial veins.

1.2 Histology

1.2.1 Cell Types of the Adenohypophysis

The endocrine cell types of the anterior pituitary gland develop from a common primordium. Cytodifferentiation occurs as a response to signaling pathways that emanate from distinct organizing centers. To develop and grow, each cell type requires additional specific transcription factors that regulate its cytodifferentiation and hormone production [14–16] (Table 1.1). Pituitary-specific transcription factor 1 (Pit-1) regulates the somatotroph, lactotroph, and thyrotroph cell types which produce growth hormone (GH), prolactin (PRL), and thyroid-stimulating hormone (TSH),

Table 1.1 Pituitary transcription factors, cell lineage, and hormone production in adenohypophysial cells

Transcription factor	Coexpression	Cell lineage	Hormone	Hypersecretion
Pit-1		Somatotrophs	GH	Acromegaly
	ER- α	Lactotrophs	PRL	Hyperprolactinemia
	GATA-2	Thyrotrophs	TSH	Hyperthyiroidism
Tpit		Corticotrophs	ACTH	Cushing disease
SF-1	ER- α, GATA-2, GATA-3	Gonadotrophs	LH, FSH, α-subunit	Most non-functional

GH growth hormone, *PRL* prolactin, *TSH* thyroid-stimulating hormone, *ACTH* adrenocorticotropic hormone, *LH* luteinizing hormone, *FSH* follicle-stimulating hormone, *Pit-1* pituitary-specific transcription factor 1, *Tpit* T-box transcription factor TBX19, *GATA-2* GATA binding protein 2, *GATA-3* GATA binding protein 3, *ER-α* estrogen receptor alpha, *SF-1* steroidogenic factor (Modified from Reference 35)

respectively. T-box transcription factor TBX19 (Tpit) regulates the corticotroph cells which produce adrenocorticotropic hormone (ACTH). The expression of steroidogenic factor 1 (SF1), estrogen receptor alpha (ER-α), and Guanine-Adenine-Thymine-Adenine binding protein 2 (GATA-2) regulates the gonadotroph cell type development. Estrogen receptor alpha assists Pit-1 to boost PRL secretion while repressing the production of GH; consequently, lactotrophs require the co-expression of both Pit-1 and ER-α for their development and function. Thyrotroph cell development requires the co-expression of GATA-2 along with Pit-1. Besides their role in pituitary gland development, the pituitary transcription factors serve as diagnostic markers of cell lineage in the classification of pituitary tumors [17–19].

Somatotrophs (GH-producing cells) make up approximately 50% of the gland, occupying mainly the lateral wings. They show strong acidophilia and GH hormone immunoreactivity. Some somatotrophs also express PRL or the α-subunit of the glycoprotein hormones [20]. ***Lactotrophs*** (PRL-producing cells) account for 10–30% of the pituitary gland cell population. In men and nulliparous women, they may represent 10% of the cell population, whereas in multiparous or lactating women, they may account for approximately 30% of the cell population [21]. They are chromophobic or slightly acidophilic, show PRL immunoreactivity, and are irregularly scattered throughout the entire anterior pituitary; but most often, they accumulate in the posterolateral rim of the lateral wings. ***Corticotrophs*** (ACTH-producing cells) reside within the median wedge of the pituitary gland. They are basophilic, periodic acid–Schiff (PAS)-positive, and immunoreactive for ACTH, as well as for other pro-opiomelanocortin (POMC) peptides. They represent approximately 15–20% of the cell population. ***Thyrotrophs*** (TSH-producing cells) occupy the anterior one-third of the median wedge and represent approximately 5% of the gland. They are slightly basophilic and strongly immunoreactive for β-TSH and the α-subunit. ***Gonadotrophs*** (FSH- and LH-producing cells) account for 10% of the cells distributed throughout the pars distalis. They are immunoreactive for β-FSH, β-LH, and their α-subunit.

The hormonal function of the adenohypophysial cell types exhibits considerable flexibility, depending on the functional demand placed on them. Reversible transdifferentiation can occur between members of the Pit-1 group as it has been documented: GH cells to PRL cells during pregnancy, and GH cells to TSH cells in hypothyroidism [22]. Pituitary cell types differ not only in their function, structure, and hormone content, but also in their morphological response to functional stimulation or suppression.

Folliculo-stellate cells, a major group of cells localized in the parenchymal tissue of the anterior pituitary, make up to 10% of the cell population in the human adenohypophysis. They have no hormonal function, but various experimental in vitro and morphological studies have documented their importance [23, 24]. To date, the functional role of folliculo-stellate cells remains a mystery. Various theories published suggest that they could be pituitary progenitor or stem cells, but whether this is true in humans, remains to be elucidated.

1.3 Pathology

1.3.1 Pituitary Adenomas

Pituitary adenomas are the most common type of pituitary disorder and account for 15–20% of all intracranial tumors. They are monoclonal, benign lesions, and are produced by genetic and epigenetic alterations that influence either proto-oncogenes, or tumor suppressor genes. The initiating cause remains unclear, but inactivation or overexpression of cell cycle regulators may be enough to trigger pituitary tumorigenesis [6]. A tumor results when an induced change gives growth advantage to a previously normal cell among other surrounding cells. Once this happens, the tumor grows by selective pressures from microenvironmental ecology [25, 26]. Tumor growth can be regarded as a Darwinian, adaptive system that grows as an evolutionary process [27]. The limited resources of the microenvironment restrict its size and its progression. Hence, natural selection in tumors, as in organisms, takes place through competition for space and resources [25, 28–30]. While the majority of pituitary adenomas are sporadic, some of them are associated with familial syndromes, exhibiting different genetic background, variable phenotype, and diverse clinical behavior [31, 32].

Pituitary adenomas may be referred to as micro or macroadenomas (if they are smaller or larger than 10 mm in diameter, respectively) and, depending on their hormonal hypersecretion, as functional or non-functional [7]. Their growth pattern may be either expansive, as in a slowly growing mass exerting pressure on the pituitary gland and the sella, or infiltrative, as a tumor spreading into the surrounding bone, dura, or cavernous sinuses. Pituitary adenomas extending into the suprasellar space may compress the optic chiasm and cause visual disturbances, a frequent clinical manifestation of macroadenomas [33].

Using the hematoxylin & eosin (H&E) stain for examining cellular and tissue structure detail, cells making up pituitary adenomas may be classified as acidophilic, basophilic, or chromophobic; this tinctorial property is not completely related to their hormonal function. The next step is to demonstrate reticulin fibers to differentiate normal adenohypophysis from hyperplasia or adenoma. The normal acinar reticular pattern will be enlarged but intact in cases of hyperplasia and will be lost in adenomas (Fig. 1.4a). The use of immunohistochemical methods that employ monoclonal antibodies against pituitary hormones helps to characterize the different functional cell type(s) in the tumor (Table 1.2). The current World Health Organization (WHO) classification (2017) of pituitary adenomas recommends the use of pituitary cell lineages along with their hormone production [17, 18, 34, 35] (Table 1.3). Accordingly, the classification makes use of a wide range of monoclonal antibodies against pituitary hormones and pituitary transcription factors. Electron microscopy is currently utilized only in difficult to diagnose cases. A clinicopathological classification, which considers proliferation markers, invasion, and size, has been recommended [36] and, recognizing the variable behavior of pituitary adenomas, a proposal to replace the term pituitary adenoma to pituitary neuroendocrine tumor (PitNET) has also been made [37].

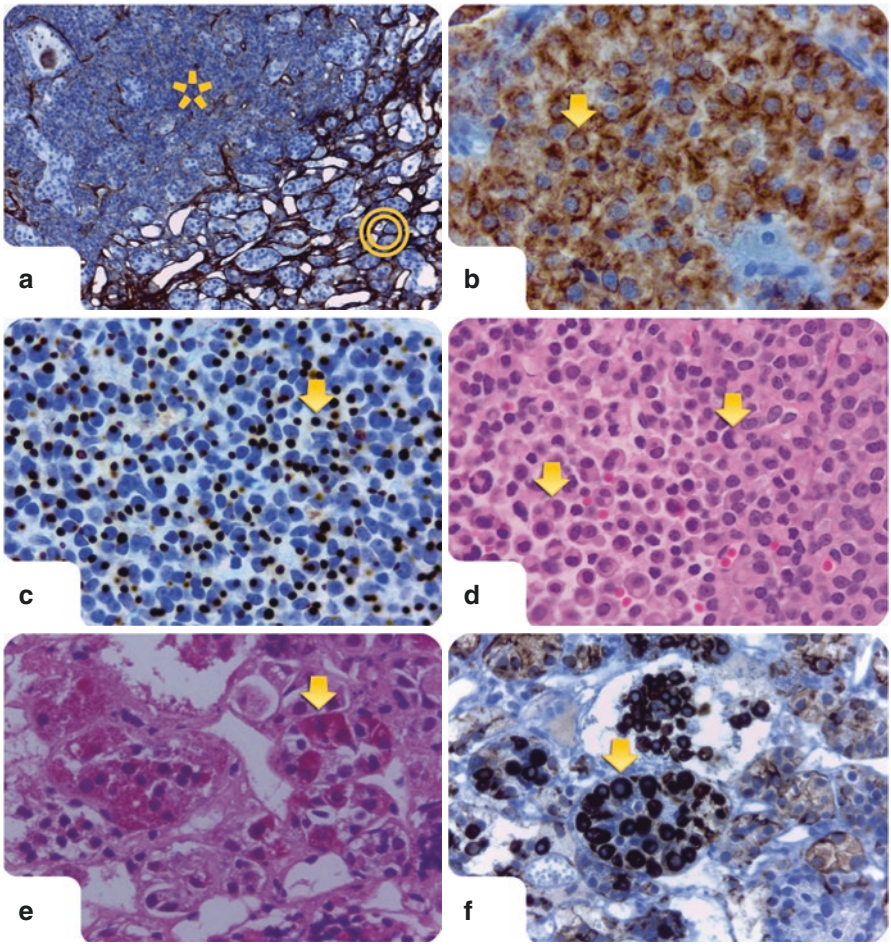

Fig. 1.4 Histologic features of pituitary pathology. (**a**) Light microphotograph showing the reticulin fiber network in both the normal pituitary gland and adenoma interface. The delicate normal acinar architecture is preserved in the normal gland (circle) but is totally broken down in the adenoma (asterisk). Stain: Collagen IV. Magnification: 10×. (**b**) Densely granulated somatotroph adenoma. Immunostaining for Cam5.2 demonstrates a strong, diffuse cytoplasmic pattern (arrow). Stain: Cam5.2 antibody. Magnification: 40×. (**c**) Sparsely granulated somatotroph adenoma. The fibrous bodies seen here are characteristic of this tumor type (arrow). Staining shows a strongly immunopositive perinuclear pattern as a result of the disruption and condensing of the cells keratin cytoskeleton. Stain: Cam5.2 antibody. Magnification: 40×. (**d**) Sparsely granulated somatotroph adenoma. Cells are chromophobic and pleomorphic on HE stain; in some of them, the fibrous bodies are apparent as spherical, paranuclear, unstained structures (arrows). Stain: Hematoxylin & eosin (HE). Magnification: 40×. (**e**) Non-tumorous pituitary gland in a patient with Cushing disease demonstrating the response of the non-tumoral corticotroph cells to glucocorticoid excess. The cytoplasm in these corticotroph cells undergoes an accumulation of keratin filaments which causes a glassy hyaline appearance (arrow). This is known as Crooke's hyaline change. Stain: Hematoxylin & eosin (HE). Magnification: 40×. (**f**) Light microphotograph showing the cytokeratin pattern seen in Crooke's cells. Massive accumulation of perinuclear cytokeratin, occurring in corticotrophs under the effect of glucocorticoid excess, is demonstrated as a strong ring-like pattern around the nucleus (arrow). Stain: Cam5.2 antibody. Magnification: 40×

Table 1.2 Morphological evaluation of pituitary adenomas

Stain	Stain pattern	Indication
Hematoxylin-eosin (HE)	Acidophilic, basophilic or chromophobic	Ancillary stain For initial classification of the tumor
Periodic acid–Schiff (PAS)	Cytoplasmic	To recognize corticotrophs and Crooke cells
Reticulin, Silver stain, Collagen IV	Acinar reticular pattern	To differentiate normal pituitary gland, hyperplasia, or tumor
Chromogranin, Synaptophysin	Secretory granules	Neuroendocrine markers
TTF-1	Nuclear	Positive in posterior pituitary gland and posterior pituitary tumors
GH, PRL, ACTH, TSH, FSH, LH, α-subunit	Cytoplasmic	To identify hormone secretion
Cam 5.2	Cytoplasmic Dot pattern Ring-like pattern	Positive in somatotrophs and corticotrophs To categorize pure somatotroph adenomas as densely or sparsely granulated To identify Crooke cells
Pit-1, Tpit, SF-1, ER-α, GATA-2, GATA-3	Nuclear	To identify cell lineage differentiation
E-cadherin	Membrane	Absent E-cadherin expression may be correlated to tumor invasiveness
Ki67, p53	Nuclear	Proliferation markers
SSTR2, SSTR5	Membrane	Predictive biomarkers for long-acting somatostatin analogs?
MGMT	Nuclear	To evaluate DNA repair pathway, DR Predictive biomarker for temozolomide treatment in aggressive pituitary adenomas and carcinomas
MSH2, MSH6, MLH1, PMS2	Nuclear	To evaluate integrity of DNA repair pathway, MMR
MPG	Nuclear	To evaluate integrity of DNA repair pathway, BER
VEGF	Cytoplasmic	To assess angiogenic growth and progression of tumor

TTF-1 thyroid transcription factor 1, *GH* growth hormone, *PRL* prolactin, *TSH* thyroid-stimulating hormone, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *ACTH* adrenocorticotropic hormone, *LMWK* low molecular weight keratin, *SSTR2* somatostatin receptor 2, *SSTR5* somatostatin receptor 5, *AIP* aryl-hydrocarbon receptor-interacting protein, *MGMT* O6-methylguanine DNA methyltransferase, *MSH2* MutS protein homolog 2, *MSH6* mutS homolog 6, *DNA* deoxyribonucleic acid, *DR* direct repair, *MMR* mismatch repair, *MPG* N-methylpurine DNA glycosylase, *BER* base excision repair, *VEGF* Vascular endothelial growth factor (Modified from reference 38)

1.3.1.1 Pit-1-Positive Tumors

Somatotroph Adenomas

Somatotroph adenomas arise from Pit-1 pituitary-cell lineage and express GH. Approximately 15% of surgically removed pituitary adenomas represent pure GH cell adenomas [38, 39]. Most of these tumors are associated with clinical signs of acromegaly, but silent forms have also been reported [40]. According to the density of GH-containing secretory granules in the cytoplasm of the cells, somatotroph

Table 1.3 Classification of pituitary adenomas

Tumor	Transcription factors	Hormones
Pit-1-positive tumors		
Somatotroph adenomas		
Sparsely granulated	Pit-1	GH (weak)
Densely granulated		GH, α-subunit
Mammosomatotroph adenomas	Pit-1, ER-α	GH, PRL, α-subunit
Mixed GH- PRL adenomas	Pit-1, ER-α	GH, PRL, α-subunit
Plurihormonal GH-producing adenomas	Pit-1, ER-α	GH, PRL, α-subunit, ß-TSH
Lactotroph adenomas		
Sparsely granulated	Pit-1, ER-α	PRL, α-subunit
Densely granulated		PRL
Acidophil stem cell adenomas		PRL, GH
Thyrotroph adenomas	Pit-1, GATA-2	ß-TSH, α-subunit
Monomorphous plurihormonal adenomas		
Silent subtype 3	Pit-1	GH, PRL, β-TSH
Tpit-positive tumors		
Corticotroph adenomas		
Densely granulated	Tpit	ACTH
Sparsely granulated		ACTH
Crooke cell adenoma		ACTH
SF-1-positive tumors		
Gonadotroph adenomas Hormone-positive	SF-1, ER-α, GATA-2, GATA-3	ß-FSH, ß-LH, α-subunit
Gonadotroph adenomas Hormone-negative		None
Polymorphous plurihormonal adenomas		
Plurihormonal adenomas	Multiple	Multiple
Transcription factor and hormone-negative adenomas		
Null cell adenomas	None	None

GH growth hormone, *ACTH* adrenocorticotrophic hormone, *PRL* prolactin, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *TSH* thyroid-stimulating hormone, *Pit-1* pituitary-specific transcription factor 1, *Tpit* T-box transcription factor TBX19, *GATA-2* GATA binding protein 2, *GATA-3* GATA binding protein 3, *ER-α* estrogen receptor alpha, *SF-1* steroidogenic factor 1 (Modified from reference 19)

adenomas can be classified as densely or sparsely granulated. A clear distinction between them is crucial for proper prognosis and treatment [41–43].

Densely granulated somatotroph adenomas are strongly acidophilic tumors with extensive immunoreactivity for GH and are often accompanied by α-subunit expression. Scattered immunopositivity for PRL and β-TSH is less frequent and not associated with hypersecretion. Immunostaining for Cam5.2 demonstrates a diffuse cytoplasmic pattern (Fig. 1.3b). Ultrastructurally, this adenoma type is comprised of cells that are like those of normal somatotrophs. A hallmark feature of densely granulated somatotroph adenomas is the large number of secretory granules dispersed throughout the cytoplasm. They occur with the same frequency in both sexes and display a slow, expansive growth, causing the typical "ballooning of the sella". They

may remain intrasellar for several years. Usually they have high expression of somatostatin receptors and respond with somatostatin analogs treatment [41].

Sparsely granulated somatotroph adenomas are usually chromophobic, with sparse and weak GH immunoreactivity. Nuclear pleomorphism may be evident, and the adenoma cells show spherical, paranuclear, unstained structures: the fibrous bodies. These fibrous bodies are strongly immunopositive for Cam5.2 in a dot pattern, characteristic of this tumor subtype (Fig. 1.3c, d). They also contain crescent-shaped cells scattered throughout the tumor, since the fiborous bodies can displace the nuclei. Sparsely granulated somatotroph adenomas, as opposed to the densely granulated type tumors, occur in younger patients. Multiple immunoreactivities for pituitary hormones are rarely noted. They tend to be macroadenomas at the time of diagnosis and are often aggressive, rapidly growing, and invasive [41]. Ultrastructurally, they show little similarity to the normal pituitary. They contain irregular-shaped nuclei, unevenly developed with varying amounts of rough endoplasmic reticulum present within the cell. Unlike the densely granulated somatotroph adenomas, this subtype contains few secretory granules which are much smaller in diameter.

Mixed somatotroph and lactotroph adenomas are made up of two distinct cell types. They are associated with acromegaly and different degrees of hyperprolactinemia. Mixed adenomas usually consist of densely granulated GH cells and sparsely granulated PRL cells. These acidophilic and chromophobic cells display immunoreactivity for GH and PRL. Ultrastructurally, two distinct features can be seen: morphology resembling that of the adenoma that produces only GH and one that produces only PRL.

Mammosomatotroph cell adenomas are monomorphous, consisting of one cell type displaying both GH and PRL immunoexpression, although immunopositivity is practically always stronger for GH. They are associated with acromegaly and hyperprolactinemia, showing biological behavior like that of densely granulated somatotroph adenomas. Ultrastructurally, these tumor cells contain both GH-containing and PRL-containing secretory granules, but they resemble adenoma cells found in densely granulated somatotroph adenomas. Mammosomatotroph adenomas contain two types of pleomorphic and heterogeneous secretory granules: large, irregular, or elongated granules; and, small to moderate-sized secretory granules that have a spherical or ovoid-shaped.

Lactotroph Adenomas

Lactotroph adenomas arise from the Pit-1 pituitary cell lineage and express mainly PRL. They are classified into three histological subtypes: sparsely granulated, densely granulated, and acidophil stem cell adenomas.

Sparsely granulated lactotroph adenomas are the most common adenoma type and they are associated with hyperprolactinemia. Lactotroph adenomas have a wide-ranging biological behavior from indolent to aggressive, and in men usually present with invasive radiological features. The PRL blood levels are generally proportional to the tumor size. Most lactotroph adenomas are chromophobic,

PAS-negative cells with a diffuse or papillary growth pattern, and exhibit strong PRL immunopositivity with co-expression of Pit-1 and ER-α. Psammoma bodies and interstitial amyloid have been documented in some cases, while cellular and nuclear pleomorphism is rarely seen. Calcification within the adenoma can be found in circa 15% of all sparsely granulated lactotroph adenoma cases. Ultrastructurally, three noticeable features are: a rough endoplasmic reticulum, a prominent Golgi apparatus, and the lateral extrusion of the secretory granules—commonly known as misplaced exocytosis.

Densely granulated lactotroph adenomas are rare and have been considered aggressive tumors. Misplaced exocytosis is the hallmark of sparsely granulated lactotroph adenomas but is rarely shown in densely granulated lactotroph adenomas. Ultrastructurally, numerous ovoid/spherical large secretory granules are clearly noted, as well as, well-developed Golgi complexes but the rough endoplasmic reticulum is less abundant, as compared to the sparsely granulated lactotroph adenoma subtype.

Acidophil stem cell adenomas are monomorphous tumors with morphological signs of PRL and GH differentiation. They are rare and are associated with hyperprolactinemia, but the serum PRL levels may be disproportionately low for their size [44]. Elevation of GH levels and acromegaly are infrequent. They are chromophobic, with moderate to strong immunoreactivity for PRL. Immunopositivity for GH is weak or negative, and Cam5.2 reveals the dot-like positivity of fibrous bodies in some cells. Ultrastructure features include an increased number of giant mitochondria (oncocytic change), small secretory granules with lateral extrusion (PRL marker), and fibrous bodies (GH marker).

Although calcification is extremely rare in other adenoma types, about 10–15% of lactotroph adenomas may display varying degrees of calcification. Dopamine agonists produce remarkable morphological changes: the nucleus becomes heterochromatic and the cytoplasm displays marked shrinkage due to the loss of the hormone-producing apparatus. As a result, PRL immunoreactivity is reduced or lost. Although these morphological changes are reversible, some portions of the tumor may maintain their suppressed features when treatment is discontinued. Long-term treatment with dopamine agonists may also cause varying degrees of fibrosis and calcification which results in the presence of psammoma bodies.

Thyrotroph Adenomas

Thyrotroph adenomas express mainly TSH and arise from Pit-1 pituitary-cell lineage. They are associated either with hyperthyroidism or inappropriately elevated levels of TSH. In some cases, they occur in euthyroid subjects. They are often macroadenomas with a tendency to invade and their morphology exhibits surprising diversity. Cells making up thyrotroph adenomas are generally chromophobic and highly differentiated, comprising elongated polar cells that form pseudorosettes around vessels. In other cases, the pattern may be diffuse with considerable nuclear pleomorphism. Another variant is markedly fibrotic. Immunoreactivity for TSH is variable; it is often patchy or scattered, rarely extensive. Scattered cells may exhibit

immunoreactivity for GH, PRL, and α-subunit. Both Pit-1 and GATA2 are expressed. In most cases, they are strongly positive for somatostatin receptor 2 (SSTR2) immunostaining. Ultrastructurally, these adenoma cells resemble normal thyrotroph cells: euchromatic nuclei, abundant rough endoplasmic reticulum, well-developed Golgi complexes, microtubules, and secretory granules located along the cell membrane.

Monomorphous Plurihormonal Adenomas

Plurihormonal adenomas produce more than one hormone. They can be monomorphous or plurimorphous, consisting of two or more different cell lineages. They include ***plurihormonal Pit-1-positive adenomas*** previously called silent subtype 3 adenomas. They are rare, but clinically significant due to their aggressive behavior. Plurihormonal Pit-1-positive adenomas can mimic lactotroph adenomas or, in some cases, may present with acromegaly. They are often acidophilic, may show mild PAS positivity, and form a diffuse or lobular pattern. Most tumor cells are immunonegative for pituitary hormones, but some of them may demonstrate scattered minor positivity. They exhibit positive nuclear expression for Pit-1.

1.3.1.2 Tpit-Positive Tumors

Corticotroph Adenomas

Corticotroph adenomas arise from Tpit pituitary-cell lineage and express ACTH and other POMC-derived peptides. They are classified into three subtypes: densely granulated, sparsely granulated, and Crooke cell adenomas.

Densely granulated corticotroph adenomas are basophilic and PAS-positive, with a sinusoidal or diffuse pattern. Immunoreactivity can be demonstrated not only for ACTH, but also for other POMC peptides (β-endorphin, β-LPH, and CLIP). Perinuclear bundles of cytokeratin filaments are demonstrated with Cam5.2 as a cytoplasmic, perinuclear pattern. Most corticotroph tumors are small microadenomas causing Cushing's disease [42, 45]. The adenomas, often measuring only a few millimeters in diameter, may be too small to be detected by imaging or to be identified at surgery. In a few cases of milder hypercorticism, Cushing's disease is produced by large, aggressive, and invasive tumors. Histologically they exhibit variable, often weak, PAS positivity, and ACTH immunoreactivity. A few cases of aggressive macroadenomas also display immunoreactivity for LH and α-subunit. In cases of Nelson's syndrome, the morphological features are like those of densely granulated corticotroph adenomas in Cushing's disease, showing few or no cytokeratin filaments.

Sparsely granulated corticotroph adenomas are composed of slightly basophilic or chromophobic cells. They are PAS-positive with patchy immunopositivity for ACTH. In some cases, they are clinically silent without Cushing's disease.

The response of the non-tumoral corticotroph cells to high levels of glucocorticoids is an accumulation of cytokeratin filaments in the cytoplasm (Crooke's change), which causes a glassy hyaline appearance with a displacement of the ACTH-positive granules to the cell periphery (Fig. 1.3e). These "Crooke cells" are

identified by using Cam5.2 immunostaining, which reveals a strong ring-like pattern around the nucleus (Fig. 1.3f). The identification of Crooke cells is of paramount importance in cases of persistent hypercortisolism after surgery, with only a non-tumoral pituitary gland identifiable in the resected tissue. The presence of Crooke cells in the non-tumoral pituitary gland confirms the previous hypercorticism and will dictate the next step of treatment. Therefore, Crooke hyalinization would be noted in: (1) non-tumoral corticotrophs adjacent to the lesion – in that case, the tumor was overlooked by the surgeon; (2) in ectopic ACTH/corticotrophin-releasing hormone (CRH) syndrome; (3) in patients with glucocorticoid secreting adrenocortical tumors; (4) in patients taking glucocorticoids (factitious Cushing syndrome). In cases of pseudo-Cushing, Crooke cells will not be present in the pituitary tissue [45].

Crooke cell adenomas are a rare type of corticotroph adenomas. They may cause Cushing's disease or may be endocrinologically silent [39]. Crooke hyalinization is not expected to develop in tumoral corticotroph cells, although a minority of them may present that alteration, identical to the Crooke cells seen in the adenohypophysis of patients with glucocorticoid excess. When this change affects more than 50% of the tumor's cells, it is diagnosed as a Crooke cell adenoma [46]. The reason why Crooke cell adenomas produce ACTH while simultaneously display Crooke hyaline changes due to increased glucocorticoid excess, is not well understood. These adenomas are usually invasive, may exhibit aggressive clinical behavior, and often recur with a low rate of success for a cure after reoperation and radiotherapy [46].

Silent corticotroph adenomas do not display functional activity and present as clinically non-functional adenomas [47]. There are two subtypes. ***Silent corticotroph adenomas subtype 1***—densely granulated—which exhibit the same morphology of corticotroph adenomas associated with Cushing's disease. They display a high propensity for hemorrhage and may present with pituitary apoplexy. ***Silent corticotroph adenomas subtype 2***—sparsely granulated—are chromophobic adenomas comprised of small cells, which exhibit only modest PAS positivity and scattered immunoreactivity for ACTH. No cytokeratin filaments are present with Cam5.2 [40, 47]. These two adenoma subtypes are probably derived from cells of the pars intermedia.

1.3.1.3 SF-1-Positive Tumors

Gonadotroph Adenomas

Gonadotroph adenomas arise from SF-1 pituitary-cell lineage and produce β-FSH, β-LH, and α-subunit. They can also express ER-α, GATA2, and GATA3. The morphology of gonadotroph adenomas is variable. Histology may reveal either polar cells forming pseudorosettes around vessels, or a diffuse pattern. Oncocytic change, exhibiting an excessive increase of number and volume density of mitochondria, is frequent. Immunoreactivity for FSH, LH, and α-subunit is variable and often patchy. In some cases, there is minimal immunoexpression of hormones and the diagnosis can only be confirmed by analyzing the transcription factors present in the adenoma.

1.3.1.4 Polymorphous Plurihormonal Adenomas

Plurihormonal Adenomas

Polymorphous plurihormonal adenomas are rare tumors, often with unique ultrastructure. The most common cell combinations making up the adenoma are GH-TSH-PRL or PRL-TSH, but other combinations of different cellular lineages have also been documented. The immunoexpression of different transcription factors will depend on the different cell lineages of the tumor.

1.3.1.5 Transcription Factor-Negative and Hormone-Negative Adenomas

Null Cell Adenomas

Null cell adenomas do not display evidence of cell lineage differentiation by pituitary transcription factors or pituitary hormones. These hormonally inactive adenomas account for approximately 1–2% of surgically removed tumors [48, 49]. They are chromophobic, and pseudorosette formation, a characteristic of glycoprotein hormone-producing tumors, may also occur. Null cell adenomas are immunonegative for adenohypophysial hormones and transcription factors. Accumulation of mitochondria can occur, and in these cases, they are referred to as pituitary oncocytomas. Characteristically, oncocytomas possess larger cells than null cell adenomas and may display acidophilia due to non-specific binding of acidic stains by mitochondria. The tumor cells are known as oncocytes. Nuclei may show signs of shrinkage, hyperchromatism, and clumping of chromosomes, a condition known as pyknosis. The pattern of immunoreactivity is equal to null cells or gonadotroph adenomas. Ultrastructurally, oncocytomas are like null cell adenomas except for the extensive accumulation of mitochondria seen in oncocytomas. All other organelles are poorly developed.

1.3.2 Pituitary Carcinomas

Pituitary carcinomas can be diagnosed only when a pituitary neoplasm gives rise to distant metastasis. They are rare, representing about 0.12–0.2% [50]. They usually present as invasive macroadenomas and appear like other pituitary adenomas. Currently, there are no accurate criteria to distinguish between an invasive adenoma and one that has the potential to produce metastases [51, 52]. Most pituitary carcinomas produce either PRL or ACTH. Pituitary carcinomas are not accompanied by specific histological features, and enhanced mitotic activity, or nuclear and cellular pleomorphism do not necessarily indicate malignancy. Neoplasms with bland features may give rise to metastasis. Carcinomas usually display a higher cell proliferation marker (Ki-67) than adenomas. Immunoreactivities of pituitary carcinomas follow the pattern of their non-malignant phenotype [52].

1.3.3 Aggressive Pituitary Adenomas

Clinically aggressive pituitary adenomas comprise a wide group of tumor types that do not respond well to therapy, are recurrent, and are generally associated with a poor prognostic outcome. About 10% of pituitary adenomas can have an aggressive clinical course [51]. Aggressive tumors can be either micro- or macro-adenomas, have the potential to invade surrounding structures, and have a higher rate of recurrence compared to their benign adenoma counterparts [53]. Some types of adenomas may suggest aggressiveness. According to WHO classification of pituitary adenomas (2017), sparsely granulated somatotroph adenomas, lactotroph adenomas in men, silent corticotroph adenomas, Crooke cell adenomas, and plurihormonal PIT-1-positive adenomas are considered as high risk (of recurrence) adenomas [17]. No single feature can provide a reliable assessment to predict aggressive behavior. To date, there is no way to diagnose well in advance an aggressive tumor; the most reliable and accepted immunohistochemical analysis is the assessment of the Ki-67 nuclear labeling. If the Ki-67 nuclear labeling index is more than 10%, the tumor may be classified as aggressive, although there is no agreement on this. Another histologic marker used to correlate tumor behavior is the p53 expression [52]. Immunohistochemical study of MGMT expression may be used as a prognostic marker for aggressive pituitary adenomas. Studies have shown that progression and recurrence of pituitary adenomas are often associated with loss of MGMT expression [54, 55].

1.4 Conclusion

The pituitary gland and the hypothalamus regulate the endocrine system. These two structures are closely related to each other, thanks to a specialized network of blood vessels and associated nerve endings. Pituitary adenomas comprise a broad spectrum of tumors; different structures around the pituitary gland will determine the possibility of their complete resection with surgery. A precise morphological characterization of pituitary adenomas will help to choose a personalized therapy.

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Physiology of the Pituitary Hormone Secretion

2

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Abbreviations

Ach	Acetylcholine
AC	Adenylate cyclase
ACTH	Adrenocorticotropic hormone
AII	Angiotensin II
ADH	Antidiuretic hormone
AVP	Arginine-vasopressin
BDNF	Brain-derived neurotrophic factor
CNS	Central nervous system

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CRH	Corticotropin-releasing hormone
CST	Cortistatin
cAMP	Cyclic adenosine monophosphate
DRDs	DA receptors
DA	Dopamine
DRD2	Dopamine receptor subtype-2
FST	Follistatin
FSH	Follicle-stimulating hormone
GHRH	GH-releasing hormone
GHSR1a	GH-secretagogue receptor 1a
GHRL	Ghrelin
GABA	γ-aminobutyric acid
GAP	GNRH-associated peptide
GnIH	Gonadotropin-inhibitory hormone
GNRH	Gonadotropin-releasing hormone
GNRHR	Gonadotropin-releasing hormone receptor
GH	Growth hormone
GPCR	Seven trans-membrane G protein-coupled receptor
GHRHR	GH-releasing hormone Receptor
hCG	Human chorionic gonadotropin
HPA	Hypothalamic–pituitary–adrenal
IGF1	Insulin-like growth factor 1
KISS1	Kisspeptin
LH	Luteinizing hormone
MNCS	Magnocellular neurosecretory cells
mTOR	Mammalian Target of Rapamycin
MC1R, MC3R, MC4R and MC5R, excluding the ACTH-specific receptor MC2R	Melanocortin receptor
MRGX2	Mas-related G-protein coupled receptor member X2
MSH	Melanocyte-stimulating hormone
MT	Melatonin
MAPKs	Mitogen-activated protein kinases
NST	Neuronostatin
NPY	Neuropeptide Y
NA	Noradrenaline
NE	Norepinephrine
NPY	Thyrotropin Releasing Hormone
OT	Oxytocin
PAC1R	PACAP type 1 receptors
PLC	Phospholipase C
PACAP	Pituitary adenylate cyclase-activating polypeptide
POMC	Precursor hormone proopiomelanocortin
PRL-R	PRL receptor

PRL	Prolactin
PKA	Protein kinase A
PKC	Protein kinase C
PitNETs	Pituitary neuroendocrine tumors
RFRPs	RF-related peptides
5-HT	Serotonin
SST	Somatostatin
SSTR	Somatostatin Receptor
SRIF	Somatotropin-release inhibitory factor
SF1	Steroidogenic factor-1
TRH	Thyrotropin-releasing hormone
T4	Thyroxine
T3	Triiodothyronine
TSH	Thyrotropin-stimulating hormone
VIP	Vasoactive intestinal peptide
VPAC1	Vasoactive intestinal polypeptide receptor 1

2.1 Introduction

The pituitary is considered one of the most important endocrine glands in the organism since it is responsible to modulate a series of critical biological processes and functions, by regulating the physiology of different tissues, organs, and other endocrine glands throughout the whole organism. Specifically, the pituitary participates in the control of multiple important patho-physiological functions such as growth, reproduction, metabolism, or stress response.

As extensively explained in Chap. 1, this complex gland is located at the base of the brain, wherein it displays a privileged location at the interphase between the central nervous system (CNS) and the rest of the organism. Indeed, this gland is strongly connected in an anatomical and physiological manner with one of the most relevant CNS areas, the hypothalamus. The pituitary is organized in two structurally and functionally distinct areas named adenohypophysis and neurohypophysis. The adenohypophysis (*adeno*, meaning "glandular") consists of three regions: *pars distalis*, *pars intermedia*, and *pars tuberalis*. The *pars distalis* (or *anterior pituitary gland*) occupies the major portion (70%) of the adenohypophysis. The *pars intermedia* is a thin band of cells between the *pars distalis* and the neurohypophysis. The *pars tuberalis* is a group of cells surrounding the pituitary stalk. The neurohypophysis, *pars nervosa* or posterior lobe, is not a glandular portion but represents a collection of axonal projections from the hypothalamus that serves as a site of secretion for neurohypophysial hormones.

In this context, the main aspects related to the physiology of the pituitary gland rely on the hormones that are produced and secreted from each of these different areas, which exhibit a plethora of pleiotropic functions to regulate the vast majority of endocrine glands, tissues, and organs in the body. In the anterior pituitary, there

are five different specialized endocrine secretory cell types: somatotrophs, lactotrophs, corticotrophs, gonadotrophs, and thyrotrophs. These cells are responsible for the synthesis and secretion of growth hormone (GH), prolactin (PRL), adrenocorticotropic hormone (ACTH), luteinizing hormone (LH), and follicle-stimulating hormone (FSH), and thyrotropin-stimulating hormone (TSH), respectively [1]. The main cell type found in the *pars intermedia* is the melanotrope cell, which secretes melanocyte-stimulating hormone or MSH. However, the *pars intermedia* is typically involuted in adulthood, being insignificant or entirely absent [2]. In the case of the neurohypophysis, the axonal projections of neurons located at the supraoptic and paraventricular hypothalamic nuclei accumulate vesicles with arginine-vasopressin (AVP) and oxytocin (OT), which are released directly from the hypothalamus to the bloodstream [3].

Some of these hormones, once are released into the bloodstream, act directly on different target tissues. In fact, GH exerts modulatory actions on the growth of all tissues of the organism, and PRL is responsible for the development of glandular *acini* in the mammary glands and the regulation of human milk proteins synthesis. Similarly, AVP acts directly on kidney and arterioles increasing peripheral vascular resistance and raising arterial blood pressure, and OT directly regulates myoepithelial cells contraction of the mammary glands as well as of the uterus, controlling the contractions during lactation and childbirth. On the other hand, ACTH, FSH, LH, and TSH are trophic hormones that act by stimulating the production and release of hormones in specific peripheral endocrine glands such as the adrenal glands (ACTH), gonads (LH and FSH), and the thyroid gland (TSH) [1].

In any case, the synthesis and/or production of these hormones at the pituitary level is tightly controlled by a plethora of central and peripheral factors that finely interact to modulate the physiologic production of these hormones (Fig. 2.1). It is

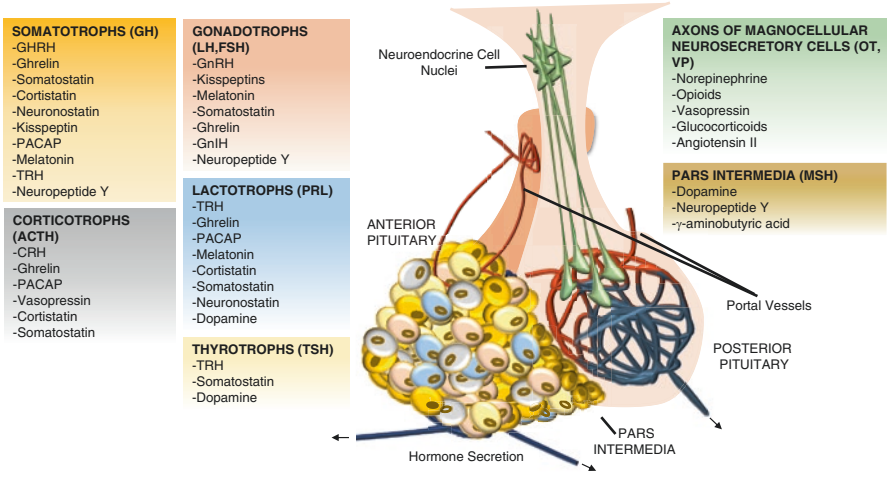

Fig. 2.1 Representative diagram summarizing the main regulators involved in the modulation of the pituitary hormone secretion of different pituitary cells

now known that each pituitary cell type exhibits a particular pattern of receptors for different signals and that the appropriate sensing, transmission, and integration of all these inhibitory and stimulatory signals is crucial to adapt the circulating levels of these hormones to the physiological necessities of the organism.

2.2 Anterior Pituitary

2.2.1 Somatotrophs

Somatotrophs, which represent the most abundant secretory cell type in the anterior pituitary, are responsible for the synthesis and release of GH. Once secreted into the bloodstream, GH acts directly through the GH receptor (GHR), and/or indirectly, by stimulating the release of insulin-like growth factor 1 (IGF1) to promote linear growth in children [4]. In addition, GH has been shown to exhibit many metabolic actions including enhanced protein synthesis, lipolysis and lipid oxidation, retention of water, phosphate and sodium and acting as an insulin antagonist [5].

GH production begins early in fetal life and its levels vary during childhood, peaking during puberty and lowering in elderly. GH is secreted in a circadian pulsatile fashion, wherein the interplay between the hypothalamic hypo-physiotropic hormones GH-releasing hormone (GHRH) and its counteracting inhibitor somatostatin (SST) plays a crucial role. Indeed, the hypothalamic control of GH secretion in mammals has long been considered as a classic paradigm of the "dual control" system of pituitary hormone secretion mentioned above. However, the daily peaks of GH secretion also vary with age and are profoundly influenced by several factors including gender, sleep, obesity, nutrition, exercise, and metabolic disorders [6]. In fact, it is now accepted that the pulsatile secretion of GH can be also modulated by diverse factors residing in the pituitary or in other regions of the CNS, as well as by additional factors arriving from peripheral organs and tissues [7], as described below.

2.2.1.1 Growth Hormone Releasing Hormone (GHRH)

GHRH is a 44 amino acid amidated polypeptide produced and secreted by the terminals of neurons located in the hypothalamic arcuate nucleus [8]. GHRH leads to the stimulation of GH production, via the hypophyseal portal system, by increasing *GH* gene expression and inducing GH release by the somatotrophs. Indeed, GHRH has been long recognized as the main stimulator of GH secretion in humans and, together with somatostatin, the central modulators of GH pulsatile secretion. In addition, GHRH also acts as a growth-stimulatory factor of somatotroph cells inasmuch as GHRH absence produces anterior pituitary hypoplasia [9]. This hypothalamic hypo-physiotropic hormone acts through a seven trans-membrane G protein-coupled receptor (GPCR) named GHRHR (GH-releasing hormone receptor), which is expressed at high levels in somatotroph cells. GHRHR is crucial for the actions of GHRH and, indeed, mutations in this receptor have been largely reported to be associated with an impairment of the physiological development of

pituitary somatotrophs [10], producing isolated GH deficiency, the most common pituitary hormone deficiency [11]. GHRH binding to GHRHR stimulates GH release by activating multiple signaling pathways, including adenylate cyclase (AC) and increasing cyclic adenosine monophosphate (cAMP) concentration, which leads to the increase of protein kinase A (PKA) activity followed by CREB activation [1, 12].

2.2.1.2 Ghrelin (GHRL)

Ghrelin, a 28 amino acid octanoylated peptide, was initially isolated from human and rat stomachs [13]. Subsequently, ghrelin expression was also found in multiple organs and tissues including the pituitary and hypothalamus [14]. Ghrelin can be found in two circulating isoforms, acylated (octanoylated) and non-acylated (which seems to lack endocrine actions but exerts non-endocrine actions) [15]. The acylated form is the endocrine functional form of ghrelin, which has been shown to act at the pituitary level but also at CNS level as an orexigenic factor. Particularly, ghrelin was rapidly recognized as a potent GH-releasing factor through its binding to the previously identified orphan GH-secretagogue receptor 1a (GHSR1a), producing a robust GH release, comparable or even higher to that exerted by the classical GH stimulator GHRH [16, 17]. The binding of acylated ghrelin to GHSR1a triggers the activation of multiple signal transduction pathways, including phospholipase C (PLC), protein kinase C (PKC), PKA, intracellular and extracellular Ca^{2+} or mitogen-activated protein kinases (MAPKs), resulting in a significant elevation of GH transcription (elicited by specific transcription factors) and GH secretion (as the result of Ca^{2+} mobilization, among other signals) [1, 18]. In addition, acylated ghrelin is able to induce an indirect, hypothalamic-mediated mechanism involving an increase in GHRH and a weak inhibition of SST neurons, promoting a complete program to stimulate GH release [19].

2.2.1.3 Somatostatin (SST)

SST or somatotropin-release inhibitory factor (SRIF) is a cyclic peptide derived from a 116 amino acid precursor that can generate two different cyclic variants by alternative post-translational processing, SST-14 and SST-28. Both peptides contain a disulfide bridge, have a cyclic structure as well as high affinity for SST receptor subtypes, and they are capable to inhibit GH release [20]. SST regulates different aspects of the endocrine system and controls neurotransmission and cell proliferation. Specifically, SST is a short half-life inhibitor of the secretion of several hormones including GH through the modulation of somatotroph cells. In particular, SST is considered the main negative regulator of GH secretion and its inhibitory function is conserved during vertebrate evolution. In mammals, SST is essential to establish and maintain pulsatility of GH secretion [21, 22]. In this sense, it has been proposed that in humans, the role of SST in the control of GH secretion seems to be mainly circumscribed to the adjustment of the magnitude of its basal and pulsatile release, while it may not be involved in the regulation of the generation of GH pulsatility [6]. Thus, although SST is undoubtedly the main inhibitory signal for GH secretion in all vertebrate groups, it is not the only one as it will be explained below,

and its role can be more complex than it was initially envisioned [20]. SST exerts its actions via interaction with different GPCR subtypes (SSTR1-5 and their splicing variants, such as SST5TMD4 or SST5TMD5) [20]. SST binding to these receptors elicits the modulation of a constellation of downstream pathways, including AC, protein phosphatases, cGMP dependent protein kinases, and calcium and other ion channels [1], resulting in changes in intracellular calcium and cAMP concentrations, and the subsequent reduction in GH secretion [20].

2.2.1.4 Cortistatin (CST)

CST is a peptide with a strong structural and functional similarity with SST. CST is predominantly expressed in inhibitory neurons of the cerebral cortex but, as well as SST, it is distributed and expressed in a wide variety of tissues, including the pituitary gland [23, 24]. Human CST is produced from the 105 amino acid precortistatin, which can be cleaved into CST-17 and CST-29. CST-17, the mainly active peptide, shares a high homology with SST-14. This strong structural similarity explains their similar capacity to bind the same family of receptors (SSTRs) and to recruit the same downstream pathways [1, 25, 26]. However, CST is also able to bind other receptors such as GHSR1a [27, 28] or MRGX2 [29], which may explain some of the dissimilar actions exerted by CST such as the induction of slow wave sleep. In any case, at the pituitary level and specifically in somatotrophs, CST can inhibit GH release through the activation of SST receptors in young males and, indeed, CST and SST show equal inhibition of the GH release induced by GHRH, ghrelin, and synthetic analogs [30]. These effects of CST seem to be independent of GHSR1a binding, suggesting a predominant role of SST receptors on the known inhibitory actions of CST in this cell type [31].

2.2.1.5 Neuronostatin (NST)

NST is a 13 amino acid neuropeptide encoded by the somatostatin gene and derived from the alternative proteolytic processing of the same 116 amino acid precursor that gives rise to SST [32]. NST is known to exert important physiological and metabolic actions in diverse tissues. Conversely, the direct biological effect on anterior pituitary function, specifically in somatotrophs, has not been extensively explored. Recent studies have shown that NST functions seem to be associated with the inhibition of basal GH and LH release. However, this hormone seems to exert different actions from those shown by SST, especially related to the modulation of hormone (GH and LH) release and gene expression in this gland [33]. NST may exert its actions via alternative GPCRs, and, particularly. Through GPR107, which is a mechanism of action clearly different from that exhibited by SST and CST [34].

2.2.1.6 Kisspeptin (KISS1)

Kisspeptin is an amidated neurohormone encoded by the *KISS1* gene that is widely known for its role in the modulation of sexual development processes, such as puberty and ovulation, through the activation of gonadotropin-releasing hormone (GNRH) neurons excitability. The product of *KISS1* gene is a 145 amino acid precursor that can generate four possible modified peptides with different lengths, 54,

14, 13, or 10 amino acids. Kisspeptin peptides can bind and activate a GPCR named GPR54 or KISS1R [35]. All the kisspeptin peptides have the same efficacy and affinity for GPR54, despite the dissimilar length. This binding leads to the activation of multiple signaling pathways, including PLC, PKC, MAPK, and intracellular Ca^{2+} mobilization, to transduce the signal, resulting in an elevation in GH secretion driven by GH gene transcription activation and GH release induction [1, 36]. The expression of kisspeptin and its receptor has been found in several tissues, including the pituitary gland, suggesting a potential role of this neurohormone in the control of additional hormones. However, the potential action of kisspeptin on somatotroph function is still controversial. Indeed, *in vivo* studies have not found any effect in GH pulses after kisspeptin administration, while in vitro kisspeptins can induce a short stimulation of GH, although lower than induced by key GH stimulators [36, 37].

2.2.1.7 Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP)

PACAP is a member of the VIP/secretin/glucagon/GHRH/GIP superfamily of peptides. It is an amidated peptide with two molecular isoforms, PACAP38 and PACAP27. These peptides bind to the GPCR PACAP type-1, which stimulates AC and subsequently increases cAMP levels in target cells [38]. PACAP is highly conserved throughout evolution and it is a potent GH stimulator in different lower vertebrate species; however, in humans, GH secretion stimulated by PACAP is very weak compared with that produced by GHRH [39]. In general, in mammals, data concerning the PACAP effect on GH release are controversial. Some studies found a stimulatory effect of PACAP, while other reports indicate that PACAP has no effect on GH secretion [40]. Interestingly, PACAP and GHRH are encoded by two closely related genes [41]. In spite of being two highly related peptides, their role on GH regulation across vertebrate evolution seems to have diverged in mammals and, while GHRH is critical for GH release, PACAP seems to play only a secondary role in the regulation of the somatotropic axis [7].

2.2.1.8 Melatonin (MT)

MT or N-acetyl-5-methoxy tryptamine is a non-peptide hormone produced by the pineal gland [42]. MT is the main driver of circadian rhythms in humans, being secreted overnight. In fact, a close relationship between MT and pituitary secretions has been proposed since the secretion patterns of some pituitary hormones follow a circadian cycle [43]. In addition, the fact that the melatonin receptor MT1 is expressed in the pituitary gland reinforces this contention [44, 45]. However, although results obtained in primate models indicate that MT directly regulates somatotroph function [45] and that MT might substantially contribute to define the daily patterns of GH, the observations made in human are controversial. In human, the age of the individual and the administration route seem to be crucial for the effect of MT on GH release. Until puberty, there seems to be a negative correlation between MT and GH secretion, promoting a diurnal growth; however, this relationship seems to be the opposite in adulthood [46–48]. In contrast, other studies have

shown that MT may not influence GH release [49] or that this effect may be different in response to acute or chronic MT administration [50, 51]. In any case, the effects of MT on GH may be mediated by MT1 through the modulation of AC/PKA and extra−/intracellular calcium pathways requiring activation of the PLC route [1, 45].

2.2.1.9 Thyrotropin-Releasing Hormone (TRH)

TRH is a tripeptide (glu-his-pro-NH2) hormone primarily produced in the paraventricular nucleus of the hypothalamus, and mainly involved in the stimulation of TSH release from thyrotroph cells [7]. However, this hormone could also exert additional actions in the modulation of other pituitary secretions. Indeed, TRH has been demonstrated to stimulate GH release in different mammalian species [7]. In humans, TRH actions have been reported in cell cultures from acromegalic patients where TRH induced GH release, while this effect is less evident in normal subjects [52]. Although these data confirm the role of TRH controlling the normal physiology of GH-producing cells, they point out that TRH has lost part of its ability to act as a primary stimulus of the somatotropic axis during the evolution. In any case, TRH actions seem to be dependent on the calcium influx through L-type calcium channels, which may be associated with PKC activation [52].

2.2.1.10 Neuropeptide Y (NPY)

NPY is a 36 amino acid peptide that exhibits widespread physiological functions in the CNS and periphery, being preferentially expressed in neurons located at the hypothalamic arcuate nucleus [53]. NPY is able to bind multiple receptors that belong to the GPCR family as do other GH-release regulators [54]. In mammals, NPY seems to elicit a stimulatory effect on GH secretion in somatotroph cells [7]. However, in healthy humans, NPY administration did not alter GH release when administered to young men [55]. Therefore, more studies should be implemented in human in order to unveil the real potential of NPY as a GH regulator.

2.2.1.11 Other Systemic/Potential Regulators

Besides all the GH regulators mentioned above, there are additional hormones and molecules that could influence the production and normal release of GH, including leptin, resistin [56, 57], estrogens [58], glucocorticoids [59, 60], and opioids [61], which have demonstrated a role as positive modulators of GH release in somatotrophs. On the contrary, IGF1, insulin [60, 62, 63], thyroid hormones [64], obestatin [65], adiponectin [57], free fatty acids [66, 67], and endothelin [1] have been shown to have a role inhibiting GH production in somatotrophs. These additional regulators are also extremely important because they are able to modify the balance between the main inhibitory and stimulatory signals mentioned above. In general, these other systemic and potential regulators are considered weak modulators of GH release; however, they could play an important role in the pathophysiology of this gland, particularly in the somatotroph cells.

2.2.2 Lactotrophs

Lactotrophs are acidophilic cells that comprise about 20% of all cells in the anterior pituitary gland. These cells mainly produce prolactin (PRL), a peptide hormone that is primarily involved in the maturation of mammary glands and the secretion of milk in association with OT, estrogen, progesterone, glucocorticoids, and other factors. However, PRL has several additional effects in both sexes, including the regulation of metabolic processes, the immune system, and the pancreatic development [68]. PRL is secreted from lactotroph cells in response to eating, mating, estrogen treatment, ovulation, and/or nursing, among other stimuli. In particular, PRL is secreted in pulses in between these events by the fine interplay between different modulators as described below [68–70].

2.2.2.1 Dopamine (DA)

DA, a classical neurotransmitter produced by different areas of the brain, is the principal regulator involved in the inhibition of PRL expression and secretion. PRL regulation by DA is mainly mediated by its binding to dopamine receptor subtype-2 (DRD2), which is the predominant dopamine receptor in lactotrophs [71, 72]. Several studies have reported that the reduction of intracellular cAMP levels represents the main mechanism by which DA suppresses PRL expression, which can ultimately lead to a dysregulation of ion channel function and a desensitization of the GPCRs that control PRL release [70]. Importantly, due to the involvement of ion channels (Ca^{2+} signaling) in this process, PRL secretion is compromised just after few seconds of DA exposure [73]. Strikingly, a slight increase of PRL secretion in lactotrophs has been reported in response to especially low concentration of DA (<0.1 nM) [74], which has been explained by different theories (e.g., prevalence of the short DA receptor isoform [75], or involvement of the Giα3 protein [76], among others). However, the stimulatory effect on PRL of DA remains controversial while the inhibitory role of this hormone has been well established and deeply demonstrated.

2.2.2.2 Thyrotropin Releasing Hormone (TRH)

As indicated above, TRH is secreted by the hypothalamus and was originally named for its action on the modulation of TSH release from the pituitary. However, it was lately shown that it could also regulate other pituitary cells, such as lactotroph cells, wherein it plays a role as one of the main stimulators of PRL secretion [77]. In fact, it has been shown that the same concentration of TRH is able to induce TSH and PRL secretion in human pituitary cells, and that TRH blockade impedes PRL secretion [77]. In the context of hypothyroidism, the low level of thyroid hormones leads to a compensatory increase of TRH. This may lead to an excess of PRL causing hyperprolactinemia [78, 79]. In lactotroph cells, TRH binds and activates the PRL receptor (PRL-R) and recruits the Gq protein that stimulates inositol phospholipid production, which activates both the PKC pathway and the release of Ca^{2+} from different compartments [77, 80]. The activation of the PKC pathway may lead to MAPK activation via ERK, but it has been shown that this pathway may be also

enhanced by TRH in a PKC-independent way [77, 80]. Additionally, it has been shown that TRH secretion is pulsatile and this can affect PRL secretion, in that PRL is more potently secreted when TRH pulse is more frequent [77]. Interestingly, DA inhibits the activation of the cAMP pathway, which is independent of TRH. However, DA may also inhibit the action of TRH in lactotroph cells, via Ca^{2+} release [81].

2.2.2.3 Ghrelin (GHRL)

As it has been described before, ghrelin is a hormone peptide of 28 amino acids whose main function is to increase GH secretion through its binding to the GHSR1a receptor. However, it has been demonstrated that ghrelin may also participate in the secretion of PRL as a stimulator factor [82]. Although the mechanisms underlying this event are not fully understood, it seems clear that ghrelin acts directly on lactotroph cells, since it may produce PRL release in dispersed normal pituitary cells [45, 83]. However, this regulation is not under the control of the changes of menstrual cycle in women, being independent of the estrogens [84]. Moreover, ghrelin seems to be less important than TRH in the stimulation of PRL secretion because: (a) the effect of ghrelin is lower than TRH, and (b) the combination of ghrelin and TRH did not show an additive/synergic effect but produced the same effect than TRH alone [85].

2.2.2.4 Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP)

The action of PACAP in lactotrophs is closely related to the cAMP and inositol phosphate pathways, which seem to regulate PKA and MAPK, respectively, leading to the modulation of *PRL* expression [68]. Similar to that found for other modulators, PACAP has been linked to PRL regulation, but its role is controversial. First, it was thought that PACAP did not participate in the regulation of PRL release; however, further studies demonstrated that PACAP mainly increases PRL secretion in rat pituitary cells [86]. Specifically, *in vitro* studies demonstrated that both PACAP and its receptor PAC1R display a stimulatory effect on PRL secretion in GH3 cells [86]. Additionally, intravenous PACAP injection may regulate PRL secretion in mammals, including human [80]. Nevertheless, PACAP did not exert similar effects in female lactating rats with actively nursing pups [86]. Moreover, PACAP knockout mice exhibit significantly reduced levels of PRL [87]. Finally, PAC1R, which is expressed in the entire anterior pituitary, but mainly present in lactotroph cells, may act independently of PACAP in improving the effect of TRH. Similarly, PACAP also increases the expression of the TRH receptor, demonstrating a clear relationship between both regulatory axes [88].

2.2.2.5 Melatonin (MT)

MT is a crucial factor in the regulation of PRL secretion, by adjusting the pattern of PRL secretion to photoperiod and conferring a circadian profile [89, 90]. Indeed, MT seems to be fundamental to modulate PRL secretion in response to sleep, but, in addition, it seems that MT may also regulate the secretion of PRL in response to sleep-independent changes caused by bright light [91]. *In vitro* studies [45, 92] have suggested that MT exerts direct stimulatory effects on PRL release, which could

contribute to the nocturnal rise of this hormone observed in humans *in vivo* [93] in that the nocturnal rise in PRL release is preceded by an increase in MT [94]. Consistently, exogenous administration of MT can enhance the nocturnal pulsatile secretion of PRL in normally cycling women [94], further supporting the contention that MT can enhance PRL secretion in humans *in vivo*.

2.2.2.6 Somatostatin (SST)

SST has been shown to play an inhibitory role on PRL secretion, which is less pronounced than the effect exerted on GH secretion. Specifically, it has been reported that SST, but not NST, is able to reduce *in vitro* the spontaneous release of PRL [33, 95]. Consistently, an inhibition of basal and TRH-induced PRL release of pituitary cells from adult rats in response to SST has been described [96], although it has been suggested that regulation of PRL secretion by SST is estrogen-dependent [97]. Moreover, several studies in human PRL-secreting pituitary neuroendocrine tumors (PitNETs) have shown that SSTR1R- and SSTR5-specific agonists and pasireotide effectively inhibited PRL secretion (but not PRL expression) [98–102], while octreotide does not alter PRL secretion [102], likely due to the fact that *SSTR5* is not highly expressed in this type of tumors [99]. Mechanistically, SST-dependent inhibition of PRL secretion could be explained by a modulation in Ca^{2+} signaling and a reduction in AC activity, inasmuch as SST fails to inhibit PRL release in the presence of the Ca^{2+} ionophore A23187 [103]. Therefore, SST inhibits PRL secretion through SSTR-dependent pathways, which appears to be receptor subtype-specific and sensitive to the presence of estrogens.

2.2.2.7 Cortistatin (CST)

The potential role of CST in the regulation of PRL secretion has been a matter of debate from its discovery. Initial studies have reported that CST, as well as SST, can inhibit PRL release from prolactinomas *in vivo* and *in vitro*, mainly due to the effect of its binding to SSTR5 [104, 105]. However, additional studies have also shown that CST can actually stimulate PRL release in rats [106] and mice [28]. Indeed, this latter study suggested that endogenous CST can indeed be considered a physiological stimulator of lactotroph function, inasmuch as circulating PRL levels are markedly suppressed in CST-KO mice [28].

2.2.2.8 Other Systemic/Potential Regulators

In addition to the classical regulators of PRL secretion described above, additional factors with the capacity to finely tune the concentration of PRL must be considered. This is the case of estrogens, which may increase PRL release. In fact, a positive regulation of PRL secretion by estradiol treatment has been reported in pituitary cell cultures from monkeys [107]. Additionally, it has been published that ET1, a peptide involved in the control of the blood pressure by blood vessel constriction, increased plasma PRL levels in men [108], likely due to modulation of Ca^{2+} signaling [109]. Moreover, different adipokines have been shown to play an important role regulating PRL secretion. In fact, both adiponectin and leptin directly increase PRL release from primate species by

modulation of Ca^{2+} and PLC/PKC signaling pathways [57]. On the other hand, besides DA, other inhibitors of PRL secretion have been identified, such as IGF1 [110] or GNRH-associated peptide (GAP), which were reported to be potent inhibitors of PRL release in primary cultures from human and rat pituitary tissues, respectively [111]. Therefore, there are many factors to be considered in the regulation of PRL expression and secretion that may act simultaneously and/or sequentially in lactotroph cells.

2.2.3 Gonadotrophs

Gonadotroph cells arise from the ventral pituitary and require the expression of steroidogenic factor-1 (SF1) and GATA2 for differentiation. These cells constitute 10% of all adenohypophysial cells and are spread out throughout the *pars distalis* and *pars tuberalis*. These cells produce two types of hormones known as gonadotropins, FSH and LH, which are heterodimeric glycoproteins composed by a common α subunit (CGA) and a hormone-specific β subunit [112, 113]. Gonadotropins play an essential role in the normal mammalian sexual maturation and reproduction, acting at gonad levels by binding to GPCRs named FSHR and LHR, respectively [114]. As mentioned above regarding other anterior pituitary hormones, the precise control of gonadotrophs is also finely regulated by hypothalamic and peripheral signals [80], as described below.

2.2.3.1 Gonadotropin-Releasing Hormone (GNRH)

GNRH is a decapeptide produced by hypothalamic neurons and secreted into the hypophyseal portal circulation to directly act on the cells of the anterior pituitary gland [115]. GNRH is the main positive stimulator of FSH and LH release from the anterior pituitary. Likewise, GnRH-(1-5), a metabolite of GNRH, has been shown to indirectly enhance LH release via activation of kisspeptin neurons inducing GNRH expression and secretion [116, 117]. Indeed, this hormone is secreted in a pulsatile manner and the variations in its frequency and amplitude produce differential effects on the synthesis and release of gonadotropins [115, 118]. Interestingly, it has been found in animal models that FSH and LH release and expression are mainly stimulated by low and high GNRH pulse frequencies, respectively [115]. Moreover, the stimulatory effects of GNRH on gonadotropins have been also established in humans and non-human primate models [36, 119, 120]. In particular, GNRH treatment can directly produce an increase of FSH and LH secretion *in vivo* and in primary pituitary cell cultures from different non-human primate models [36, 120]. All these effects are mediated through the binding to the GNRH receptor (GNRHR) [115] and are exerted through the modulation of different signaling pathways including the NOS/NO/GC/cGMP pathway and extracellular Ca^{2+} mobilization, which result in the secretion of LH in baboons [36]. Additionally, other pathways such as PKC/MAPK and cAMP/PKA have been shown to be activated by GNRH in rodent models [115].

2.2.3.2 Ghrelin (GHRL)

In addition to its actions on somatotroph and lactotroph function, acylated ghrelin has been related with inhibitory actions in gonadotroph cells from rodent, ovine, and primate models, consequently modulating reproductive function [19]. Thus, ghrelin administration decreased LH pulse frequency, but not amplitude, in adult ovariectomized rhesus monkeys [121]. In the same line, ghrelin administration inhibited LH and FSH secretion in healthy men and women [19, 122, 123]. However, the effects of ghrelin on gonadotropins expression levels and the signaling pathways involved in these actions remain to be fully elucidated.

2.2.3.3 Somatostatin (SST)

Several reports have described that SST, besides its inhibitory actions on GH, TSH, PRL, and ACTH release, can also induce the inhibition of LH and FSH secretion in humans and non-human primate models [33, 124, 125]. Thus, somatostatin reduced LH, but not FSH secretion, after 4 and 24 h of incubation in primary pituitary cell cultures from baboons. Moreover, somatostatin treatment also reduced *LH*, but not *FSH* expression, after 24 h of incubation [33]. In the same line, somatostatin inhibited FSH and LH secretion in healthy humans and reduced LH response to GNRH in healthy women [124, 125]. Regarding the signaling pathways, the effects exerted by somatostatin on gonadotropins secretion/expression were mediated through AC/PKA, MAPK, and extra-/intracellular calcium mobilization [33].

2.2.3.4 Kisspeptins (KISS1)

Kisspeptin-54 and kisspeptin-10 have been associated with an induction of FSH and LH levels in healthy humans, although these effects are less potent in comparison with the stimulatory actions of GNRH [119, 126]. Interestingly, kisspeptin-10 stimulated gonadotropins release in a different manner in women and men, showing a sexual dimorphism [127]. In line with these results, kisspeptin-10 infusion increased LH levels in rhesus monkeys [128], and also stimulated LH secretion/expression in primary pituitary cell cultures from baboons [36], being this stimulatory effect similar to that exerted by GNRH. In contrast, this peptide did not alter FSH secretion/expression levels in the same non-human primate model [36]. The signaling pathways involved in the stimulation of LH synthesis and release by kisspeptin-10 were PLC, PKC, MAPK, intracellular calcium mobilization, mTOR, and PI3K pathways [36].

2.2.3.5 Melatonin (MT)

The information regarding the effect of MT on gonadotropins secretion/expression is not entirely consistent. Specifically, exogenous MT administration has been shown to cause different effects reducing but also increasing LH levels in humans [94, 129]. In this regard, long-term administration with MT has been shown to reduce basal blood LH levels, without altering FSH levels in humans [130]. In contrast, long-term or acute MT administration did not alter gonadotropins levels in healthy men [131, 132]. On the other hand, no changes in gonadotropins synthesis

and/or release were found in non-human primate models [92, 133]. Therefore, further studies are necessary to clarify the effect of MT on gonadotroph cells.

2.2.3.6 Gonadotropin-Inhibitory Hormone (GnIH)

GnIH is a hypothalamic peptide initially discovered in birds due to its inhibitory action on LH secretion [134]. The human GnIH peptides are called RF-related peptides (RFRPs) and have been considered as specific inhibitors of gonadotropin secretion in mammals, acting through the binding to GnIH receptor (GPR147) [135]. GnIH has been demonstrated to reduce LH secretion *in vitro* in avian, ovine, and bovine gonadotroph cells [134, 136, 137]. Moreover, a recent study has demonstrated the suppression of LH levels after exogenous GnIH administration in postmenopausal women [135]. Although further studies are required to better understand the function of GnIH in humans and the signaling pathways involved in its actions, results generated in a mouse gonadotroph cell line (LßT2) showed that the effects on gonadotropin secretion are mediated through the crosstalk inhibition of the AC/cAMP/PKA/ERK pathway [138].

2.2.3.7 Neuropeptide-Y (NPY)

NPY is released to the hypophyseal portal circulation for transportation to the anterior pituitary gland where it modulates somatotroph cells function but also enhances LH release in response to GNRH [139]. Indeed, although NPY administration did not alter LH or FSH secretion in healthy men, the co-administration of NPY and GNRH produced a potentiation of LH and FSH release compared to GNRH alone [140]. However, in a different study, the administration of human NPY to the third ventricle of the brain in ovariectomized rhesus monkeys produced a striking reduction of LH secretion through the alteration of GNRH/LH secretory system, and these effects seemed to involve the mobilization of intracellular calcium [141]. Therefore, these contradictory results indicate the necessity of implementing further studies to undoubtedly define the role of NPY on gonadotropins release.

2.2.3.8 Other Systemic/Potential Regulators

Gonadotrophs function is also regulated by additional peripheral factors, in addition to the previously described above. Thus, short-term administration of glucocorticoids can reduce basal serum levels of LH and FSH in the follicular phase of healthy women [142], but not in healthy men [143]. On the same line, leptin has been demonstrated to directly increase FSH secretion *in vitro*, without altering LH secretion or *FSH*/*LH* expression, through the modulation of AC/PKA, PLC/PKC, PI3K, and extra-/intracellular calcium mobilization in two primate species [57]. Treatment with inhibins (A and B; glycoprotein hormones secreted by the granulosa and theca cells of the ovary and by the Sertoli cells of the testis [144]) can reduce FSH levels, with unclear effects on LH levels, in human fetal primary pituitary cell cultures [145]. Moreover, the reduction of inhibin B in older ovulatory women produces a monotropic FSH increase, and results in men have shown that inhibin B is the major regulator of FSH release [146]. In contrast, activins, glycoproteins belonging to the TGF-β superfamily, have been associated with a potent increase of FSH and LH

release in non-human primate models [147] and in human fetal primary pituitary cell cultures [145], mainly through the binding to different receptors (ActRII, ActRIIB, and ActIR/ALK4), and also producing the phosphorylation and translocation of SMAD proteins to the nucleus to finally regulate *FSH*/*LH* gene transcription [148, 149]. In addition, follistatin (FST; a monomeric polypeptide secreted from mature gonadal cells and considered a key regulator of activins [150]) has been associated with a decrease of basal and GNRH-stimulated LH and FSH levels in human fetal primary pituitary cell cultures, which might be due to the direct blockage of activin actions [145]. On the other hand, testosterone acts as a negative regulator of LH and FSH secretion in human and non-human primate models [151, 152]. Furthermore, the presence of endothelins has been detected in gonadotrophs cells, and its intravenous administration produced an increase of GNRH-stimulated LH and FSH levels in men probably mediated, at least in part, by calcium mobilization [109]. Likewise, the information about the effects of opioids at anterior pituitary level is quite contradictory; however, several reports have demonstrated a reduction on serum LH concentrations after a chronic and acute administration of opioids, and a reduction of FSH concentrations after a chronic administration, mainly acting through the μ-opioid receptor pathway [153–155]. Finally, NST has been recently related with an inhibition of basal, but not ghrelin or GNRH-stimulated, LH secretion in primary pituitary cell cultures from baboons [33]; however, this peptide did not alter *LH* mRNA expression levels or FSH secretion/expression levels. The signaling pathways involved in the effect of NST on LH secretion were AC/PKA, MAPK, and extra−/intracellular calcium mobilization [33].

2.2.4 Thyrotrophs

Thyrotrophs are the anterior pituitary cells responsible for producing the thyroid-stimulating hormone (TSH) and their physiology is modulated by central and peripheral factors. TSH modulates the growth of the thyroid gland and the release of thyroid hormones by stimulating the thyroid follicular cells to release thyroxine (T4) and triiodothyronine (T3). The peptide hormone TSH has two different subunit, beta and alpha chains. Remarkably, TSH, LH, FSH, and the human chorionic gonadotropin (hCG) share the same alpha subunit but the beta chain is different and confers specificity and different capabilities. Indeed, FSH acts through the cAMP second messenger system and by the IP3 signaling cascade. These processes start when TSH actives TSHR (a GPCR). The final consequence of this process is the activation of the expression of a plethora of key proteins that influence many organs, promoting growth and bone maturation, increasing the basal metabolic rate and the basal cardiac output, and promoting the maturation of the central nervous system [156].

2.2.4.1 Thyrotropin-Releasing Hormone (TRH)

TRH, a tripeptide (glu-his-pro-NH2) hormone that is primarily produced in the paraventricular nucleus of the hypothalamus, has been long established and accepted

as the main regulator of TSH release. Specifically, TRH is produced by the hypophysiotropic TRHergic neurons, which integrate the information received and modulate the synthesis and release of TRH. This hormone is necessary to increase TSH release in the anterior pituitary, through the activation of TRH receptors, a family of calcium-mobilizing GPCRs that signal by elevating calcium levels and activating PKC [157]. In particular, TRH activates TRHR1 and the consequently increased IP3 levels mobilize, in turn, intracellular calcium levels, causing TSH release [157]. It seems that TRH can stimulate TSH alpha subunit expression via the PKC-MAPK pathway, while TRH-stimulated TSH beta subunit expression involves the calcium-calmodulin pathway. However, the expression of the TSH beta subunit by TRH may depends upon either the pituitary architecture, and/or paracrine factors, since it can be lost in cell culture [158].

2.2.4.2 Somatostatin (SST)

SST is one of the main inhibitors, together with DA, of TSH secretion in normal subjects [159]. Indeed, SST is synthesized in the hypothalamus and it is transported via the portal vessels of the pituitary stalk to the GH- and TSH-secreting cells. SST can inhibit TRH-induced TSH secretion in normal adult males [160]. Similarly, SST can suppress TSH pulse amplitude and frequency [161] and inhibits TSH levels in normal volunteers and in patients with primary hypothyroidism [162]. In addition, it has been recently reported that SST can directly inhibit TSH secretion in pituitary cell cultures from normal primates [33]. Consistently, other studies have shown that octreotide and lanreotide can reduce TSH secretion and normalize T3 and T4 levels in patients with pituitary TSH-secreting adenomas [163, 164]. These effects of SST inhibiting TSH release are dependent on its binding and activation of SSTR2 and SSTR5 receptors [165], which lead to the inhibition of AC and cAMP levels and the modulation of K^+- and Ca^{2+}-channels [166].

2.2.4.3 Dopamine (DA)

The inhibitory effect of DA on TSH release has been long recognized [167–169]. Indeed, DA rapidly decreases TSH subunit secretion, possibly as a result of the stimulation of DA receptors present on the surface of anterior pituitary cells [170–172]. Consistently, DA agonists, such as l-dopa (L-3,4-dihydroxyphenylalanine) and bromocriptine, have been shown to decrease TSH secretion [173–175]. On the other hand, the administration of a therapeutic dose of metoclopramide, a DA antagonist, can enhance TSH secretion [173]. To exert these actions, DA and its agonists can act through a family of five different subtypes of DA receptors (DRD1–DRD5), which can act by classic mechanisms (AC, cAMP, etc.) or via GPCR-independent mechanisms like interactions with ion channels [176].

2.2.4.4 Other Systemic/Potential Regulators

In addition to these main TSH regulators, there are other molecules that can regulate the expression and release of TSH, including glucocorticoids, which are synthesized and released by the adrenal cortex [177]. Glucocorticoids can reduce T3 concentration in adults and preterm infants [173]. Although it is well known that

glucocorticoids activate cAMP/PKA or PKC signaling pathways and intracellular free calcium mobilization in rodents, the signaling pathways activated by glucocorticoids in humans or primates have yet to be fully elucidated [178]. Remarkably, thyroid hormones also exert a negative regulatory feedback on TSH production in that, when T3 and T4 levels are increased, they can suppress the synthesis and secretion of TSH [173]. However, the signaling pathways and mechanisms associated with the effects of thyroid hormones in humans and non-human primate pituitaries have not been fully identified yet [178]. Interestingly, a study analyzing the changes of pituitary hormones over a 24-hour period after a single subcutaneous dose of 40 micrograms/kg rhIGF1 demonstrated that IGF1 can reduce TSH levels in plasma from healthy volunteers [179]. Finally, three different studies have demonstrated that TSH can be elevated after opioids treatment [180–182]. Moreover, the use of opioids and their antagonists had profound effects in modifying the nocturnal pulses of TSH by altering the circadian rhythm of this hormone [178].

2.2.5 Corticotrophs

Corticotroph cells are mostly located in the anteromedial areas of the pituitary gland and constitute about 15–20% of all the anterior pituitary cells. These cells produce mainly ACTH, which is derived from the cleavage of the precursor hormone proopiomelanocortin (POMC) by the action of different prohormone convertase enzymes [183]. Corticotrophs are characteristically identified by their basophil staining, by their PAS-positivity due to the high glycoprotein content of the N-terminal glycopeptide of POMC, as well as by their ACTH immunopositivity. The main biological function of ACTH is to regulate the plasmatic levels of cortisol and androgens, by modulating their expression and secretion in adrenal cortex cells [55]. ACTH works through GPCRs located at the extracellular membranes on cells of the *zona fasciculata* and *zona reticularis* of the adrenal cortex. The activation of ACTH receptors leads to the stimulation of AC and, thus, the increase in cAMP production. The secretion of ACTH is influenced by diverse stimuli, as described below [183].

2.2.5.1 Corticotropin-Releasing Hormone (CRH)

CRH is a neuropeptide hormone that consists of 41 amino acids and it is secreted from the paraventricular nucleus of the hypothalamus [184]. It regulates neuroendocrine, sympathetic, and behavioral functions in response to stress. In particular, at the anterior pituitary level, CRH activates the synthesis and release of ACTH from corticotrophs, which, in turn, stimulates the secretion of glucocorticoid hormones (mainly cortisol in humans) from the adrenal cortex. In this way, CRH affects the response to stress, addiction, and depression, among others [185]. CRH acts via 2 distinct GPCRs, namely, CRHR1 and CRHR2. CRHR1 expression is prevalent in brain areas responsible for sensory and motor control, such as the cortical mantle, olfactory bulb, hippocampus, amygdala, hypothalamic nuclei, and cerebellum [186]. CRHR2 is predominant in subcortical regions, including the lateral septum, *stria terminalis*, or the ventromedial hypothalamic nucleus [185, 186]. In the

anterior pituitary, CRHR1 mediates the release of ACTH in response to CRH through the recruitment of several intracellular effectors such as cAMP and protein kinases [184, 185].

2.2.5.2 Ghrelin (GHRL)

Different studies in humans and non-human primate models have demonstrated that ghrelin regulates ACTH release by anterior pituitary cells [187–189]. Indeed, ghrelin plays an important role in the activation of central pathways mediating stress-induced food reward behavior [190] and is involved in the response to acute stressors [191, 192] by the stimulation of hormones involved in the stress response, including ACTH, AVP, PRL, and cortisol [193]. The stimulation of ACTH release by ghrelin is exerted predominantly at the hypothalamic level through AVP stimulation and indirect activation of CRH neurons [194]. However, despite abundant GHSR1a expression in the anterior pituitary, the direct effects of ghrelin on pituitary ACTH synthesis and release are minor and not consistent across species, which may be explained by differences in the molecular regulation of downstream pathways [13, 187]. In addition, the stimulatory effect of GH secretagogues on corticotroph function is sensitive to glucocorticoid feedback, as the ACTH response to hexarelin, a synthetic GH secretagogue that activates GHSR1a, is suppressed by pre-treatment with dexamethasone [13].

2.2.5.3 Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP)

PACAP has been shown to be involved in restraint stress-induced corticosterone release and concomitant expression of the genes involved in hypothalamic–pituitary–adrenal (HPA) axis activation. Indeed, it has been described that intravenous PACAP administration can regulate ACTH secretion in different mammalian species including humans [195]. Consistently, PACAP has been shown to be able to induce ACTH release from the corticotroph cell line AtT-20, which is suppressed by SST administration [196]. PACAP exerts its biological actions by coupling to different GPCRs classified into three groups based on their differential affinity for PACAP or vasoactive intestinal peptide (VIP). Thus, PACAP type 1 receptors (PAC1R) are more specific for PACAP while vasoactive intestinal polypeptide receptor 1 (VPAC1) and VPAC2 receptors present similar affinity for either PACAP isoforms or VIP [197]; however, the receptor subtypes mediating these effects on ACTH secretion have not been identified hitherto.

2.2.5.4 Vasopressin (AVP)

Vasopressin, also named as antidiuretic hormone (ADH), AVP or argipressin, is a hormone synthesized as a peptide prohormone in neurons of the hypothalamus and stored and released by the posterior pituitary gland. AVP is the major physiological regulator of renal water excretion and blood volume and is normally released in response to an increase in blood osmolality or decrease in blood volume [198, 199]. In addition, during acute stress, AVP expression and secretion rapidly increases similar to CRH and ACTH, and AVP contributes to the full ACTH response [200]. Indeed, AVP administration increases ACTH levels in healthy humans, wherein

AVP seems to enhance CRH-stimulated ACTH release [30]. In fact, it was reported that AVP from pituitary portal circulation is more important in altering ACTH levels than AVP derived from peripheral circulation. These effects seem to be mainly mediated by the V1b GPCRs, which are prominently expressed in corticotroph cells, and can modulate ACTH release [198].

2.2.5.5 Somatostatin (SST)

The role of SST in the regulation of ACTH secretion from pituitary corticotroph cells is still to be clearly clarified. Initial studies suggested that SST did not affect basal or CRH [201] or ghrelin [202] stimulated ACTH or cortisol levels in humans. However, more recent studies suggest that SST can regulate ACTH secretion, which is dependent on cortisol levels and cell milieu [203]. Moreover, it has been reported that SST knockout mice had elevated POMC expression and corticosterone levels suggesting that endogenous SST can inhibit pituitary-adrenal axis [187]. In line with this, inconsistent results have also been reported *in vitro*. Specifically, although SST did not affect basal or CRH-stimulated ACTH secretion in normal rat pituitary cells [204, 205], it inhibited CRH- and AVP-induced ACTH secretion in cultured pituitary cells derived from adrenalectomized rats and in serum-starved cultures [203]. In addition, it has been also reported that SST can inhibit ACTH release in primary pituitary cell cultures from mice [187] and primates [33, 65, 187]. These putative effects might involve the differential participation of SSTR2 and SSTR5 receptors [206–208].

2.2.5.6 Cortistatin (CST)

A limited number of studies indicate that CST may exert an important role in controlling ACTH levels. Indeed, these studies suggest that CST can directly modulate the function of different pituitary cell types, including corticotrophs, and these actions in humans and animal models are dose- and cell type-dependent and receptor-specific [165]. Moreover, it has been reported that endogenous CST may serve to suppress pituitary ACTH output in a gender-dependent fashion in that CST-KO female, but not male, mice had elevated circulating ACTH levels [28]. These effects may be, at least in part, direct, because the same study demonstrated that CST treatment inhibited ACTH release in primary pituitary cell cultures of normal mice and baboons [28].

2.2.5.7 Other Systemic/Potential Regulators

Besides the factors described above, there are other factors able to modulate ACTH secretion. As an example, the inhibition of ACTH release in response to opioids treatment has been reported in several studies [209–211], and this effect seems to be mediated through kappa-opioid receptors [212]. In the case of adipokines, it has been reported that while adiponectin decreases ACTH release, leptin and resistin increase ACTH release in primary cell cultures from non-human primates by modulating common (AC/PKA) and specific signaling pathways (PLC/PKC, Ca^{2+}, mTOR) [57]. Moreover, obestatin has been reported to stimulate ACTH secretion in mice *in vitro* (primary cell cultures) and *in vivo* as well as in baboon primary

pituitary cell cultures [65]. Finally, as expected, glucocorticoids negatively feedback to suppress their own axis, therefore suppressing ACTH secretion *in vivo* and *in vitro* [213–215]. Thus, the actions of all of these factors, among others, should be considered when analyzing the regulation of ACTH secretion.

2.3 Posterior Pituitary

The posterior pituitary or *neurohypophysis* is a particular region of the pituitary gland with a different embryonic origin [216, 217]. The posterior pituitary is directly connected to the hypothalamus via a nerve tract (hypothalamo-hypophyseal nerve tract). Posterior pituitary can be structured in three parts: *pars nervosa*, infundibular stalk, and median eminence [217]. It is composed by neuronal projections (axons) of magnocellular neurosecretory cells (MNCs), which are responsible for OT and AVP secretion into the general circulation [216]. These hormones are transported in association with neurophysins proteins along the axons of these neurons to the nerve terminals within the posterior pituitary. Particularly, OT and AVP are synthesized by different populations of MNCs in the paraventricular and supraoptic nuclei of the hypothalamus [218], displaying important central and peripheral functions. OT is a nine amino acids peptide that binds different GPCRs (OT-Rs) distributed throughout the brain, although the molecular mechanism underlying their activation remains unknown [219]. The classical physiological roles of OT are the regulation of lactation, parturition, and reproductive behavior, being essential to stimulate uterine contraction and milk secretion [216]. As such, stimulation of vagal sensory afferents in the nipple by the act of suckling triggers reflex synchronized firing of oxytocin magnocellular neurons in the neurohypophysis, and corresponding pulsatile OT release [216]. In many mammals, there is also an increase in OT secretion and in uterine responsiveness to OT during parturition [220, 221]. OT also plays a key role within the brain to control behavior in social cognition and in fear conditioning, which is important in social anxiety as well as in other disorders with impaired social functioning [222]. AVP [223] is a nonapeptide that binds to at least three different GPCR subtypes (V1a, V1b, and V2). The most important action of AVP is its antidiuretic action on the collecting ducts of the kidney [199]. In fact, AVP maintains body fluid balance by keeping plasma osmolarity and controlling liquid kidney excretion and thirst [199]. AVP is secreted in response to increased osmolality, changes in blood volume or pressure, nausea, hypoglycemia, and nicotine [199, 224]. Indeed, the main stimulus for AVP release is dehydration, resulting in an increase in plasma osmolarity, but other stimuli also influence AVP secretion, mainly sodium, the reduction in circulating blood, stress situations [199, 225], or different hormones. In general terms, increased firing frequency of oxytocin and vasopressinergic neurons opens voltage-gated Ca^{2+} channels in their nerve terminals. This, in turn, leads to transient Ca^{2+} influx, fusion of the neurosecretory granules with the nerve terminal membrane, and release of the hormone and their neurophysins proteins into the systemic circulation in equimolar quantities.

Therefore, these two posterior pituitary hormones (OT and AVP) comprise a highly related and integrated system, but they exert clearly distinct actions and their synthesis and release is differentially regulated by central and peripheral stimuli. Some of these stimuli have been described above; however, the secretion of posterior pituitary hormones is influenced by additional factors, as described below.

2.3.1 Opioids

Endogenous opioid peptides (or endomorphins) are synthesized by different precursors of the neurohypophysis (mainly POMC, proenkephalin A and prodynorphin) and they act directly on specific receptors well distributed centrally and peripherally [226]. The effect of the opioid system and its receptors in posterior pituitary hormones is strongly complex and some controversial results have been described. It is well described that OT secretion is inhibited centrally by mu- and kappa-opioid agonists or directly at the posterior pituitary via kappa-opioid receptors [42, 226, 227]. However, kappa-opioid receptors also induce a suppression of AVP, increasing diuresis status, with the consequence of high sodium levels and an increase on OT and AVP [228]. Therefore, the exact role of the endogenous opioid system on OT release has to be further explored.

2.3.2 Glucocorticoids

In general, glucocorticoid administration is able to elevate plasma AVP concentration and improve the impaired water diuresis in adrenal insufficiency. However, the specific effect of cortisol on AVP release is still controversial in that it depends on the species studied. For example, there is no clear effect of cortisol in horse AVP release, but in contrast, in other species such as sheep or importantly in human, evidence obtained indicates that, under certain circumstances, cortisol could inhibit the release of AVP [229–231].

2.3.3 Angiotensin II (AII)

AII is a peptide hormone produced from angiotensin I through the removal of two C-terminal residues. AII has been shown to act as an endocrine, autocrine/paracrine, and intracrine hormone [232]. Indeed, immunoreactive AII has been localized in magnocellular cells of the supraoptic and paraventricular nuclei, which mostly contain AVP [233]. AII has been shown to increase aldosterone secretion, act on venous and arterial smooth muscle to cause vasoconstriction, and increase AVP production and secretion in different species [232, 234, 235].

2.3.4 Norepinephrine (NE)

NE, also known as noradrenaline (NA) or noradrenalin, is a catecholamine hormone that functions as a neurotransmitter in the sympathetic nervous system [236]. The infusion of norepinephrine increases both arterial blood pressure and left atrial pressure. These changes are capable of eliciting a reflex inhibition of AVP release, which can reduce plasma AVP. However, the inhibitory effects of the sino-aortic and cardiac reflexes on AVP release seem to be offset by the direct stimulatory effect of circulating NE [236].

2.3.5 Other Systemic/Potential Regulators

There are other intermediates that could be implicated in the secretion of posterior pituitary hormones, although their direct actions have to be still fully elucidated. The vasoactive intestinal polypeptide 1 or VIP1 seems to have an effect in neurosecretory nerve endings increasing OT and AVP plasma levels after an intracarotid infusion in cats [237]. Galanin, which is mainly produced in the small intestine and is susceptible to changes in fluid homeostasis, has been suggested as a putative modulator of neurohypophysial function and AVP secretion [238]. The relaxin hormone has been also related with an inhibition of OT and AVP release into plasma in rats, suggesting a possible role of this hormone in the neurohypophysis [239]. Exposure of neurohypophysis to interleukin-1 beta has been also associated with an increase of AVP release [240].

2.4 Pars Intermedia

The *pars intermedia* is a thin band of cells between the *pars distalis* and the neurohypophysis. The main functional elements of the *pars intermedia* are the melanotroph cells. Like the corticotrophs in the *pars distalis*, melanotrophs produce POMC; however, as a result of differential post-translational processing, the main end-products derived from POMC in these cells are α-MSH, β-MSH, and γ-MSH [241]. These peptides are associated with many physiological functions through the binding and activation of four of the five subtypes of melanocortin receptor (MC1R, MC3R, MC4R, and MC5R, excluding the ACTH-specific receptor MC2R), which are widely distributed throughout the body [242]. MC1R is present in melanocytes, keratinocytes, leukocytes, and adipocytes; MC3R is present in the CNS, kidney, testis, ovary, skeletal muscle, placenta, and mammary gland; MC4R is present in the CNS; and MC5R is present in exocrine glands, muscle, and CNS [242, 243]. The most representative physiological function of MSH peptides is the stimulation of melanocytes in the skin to promote the synthesis of melanin via MC1R activation. Moreover, other important functions associated with these elements include the

modulation of energy homeostasis and natriuresis via MC3R, the regulation of synthesis and secretion of exocrine gland products via MC5R, the modulation of aldosterone release from the adrenal cortex, and the control of sodium metabolism and blood pressure [243, 244]. Most recently, α-MSH has been shown to exhibit an anorexigenic character and the capacity to regulate endothelial cell migration [245, 246]. All these receptors are GPCRs and exert their functions through AC and PKA activation [242]. Therefore, the secretion of MSH is influenced by diverse stimuli, as described below.

2.4.1 DA, NPY, and γ-Aminobutyric Acid

The control of the secretion of these peptides appears to be under tonic hypothalamic inhibition. In fact, and in line with this, melanotrophs can spontaneously secrete α-MSH when the connection between the hypothalamus and the *pars intermedia* is disrupted [247]. The specific molecular control of MSH release is well described in *Xenopus laevis*. Xenopus melanotroph cells are contacted by synaptic structures that secrete three types of neurotransmitters: DA, NPY, and γ-aminobutyric acid (GABA). All three transmitters inhibit the secretion of α-MSH but DA and NPY also inhibit POMC biosynthesis [248]. This inhibition is mediated by D_2-like, the $GABA_B$, and the NPYY1 receptor located in melanotrophs.

2.4.2 Other Systemic/Potential Regulators

Other classic neurotransmitters can induce MSH release *in vitro* including NE, serotonin (5-HT), CRH, TRH, brain-derived neurotrophic factor (BDNF), urocortin, mesotocin, and vasotocin [249]. In addition, alternative regulatory mechanisms have been described. It is known that factors produced by melanotrophs, such as acetylcholine (Ach) and BDNF, act in an autocrine way to stimulate MSH release [250]. Moreover, it has been described that the activation of calcium-sensing receptors [15] present in melanotrophs can also induce the production of MSH [251].

2.5 Conclusion

The pituitary gland is able to control a multiplicity of biological processes, including growth, reproduction, whole-body metabolism, and stress by the secretion of a variety of hormones, including GH, PRL, LH, FSH, TSH, ACTH, MSH, OT, and AVP. The synthesis and/or production of these hormones at the pituitary level is tightly controlled by a plethora of central and peripheral factors that finely interact to modulate the physiologic production of these hormones (Fig. 2.1). It is now known that each pituitary cell type exhibits a particular pattern of receptors for different signals and that the appropriate sensing, transmission, and integration of all

these inhibitory and stimulating signals is crucial to adapt the circulating levels of these hormones to the physiological necessities of the organisms.

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Conflict of Interest The authors declare that they have no conflict of interest.

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Pathogenesis of Pituitary Adenomas

3

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3.1 Introduction

Pituitary tumors are in their majority sporadic (~95%). Since the early 1990s, X-chromosome inactivation studies on pituitary tumors of different histological backgrounds (gonadotroph, somatotroph, lactotroph, corticotroph) have established that pituitary tumors are monoclonal [1–4]. This entails that one genetic hit transforms a cell that gives rise to the whole tumor. Mutations in proto-oncogenes such as *CMYC*, *RAS*, and *BRAF* are rare in pituitary tumors [5–7] (reviewed in [8]). Germline genetic defects in tumors in familiar or syndromic setting are not in the scope of this chapter. Mutations in genes that are involved in endocrine neoplasia syndromes such as *MEN1* (multiple endocrine neoplasia 1), *PRKAR1A* (Carney complex), *CDKN1B* (MEN4), and *GPR101* (X-LAG) are extremely rare in sporadic pituitary tumors (reviewed in [9]).

As we will see in more detail below, the main genetic hotspots are found in *GNAS* and *USP8* genes, mutated in ~40% and ~50% of sporadic somatotroph and corticotroph tumors respectively. The majority of pituitary tumors do not have known driver mutations. In contrast, numerous studies have reported posttranscriptional/posttranslational defects and aberrant signaling pathways that may trigger or contribute to pituitary tumorigenesis and progression. Furthermore, recent big data discoveries brought to our attention previously obscure putative mechanisms of pituitary tumor development and progression. This chapter will provide a brief

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overview on the deregulated hypothalamic and peripheral signals and the signaling networks deregulated in sporadic pituitary tumors, before addressing the genetics of sporadic pituitary tumors.

3.2 Hypothalamic and Peripheral Feedback Regulatory Circuits

3.2.1 Hypothalamic Regulation

The trophic action of hypothalamic factors on pituitary cells suggested that aberrant hypothalamic regulation might trigger pituitary hyperplasia and subsequent tumor formation. Animal models offer evidence of a hypothalamic role in pituitary tumorigenesis. Growth hormone-releasing hormone (GHRH) transgenic mice develop somatotroph hyperplasia, while female dopamine receptor type 2 DRD2 knockout mice present with lactotroph hyperplasia and tumors [10–12]. However, clinical experience does not offer similar evidence in human patients: patients with ectopic GHRH or CRH producing neuroendocrine tumors rarely present with somatotroph or corticotroph hyperplasia and do not develop tumors [13]. In the early 1990s, the establishment of monoclonality pushed the hypothalamic hypothesis of pituitary tumorigenesis aside [1–4].

Receptors for the hypothalamic peptides GHRH (GHRHR), corticotrophin-releasing hormone (CRH; CRHR1), vasopressin (V3R, V1BR), thyrotrophin releasing hormone (TRH; TRHR1, TRHR2), and gonadotropin-releasing hormone receptor (GnRH; GNRHR, also abbreviated as LHRHR) are detected in varying degrees of abundance in the different human pituitary tumor subtypes [14–18]. Dopamine receptor type 2 (DRD2, also abbreviated as D2R or D2DR) is expressed in lactotroph as well as somatotroph, corticotroph, and nonfunctioning pituitary tumors [19]. *DRD2* is alternatively spliced and in gonadotroph tumors, higher expression of the long over the short isoform has been associated with poor response to dopamine agonists [20]. Somatostatin receptors (SSTR1–5) are expressed in pituitary tumors in various combinations: somatotroph (mainly SSTR2), lactosomatotroph (SSTR2 and SSTR5), corticotroph (mainly SSTR5), and gonadotroph (mainly SSTR3). Reduced SSTR2 expression in sparsely granulated somatotroph tumors has been associated with worse prognosis [21]. Furthermore, somatotroph tumors expressing a truncated SSTR5 variant (SST_5TDM4), which does not respond to somatostatin analogs, present with more aggressive phenotype and have worse prognosis [22].

Constitutively activating mutations in the *GHRHR* are rare in somatotroph tumors [23, 24] and no activating *TRHR* and *GNRHR* mutations were reported in thyrotroph and gonadotroph tumors [25, 26]. Similarly, studies in corticotroph tumors did not reveal mutations in the coding regions of *CRHR1, V3R,* and *V1BR* [16, 27, 28]. Inactivating mutations were not reported in the *DRD2* gene encoding for dopamine receptor 2 in lactotroph tumors nor in the SSTR2 or SSTR5 genes in somatotroph tumors [29–33]. One exception is the report of a germline mutation in the *SSTR5* gene in a patient with acromegaly resistant to the somatostatin analog octreotide [34].

3.2.2 Negative Feedback Regulation

Pituitary function is tightly controlled by negative hormonal feedback loops from the periphery. Examples include the negative feedback of glucocorticoids on ACTH, thyroid hormones on TSH, and gonadal hormones on FSH/LH. Accordingly, compromised peripheral feedback may play a role in pituitary tumorigenesis through not only the uncontrolled hormone production, but also aberrant proliferation of the corresponding pituitary tumor cells. However, there is little clinical evidence supporting their tumorigenic role; thyrotroph (and in a lesser extend lactotroph) hyperplasia has been observed in few patients with primary hypothyroidism [35, 36]. Patients with primary hypogonadism or chronic sex steroid hormone treatment show no higher incidence of pituitary tumors in comparison to the general population [37–39].

Similar to the hypothalamic hypothesis, there is no circumstantial evidence that compromised peripheral negative feedback is initiating factor of pituitary tumorigenesis in humans. Genetic variations or mutations in the *NR3C1* gene are very rare in patients with Cushing's disease [40–42]. Similarly, no mutations were found in the genes encoding for TRβ and rare mutations of unknown functional significance in the gene encoding for TRα [43].

On the other hand, transformed pituitary tumor cells may develop resistance to the negative feedback regulation and this would confer survival benefits to the tumor. In fact glucocorticoid resistance in corticotroph tumors is a hallmark of Cushing's disease [44]. As glucocorticoid receptor GR is not downregulated in corticotroph tumors [40–42], this glucocorticoid resistance was attributed to elevated expression of 11 β-hydroxysteroid dehydrogenases 2 (11β-HSD2), which converts cortisol to cortisone thereby inactivating cortisol [45] and loss of Brahma-related gene 1 (BRG1), which is part of the GR repressor complex on the *POMC* promoter [46, 47]. Overexpression of the testicular orphan nuclear receptor 4 (TR4) may also promote glucocorticoid resistance as its interaction with GR abolishes GR's inhibitory effect on *POMC* transcription [48]. Finally, corticotroph tumors overexpress the GR chaperon HSP90 (heat shock protein 90) and this may impair the function of GR by preventing its maturation [49]. Indeed, the C-terminal HSP90 inhibitor silibinin was able to reverse glucocorticoid resistance and ameliorate the signs of Cushing's disease *in vitro* and *in vivo* [49].

3.3 Signaling and Cellular Processes

3.3.1 EGF & EGFR

Epidermal growth factor (EGF) is a prototype growth factor and the overexpression of its receptor EGFR is a hallmark of several human cancers. EGF is expressed in the pituitary gland where it may regulate pituitary function in an autocrine/paracrine manner (reviewed in [50]). It is noteworthy that in anterior pituitary cells, EGF alters endocrine cell function and hormone response, but is not mitogenic, choosing a cell differentiation role over its growth-promoting function [51].

EGFR expression was described in pituitary tumors with different studies reporting different prevalence depending on the method and—in case of immunohistochemical investigation—antibody used. Accordingly, EGFR overexpression was reported in clinically silent tumors compared to endocrinologically functional ones, in all subtypes of pituitary tumors, in invasive versus non-invasive, and in lactotroph and corticotroph tumors versus the nonfunctioning ones [52–56]. EGFR is predominantly expressed in corticotroph cells where EGF triggers replication and ACTH synthesis (reviewed in [57]). This is reflected by the fact that EGF triggers bromodeoxyuridine (BrdU) incorporation in dispersed corticotroph and lactotroph cells, but not in other pituitary cell subtypes [58]. The predominance of EGFR in corticotroph and lactotroph tumors indicates that the EGFR system may represent a putative therapeutic target [56, 59–61].

3.3.2 TGF-β

Members of the transforming growth factors β (TGF-β) family regulate cell differentiation, proliferation, extracellular matrix, and angiogenesis. The receptors of the family belong to the serine/threonine kinase receptor superfamily and transduce their signals via SMAD that upon phosphorylation translocate to the nucleus to regulate transcription. TGF-β1 has a predominant role in lactotroph cells where it inhibits prolactin synthesis and proliferation [62]. Accordingly, its downregulation in lactotroph tumors was hypothesized to contribute to the development of these tumors. In fact, TGF-β1 crosstalks with both estradiol and dopamine, both very important regulators of lactotroph function (reviewed in [63]). Moreover, differences in the expression of TGF-β1 in males and females may explain gender differences in the incidence of prolactinomas [63, 64].

An important pituitary development member of the TGF-β family is the bone morphogenetic protein 4 (BMP4), which is required for the formation of Rathke's pouch (reviewed in [65]). BMP4 is overexpressed in lactotroph tumors and its crosstalk with estrogen receptor via Smad induces proliferation and PRL production, suggesting a crucial role in lactotroph tumorigenesis [66]. BMP4 expression in the pituitary gland is moderate where it is predominantly located in the corticotroph cells. BMP4 is downregulated in corticotroph tumors, where it inhibits proliferation and ACTH production *in vitro* and *in vivo* [67].

3.3.3 Cytokines

The pituitary gland expresses cytokines and their receptors, which regulate hormone synthesis in an autocrine-paracrine manner [50, 68]. Interleukin-1 (IL-1) is expressed in human pituitary tumors and rat anterior pituitary where it regulates growth and stimulates the secretion of anterior pituitary hormones, with the exception of prolactin [69, 70]. Similarly, IL-2 and its receptor are expressed in all

anterior pituitary cell subtypes, but IL-2 plays a particular role in corticotroph cells where it enhances ACTH synthesis [71–73].

The gp130 cytokine family is a heterogeneous group of cytokines (interleukin-6 (IL-6), IL-11, oncostatin M, ciliar neurotrophic factor (CNTF), leukemia inhibitory factor [74], and others) whose receptors have no intracellular signaling domain and thus use the gp130 membrane protein as a common signal transducer to activate the JAK-STAT signaling pathway [75]. Leukemia inhibitory factor [74] stimulates ACTH synthesis in synergy with CRH [76–78]. LIF overexpressing transgenic mice present with high ACTH and cortisol levels, reduced sensitivity to dexamethasone and cushingoid features [79] (reviewed in [80]). In contrast, the physiological HPA-mediated stress response is attenuated in LIF-deficient animals [81, 82].

IL-6 and its receptor are expressed in pituitary tumors where they correlate with tumor size and invasiveness and may trigger pituitary cell growth in autocrine/paracrine manner [83–87]. Inhibition of the signal transducer gp130, which mediates among the others the effects of IL-6, inhibits the growth of lactosomatotroph GH3 tumors in nude mice [88]. IL-6 stimulates ACTH, GH, and PRL secretion from corticotroph, somatotroph, and lactotroph tumors, respectively (reviewed in [75] [89]). Interestingly, IL-6 was detected in the secretome of tumor-associated fibroblast isolated from pituitary tumors, and its levels were significantly higher in invasive tumors [90]. As we will see in more detail below, there is evidence that IL-6 with its autocrine action contributes to pituitary senescence [91].

3.3.4 Hedgehog Pathway

Sonic hedgehog (Shh) signaling plays an important role in pituitary development [92]. Hedgehog proteins bind to Patched (PTCH) 1 and 2, releasing them from Smoothened (SMO) and enabling them to activate the GLI transcription factors. Shh expression in the adult pituitary gland concentrates mainly in corticotroph cells, where it triggers ACTH synthesis via a GLI1-CRHR1 crosstalk [93]. The majority of pituitary tumors express PTCH1 (mainly gonadotroph) and PTCH2 (mainly corticotroph, somatotroph, lactotroph), but not Shh itself, and Shh administration in dispersed anterior pituitary cells and human pituitary tumors increases ACTH, GH, and prolactin levels [94, 95]. Shh treatment in the murine corticotroph tumor cell line AtT-20 inhibited cell growth, which together with the loss of expression in human corticotroph tumors led to the hypothesis that it may act as a tumor suppressor [93, 94]. In contrast, studies in murine pituitary explants revealed increased proliferation of Sox2+ and Sox9+ stem cells after Shh stimulation [95].

3.3.5 Wnt Pathway

Wingless (Wnt) acts in paracrine manner to control cell proliferation, differentiation, and migration during embryogenesis. After binding a heterodimeric receptor

complex, formed by Frizzled (Fzd) and LRP5/6 co-receptor protein, Wnt triggers the activation of three different intracellular molecular pathways: the β-catenin "canonical" pathway, the "calcium", and the "planar cell polarity pathway" [96]. Wnt4 and Wnt5 play an important role in pituitary cell differentiation [97]. WNT4 and its receptor FZD6 were shown to be overexpressed in somatotroph, lactotroph, and thyrotroph tumors, while the Wnt pathway inhibitor frizzled-related protein 2 (SFRP2) was shown to be downregulated [74, 98]. In corticotroph tumors, SFRP2 reduction correlated with tumor size and invasiveness and its overexpression suppressed proliferation and ACTH secretion in immortalized corticotroph tumor cells [99].

Regarding the downstream target of the canonical Wnt pathway, a study reported nuclear accumulation of β-catenin in 57% of pituitary tumors and not in the normal pituitary [100]. In contrast, other studies did not observe changes in β-catenin distribution in pituitary tumors [74, 98, 101].

3.3.6 Hippo Pathway

The Hippo pathway plays an important role in controlling stem cells proliferation and tumorigenesis. When the Hippo pathway is activated, mammalian sterile 20-like kinases 1 and 2 (MST1/2; ortholog of *Drosophila* Hippo) phosphorylate and activate the tumor suppressor homologs 1 and 2 (LATS1/2), which in turn phosphorylate and inactivate the transcriptional co-activators Yes-associated protein (YAP) and WW domain-containing transcription factor (TAZ/WWTR1) [102]. An inactive upstream pathway allows YAP/TAZ to translocate to the nucleus where they associate with TEAD transcription factors (TEA domain family members 1–4) eventually promoting stem cell proliferation and preventing apoptosis.

Mice deficient in *Lats1* develop hyperplasia of the anterior pituitary lobe, but show decreased secretion of pituitary hormones suggesting repressed endocrine differentiation [103]. Knocking down *Last1* in vitro leads to decreased anterior pituitary hormone synthesis and high YAP/TAZ expression was reported in null cell tumors, adamantinomatous, and papillary craniopharyngiomas, but not in hormone secreting pituitary tumors [104]. Human and mouse pituitary glands during embryonic and postnatal development show strong nuclear localization of YAP/TAZ exclusively in SOX2+ cells [104, 105]. As the Hippo pathway regulates SOX2+ pituitary stem cells (PSC) clonal expansion and specification, postnatal deletion of LATS kinases with subsequent upregulation of YAP/TAZ signaling may lead to loss of differentiation of SOX2+ PSC and uncontrolled clonal expansion, resulting in hormonally nonfunctioning pituitary tumors [106].

3.3.7 Cadherins

The pituitary gland expresses epithelial cadherin (E-cadherin), but reduced cytoplasmatic staining is observed in somatotroph, lactotroph, and hormonally

nonfunctioning pituitary tumors [107–111]. No mutations in the *CDH1* gene or changes in transcript levels were reported for the majority of pituitary tumors. Instead, strong nuclear staining is observed, indicating aberrant nuclear translocation as a cause of E-cadherin loss from the cell membrane [112]. In somatotroph tumors, E-cadherin protein levels correlate with SSTR2 expression and response to somatostatin analogs [111]. Furthermore, reduced cytoplasmic E-cadherin correlated with tumor invasiveness and recurrence [113]. A study reported missense mutations in the gene encoding for cadherin-related 23 (*CDH23*) in 33% (4/12) of familial pituitary tumors and 12% (15/25) of sporadic pituitary tumors versus 0.8% (2/260) of controls carrying functional *CDH23* variants [114].

3.3.8 Cell Cycle

Cyclin-dependent kinases (CDKs) and their associated activators (cyclins) promote cell cycle transition and this is inhibited by CDK inhibitors [115]. Transgenic/knockout mouse models of genes involved in cell cycle regulation present with pituitary hyperplasia and tumors (reviewed in [116]).

The tumor suppressor RB encodes for retinoblastoma, a pocket protein that binds to E2 family (E2F) transcription factors preventing the transcription of genes important for DNA synthesis [117]. Heterozygous mice with disrupted Rb present with pituitary tumors [118]. However, human pituitary tumors do not present with *RB1* mutations, with the exception of extremely rare aggressive cases [119–121]. In contrast, *RB1* gene expression is downregulated in around 1/3 of pituitary tumors, possibly due to promoter hypermethylation [122]. An E2F1 target, the high mobility group protein A (HMGA), was reported to be highly expressed in lactotroph and somatotroph tumors [123, 124].

Retinoblastoma is hyperphosphorylated and inactivated by cyclin D-CDK4/6 and cyclin E/CDK2 complexes. Cyclin D1 is encoded by the *CCND1* gene and binds to CDK4. CDK4 deficiency is characterized by pituitary hypoplasia with the somatotroph and lactotroph axes being particularly affected [125, 126]. In fact, lactotroph cells in CDK4-deficient mice cannot proliferate in response to estrogen [125]. Cyclin D1 is overexpressed in nonfunctioning pituitary tumors [127]. Nuclear cyclin D1 immunoreactivity is more prominent in aggressive and nonfunctioning subtypes, and correlates positively with the Ki67 proliferation index and tumor size [74, 127, 128]. In contrast, cyclin D1 levels in corticotroph tumors are lower compared to nonfunctioning pituitary tumors [129]. Both invasive and non-invasive pituitary tumors may present with allelic imbalance in cyclin D1 (*CCND1*, chromosome 11q13), while certain *CCND1* genotypes are associated with tumor grade [130–132]. Cyclin D3 was found to be overexpressed in gonadotroph tumors [133].

Cyclin E is highly expressed, while protein (but not transcript) levels of its CDK inhibitor p27 are downregulated/lost predominantly in corticotroph tumors [127, 134]. Genetically engineered mice overexpressing cyclin E and haploinsufficient for p27 showed increased frequency, size, and proliferation index of pituitary tumors [135]. Corticotroph tumors show loss of the transcriptional regulator

Brahma-related gene 1 (BRG1) that inhibits cyclin E expression, with concomitant high cyclin E expression and p27 loss [136]. Pharmacological inhibition of CDK2/cyclin E with roscovitine was shown to decrease ACTH synthesis in vitro and from corticotroph tumor xenografts *in vivo* [137]. Finally, cyclin A is highly expressed in recurrent pituitary tumors [138], but its expression in nonfunctioning pituitary adenomas did not correlate with invasion and proliferation [139].

CDK/cyclins are inhibited by CDK inhibitors belonging to the CIP/KIP (CDK Interacting Protein/Kinase Inhibitory Protein; representative members: p27, p21) and INK (INhibitors of CDK; representative members: p16, p18) families. As we have seen above the CDK2/Cyclin E inhibitor p27/Kip1 is downregulated or lost in the majority of corticotroph tumors [134]. Transcription of the *CDKN1B* gene that encodes for p27 is not altered and the observed downregulation is at the protein level. High EGFR levels were suggested to account for the reduced p27 in these tumors [56]. In addition, BRG1 decrease in corticotroph tumors leads to cyclin E accumulation, which in complex with CDK2 phosphorylates p27 and marks it for proteasomal degradation [136]. In line with this model, corticotroph tumors have relatively high phosphorylated p27 levels [140]. In contrast DKC1 (dyskeratosis congenital/dyskerin) that regulates p27 translation is not deregulated in the majority of pituitary tumors [141]. In addition to corticotroph tumors, p27 downregulation was also reported in other types of pituitary tumors where it correlates with invasiveness [142–144]. Mice with disrupted p27 show hyperplasia and tumors of the intermediate lobe and p27 downregulation sensitizes somatotroph cells to the proliferative action of GHRH excess in p27/GHRH transgenic mice [145–147]. Spontaneous mutation in the *Cdkn1b* gene in rats gives rise to a multiple endocrine neoplasia syndrome (MENX) that is characterized by focal non-secreting pituitary tumors [148].

Members of the INK family of CDK inhibitors like p16/INK4a and p18/INK4c are downregulated or lost in pituitary tumors [149–151]. Introducing p16 in murine pituitary tumor AtT-20 cells that do not express endogenous p16 arrested cells at G1 and reduced proliferation [152]. Loss of p16 expression is mainly observed in nonfunctioning (including silent corticotroph tumors), but not in hormone secreting such as somatotroph or corticotroph tumors [129, 153–155]. This may be due to promoter hypermethylation on the *CDKN2A* gene (that encodes for p16) that was frequently observed in gonadotroph tumors, but not as much in somatotroph or corticotroph tumors [156]. In addition to *CDKN2A*, hypermethylation was detected on *RB1* and *CDKN2B* (p15) gene promoters, with *RB1* and *CDKN2A* (p16) promoter methylations being mutually exclusive [157].

The CDK inhibitor p18 is downregulated in pituitary tumors and most prominently in corticotroph tumors [158]. Loss in the *CDKN2C* gene (that encodes for p18) was found in one-fourth of human pituitary tumors and *CDKN2C* promoter hypermethylation was reported [149, 153–155]. Concomitant loss of p18 and p27 in mice results in the development of large pituitary tumors suggesting a cooperation between these two cell cycle regulators [159].

The other member of the CIP/KIP family of CDK inhibitors is p21/CIP1. Mice deficient for both members of the CIP family, p21 and p27, present with pituitary tumors [160].

Decreased nuclear p21 immunoreactivity was described in all pituitary tumor types with the notable exception of sparsely granulated somatotroph tumors [161–163]. Lactotroph tumors were found to overexpress MIR-93, which targets the expression of *CDKN1A* that encodes for p21 [164].

3.3.9 PTTG

The pituitary tumor transforming gene (PTTG), also known as securing, regulates sister chromatid separation during cell division and its deregulation results in aneuploidy and chromosomal instability [165–169]. *Pttg* deletion *in vivo* induces p21 and disrupts the development of pituitary tumors in *Rb*+/− mice [170]. PTTG is expressed in most pituitary tumors, with higher expression positively correlating with tumor invasiveness and recurrence/regrowth [128, 171–175]. PTTG was hypothesized to promote pituitary tumorigenesis by deregulating RB1 and p21, via bFGF (FGF2) and VEGF and by activating the c-MYC oncogene [176–181]. PTTG stabilization by the RWD-containing sumoylation enhancer (RWDD3 or RSUME) may account for its abundance in pituitary tumors [182]. In somatotroph cells high PTTG expression is accompanied by increased p21 and other markers of senescence [170].

3.3.10 Senescence

Cellular senescence is an irreversible cell cycle arrest in G1 phase that occurs as a consequence of multiple stimuli, such as DNA damage, aneuploidy, oxidative stress, oncogene activation, and telomere shortening in aged tissues [183, 184]. Oncogene-induced senescence has been associated with activated p53/p21 and RB/p16 pathways [185]. Pituitary tumors show increased expression of senescence markers such as p53, p19, p21, cyclin D, and senescence-associated-β-galactosidase (SA-β-Gal), while senescence has been linked especially in somatotroph tumors [170, 186, 187]. As oncogene-induced senescence halts cell proliferation in the early stages of transformation; its presence may explain the usually benign nature of pituitary tumors (reviewed in [188]).

Senescent cells influence tumor microenvironment via secreted factors collectively referred to as senescence-associated secretory phenotype (SASP) (reviewed in [189]). The SASP cytokine IL-6 may contribute to pituitary senescence in an autocrine manner, since endogenous IL-6 inhibition decreased SA-β-gal in human pituitary tumors in vitro [91].

3.4 Genetics of Sporadic Pituitary Tumors

3.4.1 Chromosome Instability

Loss of heterozygosity (LOH) studies had revealed loss of the long arm of chromosome 11 (11q13) at the *MEN1* locus, short arm of chromosome 9, and the long arm of chromosome 10 (10q26), with LOH in 11q13, 13q, and 10q26 being more frequent in invasive pituitary tumors [190–195]. LOH at the *RB1* gene locus in the long arm of chromosome 13 (13q) was observed in invasive pituitary tumors and carcinomas [121, 196]. Pituitary tumors display gene copy number variations (CNV) with higher frequency in functioning compared to hormone inactive tumors [197–204]. Lactotroph tumors were found to display the highest CNV compared to corticotroph and somatotroph, while gonadotroph tumors showed no CNV [205]. Corticotroph tumors from pediatric patients show high chromosomal instability in ~20% of cases, which correlated with aggressiveness [206]. Overall, chromosomal alterations depend on the secretion type with secreting tumors showing higher variations compared to silent (gonadotroph, silent corticotroph) types, while no correlation was found with tumor aggressiveness [204].

3.4.2 Mutations

Mutations in oncogenes and tumor suppressor genes frequently reported in other cancers are very rare in pituitary tumors. Recent next-generation sequencing efforts reveal that only two genes, *GNAS* and *USP8*, are mutated in >5% of sporadic pituitary tumors [201, 202, 204]. No other driver mutations were found and overall the number of mutations in pituitary tumors is relatively low [201, 202, 204, 207–210].

3.4.3 GNAS

The first genetic hotspot in sporadic pituitary tumors was identified in a hypothesis-based approach, since the receptors of the aforementioned hypothalamic peptides belong to the G protein-coupled receptor superfamily and are coupled to G proteins. In the mid-1980s, studies reported elevated adenosine cyclase (AC) activity and cAMP levels in one-third of somatotroph tumors that were not influenced by GHRH and cholera toxin, but were caused by activating mutations in the gene encoding for the stimulatory G alpha subunit (Gsα) [211–213]. Somatic mutations in the *GNAS* gene (previously referred to as *gsp* proto-oncogene) are found in ~40% of somatotroph tumors, but very rarely in corticotroph and other pituitary tumors [214]. Somatotroph hyperplasia and tumors are observed in the context of McCune–Albright syndrome, a multi-organ mosaicism disorder that is caused when somatic *GNAS1* mutations occur in post-zygotic state (reviewed in [215]).

In contrast to *GNAS,* mutations in the genes encoding for G protein subunit alpha i2 (*GNAI2*; also known as *GIP2*) and Gq alpha subunit (*GNAQ*) are very rare in pituitary tumors [214].

3.4.4 USP8

Whole exome sequencing in corticotroph tumors led to the discovery of a single somatic mutational hotspot in the gene encoding for ubiquitin specific protease 8 (USP8) [216, 217]. Follow-up sequencing studies revealed that ~50% of corticotroph tumors (including cases of corticotroph tumor progression after bilateral adrenalectomy also known as Nelson's syndrome) have somatic *USP8* mutations [216–221]. Notably, *USP8* mutations are found exclusively in corticotroph tumors and not in any other pituitary tumor subtype nor in ACTH-secreting ectopic neuroendocrine tumors [222]. A germline USP8 mutation was identified in a female pediatric patient presenting with corticotroph tumor [223].

USP8 is a deubiquitinase that cleaves ubiquitin from target proteins, rescuing them from degradation. The mutational hotspot identified in corticotroph tumors is located in exon 14 and prevents the binding of 14-3-3 proteins, thereby enabling the cleavage to a highly catalytically active C-terminal fragment [216]. Overactivated USP8 mutants rescue ligand-bound EGFR from the lysosome and potentiate *POMC* expression and ACTH synthesis [216]. USP8 mutant corticotroph tumors have higher *POMC* levels [219]. USP8 mutants have less potent effect on cell growth in vitro, which is reflected by the relatively smaller size and lack of invasion of *USP8* mutant corticotroph tumors [216–218].

Overall, corticotroph tumors carrying USP8 mutations are usually smaller, occur mainly in female patients and may be hormonally more active, as reflected by higher basal and postoperative cortisol levels [218]. Patients with USP8 mutant tumors show worse postsurgical outcome as reflected by the lower prevalence of adrenal insufficiency after surgery [218]. Indeed, in these patients, the frequency of recurrence after postsurgical biochemical remission is higher and occurs earlier [221]. Interestingly, USP8 mutant corticotroph tumors display higher SSTR5 levels suggesting that they may respond favorably to pasireotide [204, 219, 224].

3.4.5 USP48

Whole exome sequencing in USP8-wild type corticotroph tumors revealed a second somatic mutational hotspot in the gene encoding for the USP48 deubiquitinase in about 10% of corticotroph tumors [225, 226]. USP48 is a deubiquitinase first identified in the rat brain as synUSP [227]. The pathogenic mutations are mainly concentrated on a single amino acid (Met415) and result in amino acid exchange of Met415Ile or Met415Val [226]. Mutant USP48 showed modest stimulatory action

on basal but potentiated CRH-induced ACTH synthesis [225]. One of the USP48 clients is Gli1 and knocking down Gli1 in corticotroph tumor cells abolished its stimulatory effect suggesting that mutant USP48 may use this mechanism to sensitize corticotroph tumor cells to the stimulatory action of CRH [93, 225].

3.4.6 SF3B1

Whole genome sequencing on lactotroph tumors identified a somatic mutational hotspot in the *SF3B1* gene encoding for splicing factor 3 subunit B1 [228]. The *SF3B1 R625H* hotspot mutation was found in ~20% of lactotroph tumors (but no other pituitary tumor type) and patients with *SF3B1* mutant tumors were more frequently male, had higher prolactin levels (relative to tumor size), and shorter progression-free survival; no statistically significant differences were found with tumor invasion and size [228]. SF3B1 is a component of the U2 small nuclear ribonucleoproteins (snRNP) complex that is involved in pre-mRNA splicing and one of SF3B1 splicing targets is *ESRRG* (Estrogen Related Receptor Gamma). The mutant SF3BP1 results in cryptic ESRRG, which has high affinity for the transcription factor Pit-1 that drives pituitary *PRL* gene expression, resulting in prolactin overproduction. Interestingly, inhibiting SF3B1 with pladienolide-B significantly reduced cell viability in pituitary tumors in vitro [229].

3.4.7 RAS-BRAF

Activating *RAS* mutations are rarely reported and only in highly invasive pituitary tumors [5–7]. The most common *BRAF* mutation V600E was very rarely described in nonfunctioning pituitary tumor [230, 231]. A recent study in an Asiatic cohort reported the *BRAF V600E* mutation in 16% of corticotroph tumors, but this was not replicated in a multicenter study that found the mutation in only one out of 94 corticotroph tumors [225, 226].

3.4.8 TP53

TP53 mutations were very rarely described in pituitary tumors and only exclusively in invasive pituitary tumors and carcinomas [232–235]. This extreme rarity of *TP53* mutations in pituitary tumors was challenged by recent next-generation sequencing studies that revealed pathogenic *TP53* mutations in ~30% of *USP8*-wild type corticotroph tumors, suggesting that they may be more frequent in certain tumor subcategories than previously thought [225, 236].

Mutations in *CDKN1B* (p27), *CDKN2A* (p16/INK4a), and *CDKN2C* (p18/INK4c) are rare in sporadic pituitary tumors [149–151, 163, 225, 237–241].

3.4.9 Other

The antiproliferative response to glucocorticoids was linked to the tumor suppressor CABLES1 (CDK5 and ABL1 enzyme substrate 1) [136]. Corticotroph tumors have low CABLES1 expression, and this may account for the decreased p27 protein levels observed in these tumors. Mutations in the *CABLES1* gene were described in four female patients (2 young adults and 2 children) with large corticotroph tumors [242].

3.4.10 Noncoding RNAs

Noncoding RNAs do not encode proteins and are classified into microRNAs (miRNAs) and larger long noncoding RNAs (lncRNAs) (reviewed in [243]).

MicroRNAs (miRNAs) are 22 nucleotides long RNAs that are expressed at defined genomic loci and regulate gene expression posttranscriptionally by cleaving mRNAs or repressing their translation (reviewed in [244]). Pituitary tumors show aberrant expression of miRNAs (reviewed in [245]). The miRNA let-7 (that targets *HMGA2*) is downregulated in lactotroph, corticotroph, and gonadotroph, but not in somatotroph tumors, and correlates with high tumor grade [246]. Other "tumor suppressor" miRNAs such as miR-15a and 16-1 (that target cyclin D) are downregulated in lactotroph, somatotroph tumors, and their expression is negatively correlated with tumor size [247]. Corticotroph tumors also show downregulation in miR-15a and 16-1 as well as let-7a, miR-21, miR-141, miR-143, miR-145, and miR-150; no correlations were found with tumor size or remission after surgery, with the exception of miR-141 that correlated with higher remission rate [248]. Somatotroph tumors show downregulation of miRNAs (miR-34b, miR-326, miR-432, miR-548c-3p, miR-570, and miR-603) that target *HMGA1/2* and *E2F1* [124]. Nonfunctioning tumors have downregulated miR-149-3p, miR-130a-3p, and miR-370-3p compared to hormone-secreting pituitary tumors [249]. In contrast, miR-107 (that targets *AIP*) is upregulated in somatotroph and nonfunctioning pituitary tumors [250]. Somatotroph tumors also show upregulated miR-26b and miR-128 that regulate *PTEN* and subsequently the AKT survival pathway [251]. Nonfunctioning tumors show upregulation of miR-128a, miR-155, and miR-516a-3p (that target *WEE1*) and miR-135a, miR-140-5p, miR-582-3p, miR-582-5p, and miR-938 (that target *Smad3*) with expression levels correlating with tumor size [252, 253]. Corticotroph tumors have upregulated miR-122, miR-493 (target E2F1), and miR-26a (targets protein kinase Cδ) [254, 255].

In addition to miRNAs, large numbers of long (~200 nucleotide) noncoding RNAs (lncRNAs) are transcribed that regulate genome organization and mRNA stability and translation (reviewed in [256]). Pituitary tumors show aberrant expression of lncRNA that act in tumor-suppressing or growth-promoting manner (reviewed in [257]). The maternally expressed gene 3 (MEG3) lncRNA that

functions via p53 is lost in gonadotroph tumors and negatively correlates with invasion [258]. The lncRNA H19 is downregulated in lactotroph and other pituitary tumors and correlates negatively with tumor volume probably; H19 may suppress tumor growth via the mTOR-4E-BP1 pathway [259]. Lactotroph tumors also showed decreased expression of CLRN1-AS1, which regulates the WNT/b-catenin signaling cascade [260]. In contrast, Hox transcript antisense intergenic RNA (HOTAIR) was upregulated in nonfunctioning pituitary tumors and this correlated with invasive behavior [261]. Interestingly, a lncRNA, MIR205HG, was shown to regulate growth hormone and prolactin by regulating the transcriptional activity of Pit1 [262].

Circulating miRNAs were proposed as potential biomarkers in pituitary tumors [263]. Indeed, late postoperative plasma miR-143–3p levels were decreased in patients with gonadotroph tumors suggesting its usefulness as a biomarker of successful surgery [264].

3.5 Conclusion

Pituitary tumorigenesis is complex and cannot be attributed to a single factor. Mutations in oncogenes and tumor suppressor genes frequent in cancer are rarely found in pituitary tumors. Few driver mutations were identified mainly in the *GNAS* and *USP8* genes mutated in somatotroph and corticotroph tumors respectively. Additional mutational hotspots were found in the *USP48* in a smaller fraction of corticotroph tumors and in the *SF3B1* in a fraction of lactotroph tumors. Posttranscriptional/−translational alterations in cell cycle and growth factor signaling undoubtedly play an important role, but the causative genetic events remain in many cases obscure. The use of pangenomic techniques has shifted our attention to previously unconsidered processes and highlighted the fact that when it comes to pituitary tumors one size does not fit all and that different histological subtypes of pituitary tumors are distinct tumor entities each with their own pathogenetic mechanisms.

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Genetics of Pituitary Adenomas

4

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Abbreviations

3 Pa	Pituitary adenomas and paragangliomas or phaeochromocytomas
ACTH	Adrenocorticotrophic hormone
AHR	Aryl hydrocarbon receptor
AIP	Aryl hydrocarbon receptor-interacting protein
BRAF	Proto-oncogene B-Raf
cAMP	Cyclic adenosine monophosphate
CDH23	Cadherin-related 23
CGH	Comparative genomic hybridisation
EGFR	Epidermal growth factor receptor
FIPA	Familial isolated pituitary adenomas
GH	Growth hormone
GHRH	Growth hormone-releasing hormone
HSP90	Heat shock protein 90
IGF-1	Insulin-like growth factor 1

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MEN1	Multiple endocrine neoplasia type 1
MEN2A	Multiple endocrine neoplasia type 2A
MEN2B	Multiple endocrine neoplasia type 2B
MEN4	Multiple endocrine neoplasia type 4
MGMT	O6-methylguanine-DNA methyltransferase
microRNA	Micro ribonucleic acid
NF1	Neurofibromatosis type 1
NFPA	Non-functioning pituitary adenoma
PA	Pituitary adenoma
PI3K	Phosphoinositide 3-kinases
PIT-1	Pituitary-specific POU-class homeodomain transcription factor
PitNET	Pituitary neuroendocrine tumour
PKA	Protein kinase A
POMC	Proopiomelanocortin
PTEN	Phosphatase and tensin homolog
SDH	Succinate dehydrogenase
USP48	Ubiquitin-specific peptidase 48
USP8	Ubiquitin-specific peptidase 8

4.1 Introduction

Pituitary adenomas (PAs) or pituitary neuroendocrine tumours (PitNETs) are mostly benign tumours located in sella turcica, deriving from the anterior lobe of the pituitary [1, 2]. They are relatively common in the general population in autopsy and magnetic resonance imaging (MRI) studies, but in 0.1% of cases they are clinically relevant [3–8]. In the last few years, using modern scientific and molecular methods, several novel genetic mutations have been identified that can predispose to pituitary adenomas. Moreover, genetic testing is getting more easily available for patients in routine clinical practice worldwide.

Hereditary pituitary adenoma can manifest solely as an isolated tumour, without any other clinical manifestations, like familial isolated pituitary adenoma (FIPA). Pituitary adenoma can be also a part of a syndromic disorder such as Multiple endocrine neoplasia type 1 (MEN1), Multiple endocrine neoplasia type 4 (MEN4), Carney complex, McCune-Albright syndrome, pheochromocytoma-pituitary adenoma (3Pa) or DICER1. In some cases, pituitary adenomas in a familial setting are characterised by an earlier onset of the disease, more aggressive course and resistance to medical treatment [9].

In this chapter, we focus on clinical aspects of PAs with germline mutations. Genetic testing in family members could result in earlier recognition of the disease leading to more favourable outcomes. Careful selection of cases is key to keep patient anxiety and medical care costs under control [10]. Additionally, genetic results may influence family planning and reproductive choices,

favouring the suggestion that genetic counselling should be widely available for PA patients [11].

4.2 Epidemiology

Pituitary adenomas are the third most common neoplasms of central nervous system after meningiomas and gliomas, representing about 15% of cases [12, 13]. Most of PAs develop sporadically, but in 5% they may occur in a familial setting. The incidence of pituitary adenomas rises with age, with a peak of diagnosis between 30 and 60 years old [14]. Many of them constitute incidental findings during imaging scans performed due to other reasons [15]. Although during childhood and adolescence pituitary tumours are very rare, the genetic background should be considered in almost every case as it is responsible in a higher proportion, especially among somatotrophinomas [16] where up to 46–49% of gigantism cases have identifiable genetic cause [16, 17]. In adults, however, the genetic cause is present only in a minority of patients. The most common pituitary adenomas in early childhood are corticotroph adenomas [18], while in adolescence, prolactinomas and somatotroph adenomas are most frequent [19, 20]. In adulthood, clinically non-functioning adenomas (NFPAs) has taken over the most frequently encountered pituitary adenomas from prolactinomas in terms of frequency, probably due to better and more frequently used imaging of the population [13, 21]. Non-functioning pituitary adenomas and prolactinomas are followed by somatotroph adenomas, corticotroph adenomas and thyrotroph adenomas [22].

4.3 Pathogenesis

Pituitary adenomas develop from the hormone-secreting anterior pituitary cell types, such as somatotroph, lactotroph, corticotroph, gonadotroph and rarely from thyrotroph cells or their precursors. Several different factors that lead to pituitary tumorigenesis have been described but in most cases, molecular pathogenesis and epigenetic mechanisms are still unknown [23–28]. The impact of environmental factors is also questionable. Some data suggest that in highly polluted areas the course of somatotrophinomas is more aggressive; further, independent studies are required to confirm these data [29–31].

PAs are predominantly benign tumours with clinical symptoms caused by hormonal hypersecretion or mass effects leading to visual field disturbances or hypopituitarism. In some cases, PAs can be locally aggressive with invasion of the surrounding sinuses and putting pressure on the cranial nerves. Progression of pituitary tumours to true malignancy with distant metastases is very rare, found in about 0.2% of all pituitary adenomas, but aggressive tumours occur more frequently, reported to account from 2.5% to 10% in surgical series [32] with often similar pathological characteristics [33].

Recently a change has been suggested to address adenohypophyseal tumours as pituitary neuroendocrine tumours (PitNETs). This suggestion has been met with some controversy. The International Pituitary Pathology Club suggests that the hormone-producing cells of the pituitary are a part of the neuroendocrine system and propose to replace the word 'adenoma' by 'neuroendocrine tumour' to highlight the similarity with other neuroendocrine neoplasms [34]. However, others note the risk of aligning pituitary adenohypophyseal tumours to other neuroendocrine tumours would mean unnecessary anxiety in patients and physicians less familiar with the disease [35–40]. Further discussion is welcome on this issue.

The 2017 WHO classification emphasises the diagnostic role of immunohistochemistry for adenohypohyseal hormones and their cell lineage-specific transcription factors [33, 34, 41]. Pangenomic classification of pituitary tumours supports the key role of transcription factors and showed association between POU1F1/PIT-1 lineage and DNA hypomethylation, the transcription of differentiation markers, transposable elements and chromosomal instability [42].

Sporadic PAs seem to be monoclonal in origin [43]. X-chromosome inactivation studies have shown that they arise from a single somatic pituitary cell, although recurrent tumours may show an evolved and slightly different clone from the original tumour [44]. Monoclonal character is supported by observed long-term hormonal remission after successful tumour resection without changes in the surrounding tissue [45–48]. In patients with germline mutations multiple separate adenomas may arise from the pituitary tissue, for example, in aryl hydrocarbon receptor-interacting protein (*AIP)* mutation-positive patients [49]. Pituitary hyperplasia can be characteristic in patients with germline mutations, such as Carney complex, McCune-Albrigth syndrome, X-inked acrogigantism (XLAG), and rarely has been seen in *AIP* cases as well [50]. Genetic alterations associated with PitNETs can be germline, mosaic or somatic (Fig. 4.1).

4.3.1 Somatic Mutation

In general, sporadic pituitary adenomas have lower somatic mutations rate than other malignant tumours. *GNAS* gene mutation is the first reported and it is the most frequent genetic cause of sporadic growth hormone (GH)-producing tumours. It can be found in up to 40% of cases. This modification of the alpha subunit of the G-protein leads to loss of the GTPase activity while retained adenylyl cyclase stimulation resulting in sustained cyclic adenosine monophosphate (cAMP) production and protein kinase A (PKA) activation. This results in an uncontrolled hormone production and cell proliferation [51]. Another common somatic mutation causing sporadic adrenocorticotrophic hormone (ACTH)-producing adenomas was found in gene encoding ubiquitin specific peptidase 8 (USP8) [52, 53]. It results in loss of deubiquitination of epidermal growth factor receptor (EGFR) and consequently increased EGFR recycling to the cell surface, which leads to proopiomelanocortin (POMC) gene transcription. The frequency of *USP8* mutation in corticotrophinomas ranges between 36% and 60% [52, 54, 55]. Several studies, including the recent comprehensive assessment, support the role of somatic *GNAS/USP8* mutation status in predicting treatment

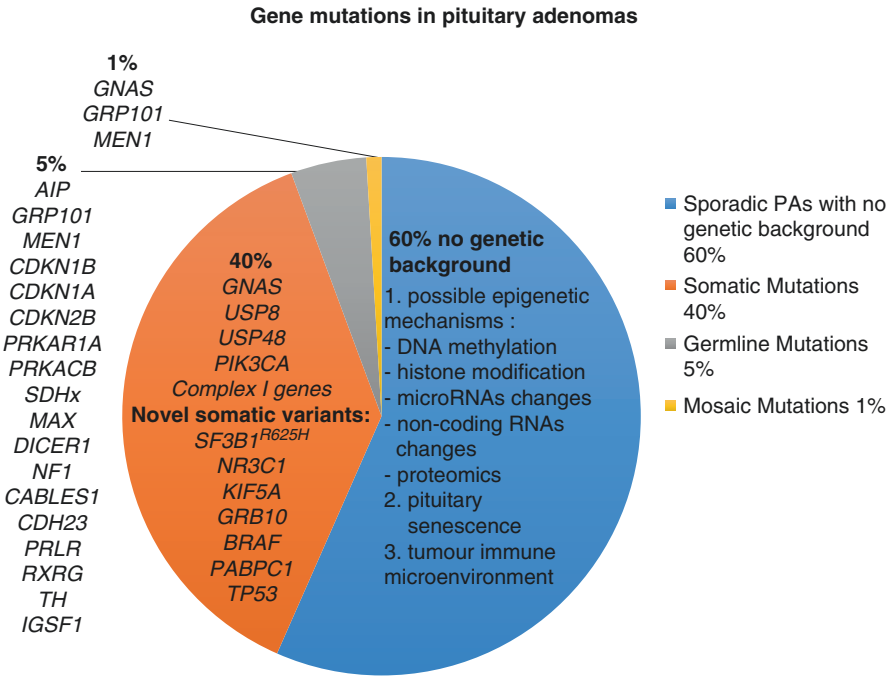

Fig. 4.1 Genetic origin of pituitary adenomas (adapted from Caimari et al. 2016 [64]) Data on PIK3CA and complex 1 genes needs to be confirmed in further publications

response [42]. In 30% of all pituitary adenomas single nucleotide or gene mutations in the catalytic subunit of PI3K have been presented. The clinical behaviour of tumours harbouring this mutation might be more aggressive [56]. However, in Cowden Syndrome with germline mutation of PI3K-PTEN-AKT, the increased prevalence of pituitary adenomas has not been observed [57]. Recent studies reported novel somatic variants [42, 58–60] which could provide new insight into pituitary tumorigenesis. Somatic hotspot mutation in splicing factor 3 subunit B1 (*SF3B1)* has been found in 20% of prolactinomas [61], in a recent study, although not reported in [42]. This recurrent variant results in aberrant splicing of ESRRG, a member of the oestrogen receptor-related receptor family, leading to increase PIT-1 activation and abnormal prolactin secretion and lactotroph proliferation. Other reported novel somatic mutations are *NR3C1, BRAF, USP48 PABPC1,* and *TP53* mutations found in corticotroph pituitary tumour, *GRB10* mutation identified in somatotroph adenomas or *KIF5A* mutation associated with lactotroph adenomas [58–60, 62, 63].

4.3.2 Germline Mutations

Germline mutations are defined as any changes in germ cells that are inherited from the parents or arisen in the fertilised egg, therefore every single body cell is affected. Their prevalence is evaluated to be around 5% of all PAs [64]. Germline mutations

are responsible for syndromic diseases such as MEN1, MEN4, Carney complex, *SDH*-related familial PAs, DICER1 syndrome, NF1, or due to apparently isolated pituitary adenomas due to *AIP* mutation or *GPR101* duplication.

4.3.3 Mosaic Mutations

Mosaicism means that the patient's body consists of at least two different genotypes; one without any gene mutations and other which is a result of genetic changes. If the germ cells (i.e. sperm or ova) harbour an alternative clone, then germline mosaicism can occur with the possibility of affected children.

In pituitary adenomas, somatic mosaicism occurs only in about 1% of patients [64]; *GNAS* mutation in McCune–Albright syndrome, *GPR101* duplication in XLAG and recently *MEN1* mosaicism has also been described [65]. The phenotype in these cases is dependent on the cell type and the number of affected tissues.

Germline mosaicism is relatively rarely diagnosed among endocrinological diseases, but has been described, for example, in MEN1 syndrome [66] and Carney complex [67].

4.3.4 Epigenetic Mechanisms

In a significant proportion of cases, genetic background of pituitary tumorigenesis is still not fully understood. Recent studies found association between epigenetic mechanisms and clinicopathological features of pituitary tumours. Epigenetic alterations may occur at chromatin level such as DNA methylation and histone modification or via non-coding RNAs, microRNAs or proteomics, and we refer to a recent comprehensive review on this topic [68]. Moreover, the tumour microenvironment, including fibroblasts and immune cells, may also influence pituitary tumorigenesis [69–72].

4.4 Clinical Presentation

4.4.1 Local Symptoms

The growing pituitary tumour causes pressure on surrounding tissue and its effects depend on the size of the lesion. Generally, this 'mass effect' is frequently associated with macroadenomas (>10 mm).

Headaches – occur in up to 37.5–72% of cases [73, 74], but their clinical presentation is heterogeneous. Headaches can be chronic, and often the sign which leads to investigations and diagnosis. Stretching of the meninges is one of the proposed mechanisms of headaches, but majority of studies found no association between tumour size and the presence of cephalgia [75, 76]. Pituitary tumours may invade cavernous sinuses where the trigeminal nerves are located. In this case, headache might be characterised by a constant, uni- or bilateral frontal or midface pain [76].

Indirect mechanisms are also suggested, probably due to dysfunction of the pituitary axis. Patients with somatotroph adenomas and prolactinomas are more prone to headaches than other tumour types [77]. Release of cytokines or neuroendocrine hormones may play a role in the initiation of headaches [73, 74, 78]. Headaches could also be acute severe headaches (with or without associated previous chronic headache). Acute, severe pain accompanied by photophobia or stiff neck may represent pituitary apoplexy, which in some cases could require emergency surgery. *AIP* mutation-positive patients are especially susceptible to pituitary apoplexy, even with a childhood onset [79].

Visual field disturbances – Pituitary adenomas are the most common cause of the chiasmal syndrome. Clinical symptoms are present more frequently when the optic pathway is displaced more than 3 mm from its original position. Based on a recent study, true bitemporal hemianopsia occurs much rarely than other visual field disturbances; the most frequent is bilateral temporal visual field defect, either pure or mixed (43% of patients). If the compression of chiasma is asymmetric, it is likely to manifest with asymmetric visual field defect [80, 81].

Compression on healthy pituitary tissue – leads to a progressive hormonal pituitary insufficiency. Usually first appearing hormone deficiency involves GH axis, followed by gonadotroph, thyrotroph and corticotroph axis. Thus the first visible symptoms are those of hypogonadism, due to gonadal insufficiency or hyperprolactinemia caused by 'stalk effect' [82]. Special attention must be paid by physicians to the possibility of pituitary-adrenal axis failure which can be a life-threatening condition.

4.4.2 Hormonal Excess Symptoms

Hormone-secreting tumours and those with a genetic background arise more often in childhood and adolescence. Depending on the age of onset, symptomatology may be different.

4.4.2.1 GH Excess

Growth hormone (GH) is a peptide which stimulates the IGF-1 production in all the peripheral tissues, while circulating IGF-1 is mainly arising from the liver. When the overproduction of GH starts before epiphyseal closure, increased growth velocity resulting in tall stature can be the most prominent manifestation. The development of abnormal tall stature could be further aggravated by hypogonadism and therefore delayed epiphysis closure. The hypogonadism can result from (a) damaged LH/FSH secretion due to the tumour's mass effect, (b) prolactin secretion from the tumour itself inhibiting gonadotrophin secretion via kisspeptin or (c) the tumour pressing on the talk resulting in reduced dopamine input from the hypothalamus and increased prolactin levels. In adulthood, soft tissue swelling influences patient's physical appearance and leads to changes in facial features, hand, feet, nose, prognathism, macroglossia, sleep apnoea, skin tags and teeth separation. Common metabolic complications of GH-axis overactivation are insulin resistance, hyperglycaemia, hypertension and increased triglyceride level. Other features observed in patients with

acromegaly are arthropathy, carpal tunnel syndrome, organomegaly, thyroid goitre, colorectal polyps, sweating and many others [16, 17, 83–94]. Apart from somatotropinomas, cause of GH excess can be GHRH-secreting pancreas or bronchial neuroendocrine tumours, altered GH regulation in NF1-associated optic pathway gliomas, pituitary hyperplasia without a tumour and sometimes normal-sized pituitary gland (for example, in Carney complex, McCune-Albright syndrome or XLAG) and the extremely rare ectopic GH secreting tumours. Additionally, deficiency of the X-link immunoglobulin superfamily member 1 (IGSF1), associated with central hypothyroidism, hypoprolactinaemia, GH deficiency in childhood and macroorchidism, may result in adulthood in somatotrope neurosecretory hyperfunction in humans [95]. Adult patients harbouring *IGSF1* loss of function mutation present acromegalic facial features as well as organ consequences following GH excess but not tall stature.

4.4.2.2 ACTH Excess

Under normal conditions, corticotrophin-releasing hormone (CRH), produced in the hypothalamus, increases corticotroph cell POMC transcription and ACTH synthesis, resulting in adrenal cortex cortisol synthesis. Autonomous ACTH secretion from a tumour results in excess cortisol levels and impaired or lost negative feedback. Long-term exposure to cortisol leads to characteristic clinical signs of Cushing's syndrome. ACTH excess presents differently in children and adolescents than in adults. Pathognomonic features are short stature with subnormal growth velocity and unexplained obesity [96]. In adults, central obesity with proximal myopathy and moon face are characteristic. In physical examination physicians should pay attention to skin lesions like facial plethora, violaceus striae (unusual in children <7-year-old), acanthosis nigricans due to insulin resistance and signs of hyperandrogenism, such as hirsutism or acne. Metabolic complications present as hypertension, diabetes mellitus or glucose intolerance and dyslipidaemia. Amenorrhea, menstrual dysfunction, hypogonadism, osteoporosis, susceptibility to infections and psychological complaints like depression, anxiety and irritability are also common [19, 97–103].

4.4.2.3 Hyperprolactinaemia

Elevated prolactin level among patients with pituitary adenoma might be due to two main reasons (if not due to a medication-induced hyperprolactinaemia): prolactin-secreting tumour or a presence of large lesion which can cause a 'stalk effect' [82]. Hyperprolactinaemia inhibits periodic gonadotropin-releasing hormone (GnRH) secretion via a pathway including kisspeptin. In children and adolescents, it can cause delayed puberty and growth arrest. Galactorrhoea may occur at any age in both genders, but most common in adult females. In females after menarche, the most common symptom of hyperprolactinaemia are menstrual irregularities. In males, decreased libido and erectile problems might be the first symptoms, and hypogonadism can result in gynaecomastia. Generally, symptoms are more easily recognised in women. Diagnosis of hyperprolactinaemia is challenging among elderly male or postmenopausal female patients as they are not aware of symptoms of hypogonadism [104]. Hyperprolactinaemia-induced long-term hypogonadism decreases bone density and can lead to osteoporosis. In some studies, patients with prolactinoma and concomitant hypogonadism have a higher risk of vertebral

fractures [105, 106]. They also might develop metabolic syndrome since hyperprolactinemia promotes weight gain and has adverse effect on glucose metabolism and lipid profile [107, 108].

4.4.2.4 TSH Excess

Elevated thyroid-stimulating hormone (TSH) levels due to pituitary adenoma are rare. Elevated thyroxine levels in the context of inappropriate normal or increased TSH levels are the key with independence of the presence of symptoms and signs of thyrotoxicosis. Indeed, in 90% of cases biochemical hyperthyroidism is present, but clinical symptoms are observed only in 67% of patients. In biochemically silent cases only tumour immunohistochemistry analysis helps to classify the type of tumour [109]. In 25–42% of patients co-secretion of other pituitary hormones is noted, often GH and/or prolactin [110, 111].

4.4.2.5 LH/FSH Excess

About 80–90% of gonadotroph adenoma are silent without any biochemical manifestation and symptoms appear due to the mass effect of the tumour [112]. In the minority of cases clinical complaints may manifest as precocious puberty in children, menstrual irregularities, enlarged ovaries, ovary hyperstimulation in women and enlarged testicles in male patients [113].

4.4.3 Clinical Features Suggesting Genetic Background

In all patients harbouring a pituitary adenoma, thorough family history, 4 generation family tree and detailed physical examination should be performed (Table 4.1). It helps with differential diagnosis and guides genetic testing.

4.4.4 Pituitary Adenomas Associated with Hereditary Diseases

4.4.4.1 Familial Isolated Pituitary Adenomas (FIPA)

Familial isolated pituitary adenoma is an inherited condition, defined as a presence of two or more cases of PA among family members without any other associated tumours [115]. The genetic background of FIPA families is heterogeneous. While the majority have no identifiable mutation, around 20% of cases harbour an *AIP* mutation, with rare cases and kindreds with *GPR101* duplications [116, 117].

AIP

AIP, located at chromosomal region 11q13.2, acts as a tumour suppressor gene in the pituitary. Being a co-chaperone, the AIP protein interacts with numerous other binding partners, including the heat shock protein family, nuclear or growth factor receptors and viral proteins. As a consequence, it influences a wide spectrum of molecular pathways [118, 119]. Patients usually inherit a loss-of-function mutations (majority resulting in truncated proteins while missense variants have shortened half-life [120]) and the second copy is lost in the tumour tissue as seen with loss of

heterozygosity (LOH) studies [121, 122]. Up to now, all cases had germline mutation (most inherited with one published de novo case [83]) with over 100 different gene variants described. The phenotype presents as a spectrum between young-onset aggressive disease and mild cases not needing treatment [79, 83, 115, 123, 124].

Around half of *AIP* mutation-positive families are homogenous with the vast majority having somatotrophinomas or somatolactotrophinomas [28, 124–126]. Among the heterogeneous family the most common combination is the co-existence

Table 4.1 The most common extra pituitary manifestations of hereditary systemic disorders associated with abnormal pituitary hormones

Organ	Manifestation	Gene and disease
Skin	angiofibromas (85%), collagenomas (70%), lipomas (30%)	MEN1
	mucosal fibromas (95-98%)	MEN2B (95-98%)
	lentiginosis (70%) cutaneous myxomas (50%)	Carney complex
	cafe au-lait spots	McCune-Albright syndrome, NF1*
	skin neurofibromas, crow syndrome	NF1*
Thyroid	goiter, thyroid tumour	DICER1, McCune-Albright syndrome
	medullary carcinoma	MEN2A (90%) MEN2B (90%), MEN4, NF1*
	thyroid tumours (75% benign, 25% malignant)	Carney complex
Parathyroid	Parathyroid adenoma	MEN1 (95%), MEN2 (up to 30%), MEN4, Carney complex, CDC73**
Chest abnormalities	cardiac myxomas	Carney complex (up to 40%)
	pleuropulmonary blastoma	DICER1 (70%)
Pancreatic tumour	neuroendocrine tumour	MEN1 (30-70%), MEN4, VHL, McCune-Albright
Renal manifestations	cystic nephroma	DICER1
	renal tumour	SDHx
Adrenal gland manifestations	PPNAD (up to 60%)	Carney complex
	Adrenal Cushing's syndrome	McCune-Albright syndrome
	phaeochromocytoma/paraganglioma	SDHx, MAX, MEN2A (40-50%), MEN2B (50%),
	adrenocortical tumours	MEN1 (40%), MEN4
Gonadal abnormalities	precocious puberty	McCune-Albright syndrome
	gonadal tumours (mostly calcifying Sertoli cell tumours)	Carney complex (up to 41%)
	Sertoli-Leydig cell tumour	DICER1
Bone manifestations	fibrous dysplasia	McCune-Albright syndrome
Central nervous system	Schwannomas	Carney complex (10%), NF1*
Eye abnormalities	optic pathway glioma, Lish nodules	NF1*
	visual loss due to facial fibrous dysplasia	McCune-Albright syndrome

of acromegaly with prolactin-producing tumour (37% of patients) [79, 115, 121, 124]. Usually patients are diagnosed with macroadenoma and age at disease onset is before 30 years. Penetrance is around 20–23% [79, 124]. Genetic screening of *AIP* mutation should start at not later than 4 years of age.

GPR101 Duplication

The *GPR101* gene microduplication on chromosome Xq26.3 was recently found as an infant cause of gigantism, named X-linked acrogigantism (XLAG) [17, 117]. It is a very rare condition and less than 40 patients have been described so far [127]. Mostly *GRP101* mutation occurs sporadically but a few familial cases have also been found. Germline mutation was described only among female patients whereas somatic mutation has been identified in four male patients. In three families, mother-to-son transmission with full penetrance [128] has been observed representing familial form of the disease. XLAG is characterised by excessive GH production before the age of 5 years, some acromegalic features (facial coarsening, prominent mandible, enlarged extremities) and increased appetite. Pituitary tumours are mostly macroadenomas with mixed GH and prolactin secretion. In about 25% of cases pituitary hyperplasia is observed. Preimplantation genetic diagnostic and prenatal *GPR101* testing for a pregnant patient with XLAG should be offered due to 50% chance of transmitting the mutation.

Other

The phenotypes of *AIP* and *GPR101* mutation-negative families are different. They have more varied tumour types, and the age of onset is similar to sporadic pituitary adenomas in the 4-fifth decade. Homogenous families represent about half of the kindreds with somatotrophinomas and prolactinoma families being the most common, while in the heterogeneous families acromegaly-prolactinoma and prolactinoma-NFPA combinations are the most common [79, 124]. Other genes with germline variants have been suggested as causing pituitary adenoma (*CDH23* [129] and *CABLES1* [130]), but more data are needed to confirm these results.

4.4.4.2 Multiple Endocrine Neoplasia Type 1 (MEN1)

Multiple endocrine neoplasia type 1 is a rare genetic disorder with an autosomal dominant inheritance. In most cases it is caused by an inactivating mutation in the *MEN1* gene, located on the 11q13 chromosome. This gene plays an important role as a tumour suppressor, and it encodes the protein menin, which is responsible for regulation of transcription, cell apoptosis and epigenetic changes [131–133]. Most of the *MEN1* mutations appear in a familial setting, but in 10% of cases are de novo and a few mosaic mutations have been observed. Somatic *MEN1* mutations are common in sporadic parathyroid tumours (1–18%) and pancreatic-gastrointestinal tumours (16–38%), but not in pituitary adenomas [134, 135]. The disease has a high (up to 95%) overall penetrance, increasing with age. In 17% of cases, MEN1-associated tumours manifest before the age of 21. Overall the prevalence of MEN1 is estimated to be 1:30,000 [131]. Characteristic main tumours are parathyroid adenoma, pancreatic neuroendocrine tumour, and pituitary adenoma. Some cutaneous

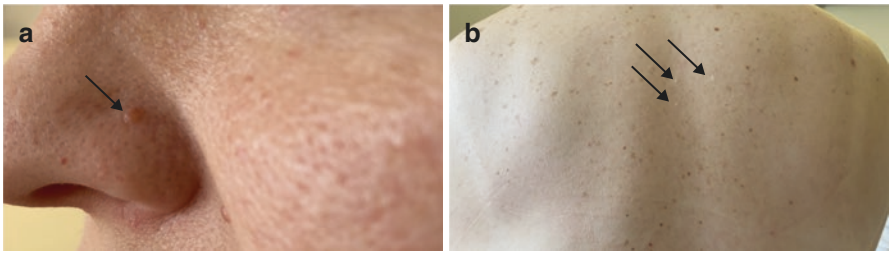

Fig. 4.2 Clinical presentation of skin changes in an MEN1 patient: (**a**) angiofibromas (**b**) lentigines and collagenomas. Courtesy of Prof. Alicja Hubalewska-Dydejczyk, Department of Endocrinology, Medical College, Jagiellonian University, Cracow, Poland

tumours, e.g. angiofibromas, collagenomas and café au lait macules have been associated with MEN1 (Fig. 4.2). The diagnosis of MEN1 is based on clinical, familial and genetic criteria: (1) a presence of two or more characteristic MEN1 tumours, (2) occurrence of one MEN1-associated tumour and first-degree family member with a clinical manifestation of MEN1, (3) asymptomatic patient with a germline *MEN1* mutation, who has not yet developed clinical, radiological or biochemical abnormalities [135].

Pituitary adenomas occur in 30% (range 15–50%) of patients with MEN1 syndrome, predominantly in the fourth decade of life, but various ages of onset have been noted in the literature (from 5 years to 90 years) [135–137]. The most common subtype is prolactinoma (60%), followed by NFPA (15%), somatotrophinoma (10%), coricotrophinomas (5%) and thyrotrophinomas (1%) [135, 138, 139], although recent studies have shown increased numbers of NFPA among asymptomatic patients as a result of clinical screening [140]. Pituitary adenoma as a first MEN1 manifestation appears more frequently in childhood and adolescence (21%) than among adults (15–20% of cases) [137]. PAs in symptomatic MEN1 patients are noted to be invasive macroadenomas with a low response rate to medical treatment compared to sporadic cases. Interestingly, tumours found by screening were mostly stable non-functioning microadenomas [140]. Pituitary adenomas associated with MEN1 have more frequently plurihormonal profiles and are more often characterised by the presence of multiple pituitary tumours within the same gland than sporadic cases [131, 139, 141, 142]. Genetic screening test should search for sequence variation or large deletions [131, 143]. It is suggested to start genetic screening at the age of 5 years.

4.4.4.3 Multiple Endocrine Neoplasia Type 4 (MEN 4)

Multiple endocrine neoplasia type 4 (MEN4) is a systemic disease, first described in 2006 [144], caused by *CDKN1B* gene mutation, with a phenotype similar to MEN1 syndrome. The *CDKN1B* gene, located on chromosome 12q13, encodes p27, which regulates the cell cycle and acts as a tumour suppressor. Genetic testing should be performed in the absence of *MEN1* mutation. The penetrance has not been determined due to the small number of identified patients. To date, only 20 cases

and eight pituitary tumours among adults have been noted [145]. In rare MEN1-like patients without identifiable germline *MEN1* mutations, other *CDKI*s pathological variants (p15 [*CDKN2B*, 1%], p18 [*CDKN2C*, 0.5%], p21 [*CDKN1A*, 0.5%]) have been found [146]. Moreover, 3 cases with *CDC73* gene mutation [147–149] (responsible for hyperparathyroidism-jaw tumour syndrome) and 2 cases with *CASR* mutation [147, 149] (causing familial hypocalciuric hypercalcemia) with MEN1-like phenotype have been described.

4.4.4.4 Multiple Endocrine Neoplasia Type 2 (MEN2)

MEN2 is divided into two subtypes: MEN2A and MEN2B. It is caused by *RET* mutation located on chromosome 10q11.21. The prominent features are medullary thyroid carcinoma, phaeochromocytoma and hyperparathyroidism for MEN2A or medullary thyroid carcinoma, pheochromocytoma, marfanoid feature, mucosal fibromas for MEN2B. An activating mutation of the *RET* gene results in increased stimulation of several protein kinase pathways. Pituitary tumour in MEN2 syndrome occurs very rarely; to date, only four patients with *RET* mutation and PA have been described [150–153]. It is unclear if these represent coincidence or there is a casual relationship.

4.4.4.5 Carney Complex

Carney complex is a rare, heterogeneous, systemic disorder with an autosomal dominant inheritance. It was first described in 1985 by Professor Carney as a "complex of myxomas, spotty pigmentation, and endocrine overactivity" [154]. Around 70% of cases occur in a familial setting, and the genetic mutation is caused by a germline inactivating mutation mainly in the *PRKAR1A* gene, located on 17q22–24 locus [155, 156]. This gene encodes the regulatory subunit type 1α of protein kinase A. To date over 140 *PRKAR1A* mutations have been described, referred to as Carney complex 1. Another affected locus is noted on chromosome 2p16 (Carney complex 2 locus), but the gene responsible for this mutation has not been found. Recently, other PKA gene subunit including *PRKACB* on chromosome 1 [157] and *PRKACA* on chromosome 19 [158, 159] (associated with adrenal hyperplasia) have been described in patients with Carney complex.

Genotype-phenotype correlation has been observed in Carney complex patients. If the mutation involves exon deletions, then acromegaly, myxomas, lentigines and psammomatous melanotic schwannoma (PMS) develop more frequently [160]. Patients with large deletion develop Carney complex features earlier [156] and more severe [67]. The study with the largest number of Carney complex patients showed female predominance (63%) [161]. To confirm the Carney complex diagnosis, the occurrence of two major criteria is required or one main criterion concomitant with a known inactivating *PRKAR1A* mutation or affected first-degree relative [162]. Symptoms may manifest at every age, but the peak of Carney complex diagnosis is 20 years old [163]. Skin lesions can occur at birth, then the most common tumours in childhood and adolescence are cardiac and cutaneous myxomas (Fig. 4.3). Pigmented nodular adrenocortical disease (PPNAD) is the most frequent endocrinological dysfunction among Carney complex patients and usually manifests in the

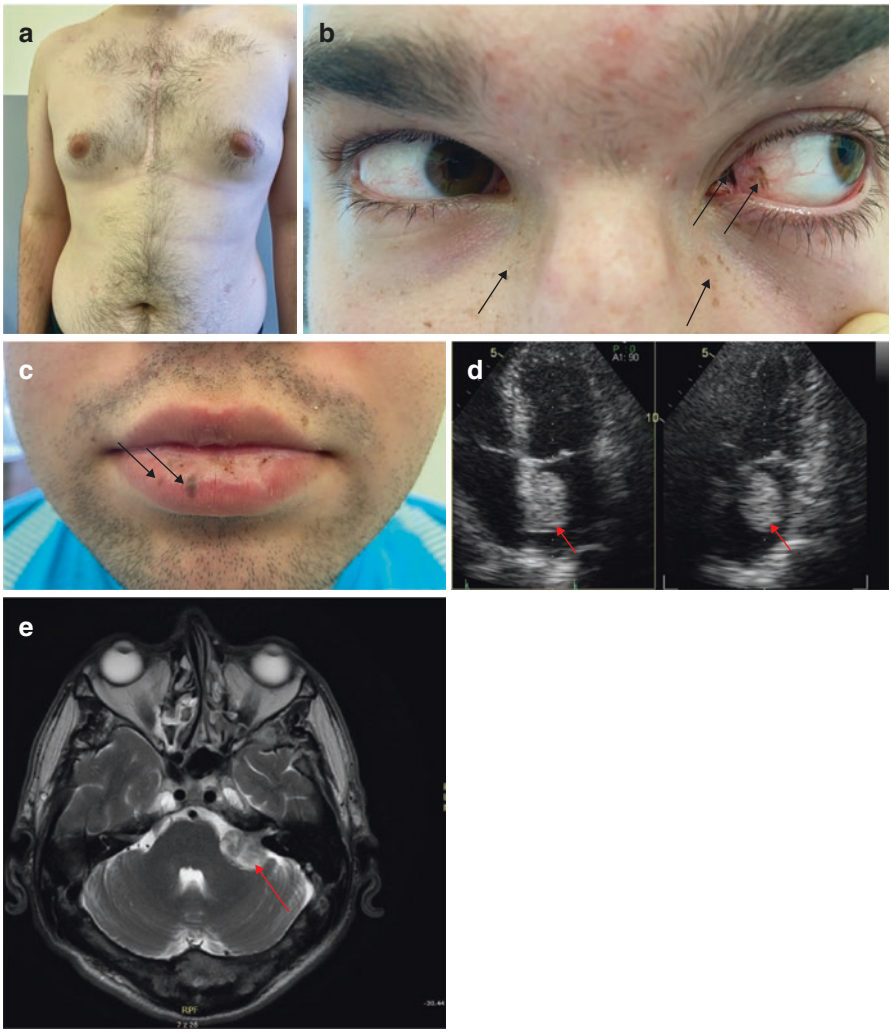

Fig. 4.3 (**a**–**c**) Clinical presentation of a patient with Carney complex with *PRKAR1A* mutation: (**a**) 20-year-old adult male patient with scarfs after cardiac myxoma surgery and bilateral adrenalectomy due to PPNAD; (**b**) lentigines on the vermilion of the lips; (**c**) pigmented lesions in the left eye and lentigines on the face. (**d**, **e**) Radiological features of Carney complex (**d**) Cardiac myxoma in the left atrium*; (**e**) Vestibular nerve schwannoma. Courtesy of Prof. Alicja Hubalewska-Dydejczyk, Department of Endocrinology, Medical College, Jagiellonian University, Cracow, Poland. *Courtesy of Prof. Agnieszka Olszanecka, Department of Cardiology, Medical College, Jagiellonian University, Cracow, Poland

second or third decade of life as ACTH-independent Cushing Syndrome. Pituitary adenomas develop in 12% of cases, but in 75% of patients asymptomatic elevation of GH and IGF-1 appears due to pituitary hyperplasia [161]. As far as pituitary manifestations are concerned, no predominance among sexes has been noted. PA

may manifest as a single lesion or multifocal tumours, usually in the third decade of life [163]. Somatotrophinoma or somatotroph and/or lactotroph hyperplasia are the most common pituitary manifestations; prolactinomas are rarely observed [164]. In recent studies, two patients with *PRKAR1A* mutation and corticotrophinoma have been noted [165, 166]. Additionally, an occurrence of pituitary apoplexy with presumed GH adenoma in a 14-year-old Carney complex patient has been described [167].

4.4.4.6 McCune-Albright Syndrome

McCune-Albright syndrome is characterised by the occurrence of three main classic features: polycystic fibrous dysplasia, precocious puberty and café au lait spots (Fig. 4.4). This rare disorder with prevalence between 1/100.000 and 1/1.000.000 [168] is caused by an activating mutation of the *GNAS* gene, located on chromosome 20q13. It encodes the guanine-nucleotide-binding protein α-subunit, and its mutation results in constant activation of adenylate cyclase. The most frequent *GNAS* mutations in McCune-Albright syndrome are R201H or R201C [169–171]. They appear first at an early postzygotic stage and leads to somatic mosaicism. The phenotype of patients depends on affected tissues [172–174]. Theoretically, if the *GNAS* mutation involves germline cells the disease could be inherited, but probably those cells would be non-viable, as to date no familial case has been described. The most common pituitary disease related to McCune-Albright syndrome is a somatotroph adenoma (in one-third of cases) or pituitary hyperplasia [175]. PCR sequencing of samples of affected tissue or peripheral blood lymphocytes should be performed [176].

4.4.4.7 The Three P Association (3 Pa)

SDHx

Association of pituitary adenomas with paraganglioma/pheochromocytoma was first described in 1952 by Iversen [177]. Relatively recently the phaeochromocytoma/paraganglioma gene has been linked to a predisposition to pituitary tumorigenesis. The co-existence of the above tumours, also known as 'the three P association (3 Pa)', is a rare condition, caused by germline mutation of the *SDHx* gene which consists of several subunits (*SDHA, -B, -C, -D or SDHA2F*) [178–180]. To date, <100 cases have been described worldwide. Based on the available data, a PA related to *SDHx* mutation could manifest with different phenotypes within the same family (somatotrophinoma, prolactinoma, or NFPA). The prevalence of PitNETs in SDHx is very low (<1%). They usually present as macroadenomas and require a multimodal approach. In a histopathological analysis, PA related to *SDHx* presents with an interesting phenotype with intracytoplasmic vacuoles which can correspond to the presence of autophagic bodies [179]. Pituitary adenomas can be the first or only manifestation of *SDHx* mutation [181]. Most recently, pituitary carcinoma has been noted in a patient with a medical history of paraganglioma and *SDHB* mutation [182]. In addition, the association of pituitary tumours and phaeochromocytoma/paraganglioma appears also in other hereditary syndromes caused by *MEN1*, *RET*, *VHL* and *MAX* mutations [179].

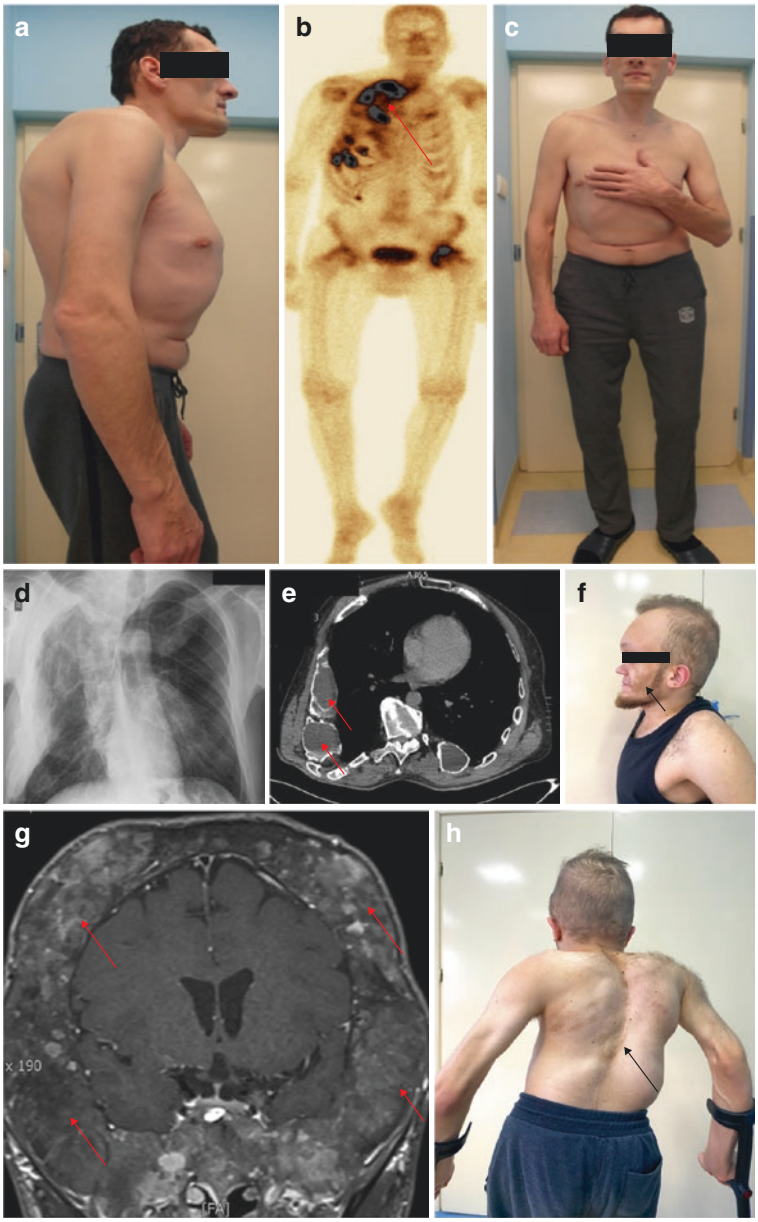

Fig. 4.4 (**a–e**) Clinical presentation of a patient with McCune-Albright syndrome with tall stature: (**a**) deformity of the thorax and acromegalic facial features; (**b**) extensive and asymmetric fibrous dysplasia in the right ribs in bone scintigraphy; (**c**) acromegalic features in McCune-Albright syndrome: tall stature (192 cm), hand enlargement; (**d**) skeletal deformity in chest radiography; (**e**) fibrous dysplasia in CT scan. (**f–h**) Clinical presentation of an adult patient with McCune-Albright syndrome with precocious puberty and GH excess: (**f**) cafe au lait spots on the face, (**g**) fibrous dysplasia involving skull base, (**h**) skeletal deformity, cafe au lait spots. Courtesy of Prof. Wojciech Zgliczyński and Dr. Maria Stelmachowska-Banaś, Department of Endocrinology, Bielański Hospital, Warsaw, Poland

MYC-Associated Factor (MAX)

MYC-associated factor X plays an important role as a tumour suppressor gene. This germline mutation was first found in a familial pheochromocytoma and paraganglioma with aggressive behaviour. The *MAX* gene, located on chromosome 14q23.3, interacts with other parts of MAX-MLX network, and together they are responsible for integration of cellular signals and modulate gene expression [183]. The combination of phaeochromocytoma/paraganglioma related to *MAX* mutation with pituitary adenoma is extremely rare—to date, only single cases with somatotrophinoma and prolactinoma have been described [184, 185]. Patients with germline *MAX* mutation may also develop other systemic manifestations like renal oncocytoma or lung cancer [186]. Most of the *MAX* mutations occur in specific helix-loop-helix-leucin-zipper domain (bHLHZ) and casein kinase II phosphorylation sites. Loss of function mutation or deletion of *MAX* is associated with tumorigenesis involving neuroendocrine cells and correlated with metastatic potential [186, 187]. Assessing gene copy number profiling may have an important role in determining clinical risk [188].

4.4.4.8 Neurofibromatosis 1 (NF1)

Neurofibromatosis type 1 is an autosomal dominant systemic disorder caused by inactivating mutation of neurofibromin, predisposing to benign and malignant tumours with a prevalence estimated around 1: 2500–1: 3500 live births. The most common characteristic features are cutaneous neurofibromas, cafe-au-lait skin lesions, intertriginous freckling [189, 190], Lisch nodules and brain tumours, including the most common optic pathway glioma (Fig. 4.5). Based on a study with 64 children with NF1 and optic glioma, 10% of patients with NF1 and optic glioma have clinical features of GH excess with elevated GH and IGF1 levels. They had no visible pituitary lesion [191]. The cause of GH excess in patients with optic gliomas could be loss of hypothalamic somatostatinergic inhibition or abnormal GHRH secretion from the tumour. As optic pathway gliomas do not stain positive for GHRH, the loss of somatostatin drive is a more likely explanation [192, 193]. Somatostatin analogues are an effective treatment. Recently a 68-year-old patient with somatotrophinoma, hyperparathyroidism, follicular thyroid carcinoma associated with *NF1* mutation and no *MEN1* mutation has been described [194]. The pituitary tumour tissue showed no loss of the wild-type allele of the *NF1* gene, but harboured a somatic *GNAS* R201C mutation. These data suggest that the rare case reports of PitNETs and NF1 could be coincidence of these relatively common diseases (PitNET 1:1000, NF1 1:2500–3500 of the general population) in the same patient.

4.4.4.9 DICER1 Syndrome

DICER1 syndrome is caused by a gene located on chromosome 14q32.13, which plays an important role in microRNA processing and DNA damaging. Dysregulation in the *DICER1* gene, which encodes ribonuclease, leads to tumorigenesis. Inheritance is of an autosomal dominant type with a variable penetrance. Almost every tumour develops a second, somatic *DICER1* mutation

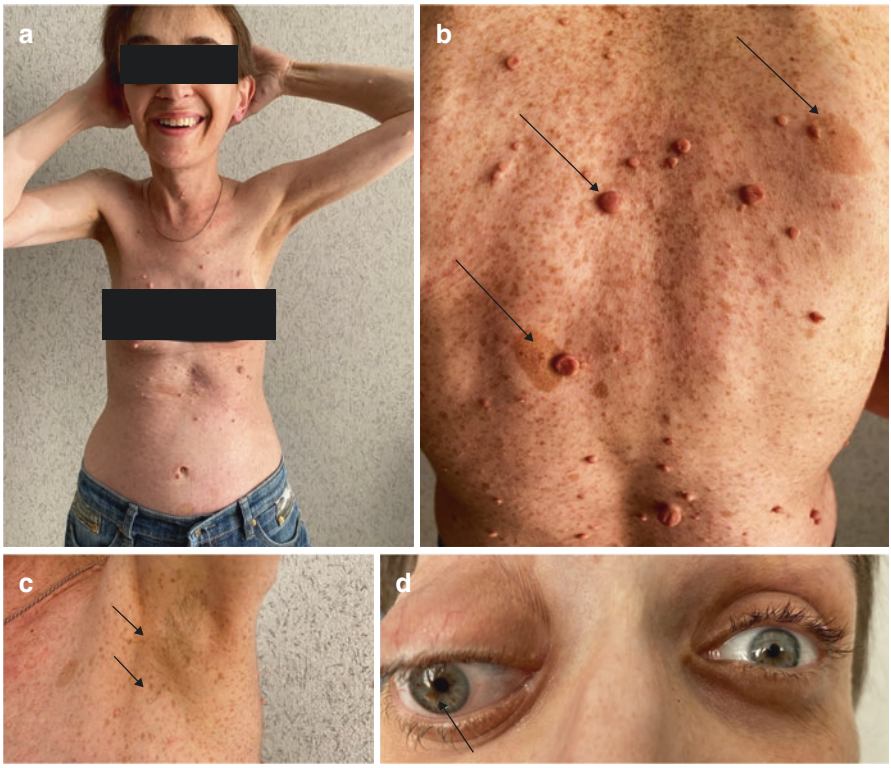

Fig. 4.5 Clinical presentation of a patient with neurofibromatosis type 1: (**a**, **b**) multiple neurofibromas and cafe au lait spots; (**c**) Crowe's sign; (**d**) Lisch nodules and right orbital tumour. Courtesy of Prof. Alicja Hubalewska-Dydejczyk, Department of Endocrinology, Medical College, Jagiellonian University, Cracow, Poland

in the RNAse IIIa or IIIb domain [195]. DICER1 syndrome presents a high variability of aggressive tumours, the most frequent being the very malignant pleuropulmonary blastoma (PPB), which occurs in over 50% of cases. Multiple endocrine manifestation involving the thyroid, ovaries and pituitary gland may also occur. In animal models, *DICER1* mutations lead to anterior pituitary dysmorphology and hypoplasia. Pituitary blastoma has been described in a few cases. These tumours manifest before the age of 2 years and in 70% of cases, Cushing's disease is observed, and the mortality rate is very high (40%) by the second year of life [196, 197].

Recently one case of a 50-year-old woman with confirmed *DICER1* mutation who developed a microprolactinoma has been reported [198]. It is controversial if *DICER1* mutation could lead to a prolactin-producing adenoma or if it is only a coincidence. Confirmation of *DICER1* mutation requires identification of heterozygous germline *DICER1* variant.

4.5 Patients with Pituitary Adenomas

Patient assessment should start with careful history and family history (Fig. 4.6). Family tree is drawn for three generations. A negative family history does not exclude germline mutation due to de novo mutations or low penetrance diseases. Detailed physical examination, biochemical and imaging assessment can follow (Table 4.2 presenting specific features by type of pituitary adenoma).

4.5.1 Somatotroph Adenoma

In 2017, the WHO defined somatotroph adenoma as a tumour that expresses mainly GH and develops from PIT-1 cell lineage. The most common subtype is a densely granulated variant, followed by sparsely granulated, mammosomatotroph, mixed somatotroph-lactotroph and acidophil stem cell adenoma [41]. Furthermore, pangenomic classification of pituitary tumours revealed gonadotroph markers (SF1) expression in a subset of somatotroph tumours (*GNAS* wild type) [42]. GH immunoreactive tumours without clinical features occur in about 2% of cases and are classified as silent somatotroph adenomas [199].

GH-producing adenoma is the most common cause of acromegaly and gigantism, with a prevalence of 70–240 cases per million, rising with age; the peak of diagnosis is estimated to be 45 years old [200–202]. In childhood and adolescence somatotroph adenoma constitutes only 2% of all types of pituitary adenomas, but, in up to 46–49% genetic mutations are responsible for its occurrence [16, 17]. Additionally, at a younger age its behaviour is more aggressive: over 70% are macroadenomas and 30% are invasive.

IGF-1 blood measurement is used as a screening test for acromegaly. Usually normal concentrations exclude acromegaly and gigantism with high probability, while its elevation constitutes a reason for further diagnostics. The gold standard for diagnosis is the oral glucose GH suppression test [203]. Recent study showed that the main determinants of GH nadir after glucose load are BMI, sex and oestrogen in oral contraceptives. Moreover, GH nadir concentration was significantly lower among healthy subjects than cut-off values used in current acromegaly guidelines [204]. During adolescence physiological insulin resistance should be taken into consideration when interpreting GH and IGF-1 results. Clinical features of GH excess are very typical, but a delay in diagnosis remains considerable, amounting on average 5 years (but also delays of 15 or even 25 years have been reported) [201]. Prolactin concentration can be elevated in 16–27% of patients, due to co-secretion by the tumour cells or compression of the pituitary stalk [205, 206]. First-line treatment is surgical resection of the pituitary tumour. In patients with persistent disease following operation, adjuvant therapy is indicated and includes first-generation somatostatin analogues (SSA), pasireotide, pegvisomant, dopamine agonists and even reoperation or radiation [203].

XLAG (age of onset < 5 years) – In infant-onset somatotrophinoma, an X-linked acrogigantism diagnosis should be considered. This recently described disease is

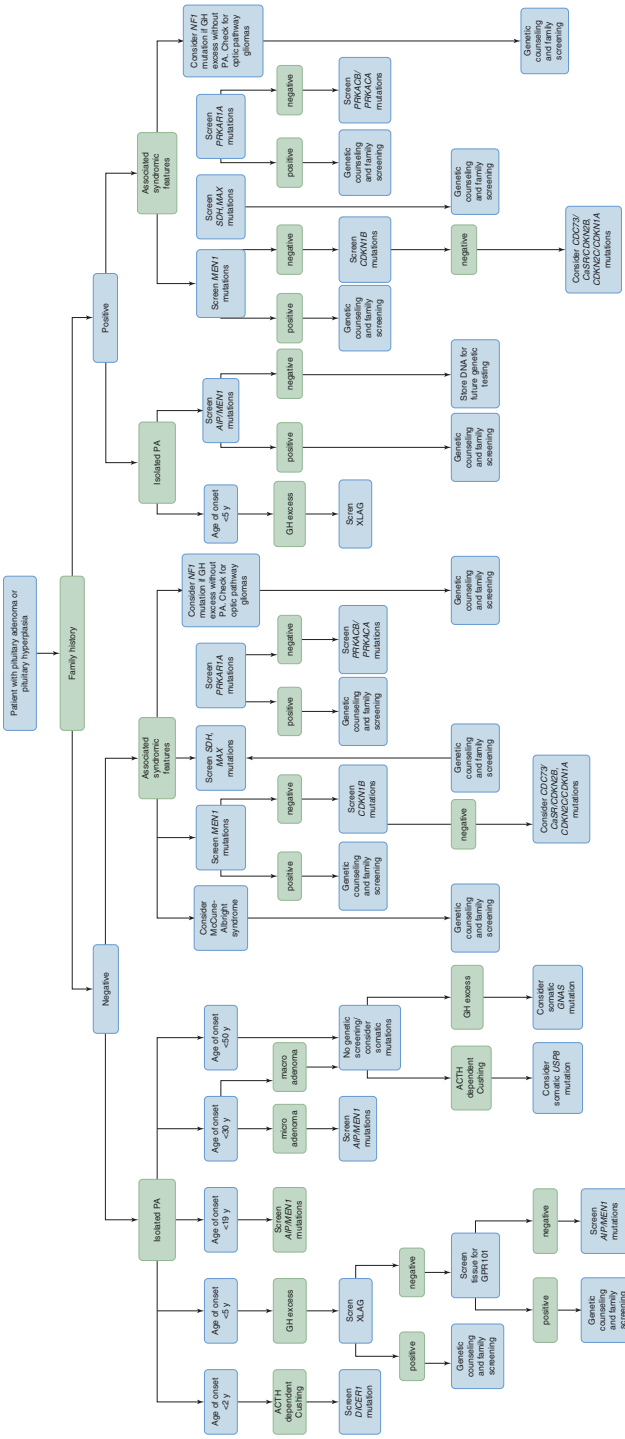

Fig. 4.6 Suggested algorithm of genetic counselling and screening depending on the family history, pituitary tumour type and age of onset of isolated adenoma

Table 4.2 Description of specific features, mean age of diagnosis and genetic alteration in syndromes by type of pituitary adenoma

Type of PA	Syndrome	Genetic alteration	Mean age of diagnosis	Specific features
Somatotroph adenoma	X-linked acrogigantism, FIPA Patients	GPR101 duplication	first years of life (<5 years)	female predominance, pituitary hyperplasia or tumour, males can be mosaic or familial
	AIP mutation, FIPA Patients	AIP mutation	2nd decade of life (<30 years)	male predominance, reduced SSTR 2 expression
	McCuneAlbright syndrome	mosaic GNAS mutation	2nd decade of life	male predominance, pituitary hyperplasia, prolactincosecretion
	Carney complex	PRKAR1A, PRKACB	3rd decade of life	no gender predominance hyperplasia (majority) or tumour
	Sporadic somatotrophinomas (GNAS mutation)	somatic GNAS mutation	3rd decade of life	smaller size, good response to medical treatment with SSA
	3Pa	SDHx mutation	single cases	macroadenomas
	MEN1	MEN1 mutation	4th decade of life	2nd most common MEN-1 related pituitary tumour, female predominance
	MEN4	CDKN1B mutation	single cases	single cases
Corticotroph adenoma	DICER1 syndrome	DICER1 gene mutation	<2-year-old	pituitary blastoma, slight female predominance
	FIPA Patients	AIP mutation	2nd decade of life (<30 years)	male predominance, no AIP positive case
	Carney complex	PRKAR1A mutation or PRKACB duplication	single cases	single cases, differential diagnosis of ACTH-independent PPNAD
	Sporadic corticotrophinomas USP8, USP48, BRAF mutation	somatic USP8, USP48, BRAF mutation	3rd decade of life (10 years earlier)	female predominance, smaller size, greater SSTR5 expression
	MEN1	MEN1 mutation	4th decade of life	female predominance, differential diagnosis of ACTH-independent CS
	Sporadic corticotrophinomas CABLES1	CABLES1 mutation	single cases	Simplex cases (4 female patients with macro adenomas)
	MEN2	RET mutation	single cases	connection with RET gene is questionable
	MEN4	CDKN1B	single cases	single cases
Lactotroph adenoma	AIP mutation, FIPA Patients	AIP mutation	2nd decade of life (<30 years)	male predominance
	MEN1	MEN1 mutation	4th decade of life	Most common adenoma type, female predominance
	3Pa	MAX mutation, SDHx mutation	various age	aggressive behaviour
Thyrotroph adenoma	AIP mutation, FIPA Patients	AIP mutation	4th decade of life	mixed tumours, mostly GH+ TSH
	MEN1	MEN1 mutation	4th decade of life	single cases
	thyroid receptor beta	THRB mutation	single cases	single cases
NFPA	AIP mutation, FIPA Patients	AIP mutation	2nd decade of life (<30 years)	male predominance
	MEN1	MEN1 mutation	4th decade of life	female predominance, microadenomas during clinical screening

caused by duplication of the *GRP101* gene located at X26.3 and leads to overexpression of an orphan G protein-coupled receptor (GPCR) [207, 208]. This mutation can be germline or sporadic [17]. In all patients the most prominent feature is an accelerated growth velocity before 4 years of age, with the peak age of diagnosis around the first year. The XLAG prevalence is from 4.4% to 10% in gigantism cases [208], with a female predominance. These GH pituitary tumours are significantly more often macroadenomas with suprasellar extension and cavernous sinus invasion. The literature describes pituitary hyperplasia in around 25% cases. Hyperprolatinaemia can occur in up to 85% patients [17]. Genetic testing should be performed using standard comparative genomic hybridisation (CGH) array, but in negative cases with a suggestive phenotype, alternative methods such as CNV ddPCR for GPR101 or HD-aCGH should be considered. On suspicion of a mosaic XLAG mutation, analysis of affected tissue should be performed.

***AIP* mutation (age of onset usually < 30 years)** – *AIP* is a well-known gene involved mainly in early-onset acromegaly and gigantism. *AIP* mutation may occur in 40% of gigantism patients [50, 79, 125]. Typically, clinical manifestations are observed in the second decade of life. Usually patients harbour macroadenomas, and age of the disease onset is before 30 years [79, 125, 209]. Males are slightly more represented, which could be due to ascertainment bias as males have taller stature and later puberty than females. Characteristic for *AIP*-related PA is a more aggressive behaviour than sporadic PA, specifically a larger tumour size (90%), suprasellar extension (50%), local invasiveness, invariably sparsely granulated cytokeratin pattern and higher risk of childhood-onset pituitary apoplexy. These features are responsible for an increased recurrence rate. *AIP* cases are mostly resistant to the first generation of somatostatin analogues and possibly somewhat better response to pasireotide [210]. Somatostatine receptor (SSTR) type 2 expression is not reduced in our studies [211], which is also supported by an animal model data [212]. Double adenomas [49] or pituitary hyperplasia [50, 213] has also been described.

MEN1 syndrome – Somatotrophinoma related to *MEN1* mutation occurs in about 10% of cases. In the absence of GPR101 duplication and *AIP* mutation, genetic testing for MEN1 could be undertaken. MEN1-associated somatotroph adenomas are mostly macroadenomas with local invasiveness and a poorer medical treatment response [135–137]. In MEN1 cases we specifically need to be aware of GHRH-secreting pancreas lesions causing GH excess and in children gigantism.

Somatic *GNAS* mutation – One of the most common genetic causes for isolated somatotroph adenomas is a sporadic activating guanine nucleotide-binding protein (*GNAS*) mutation. It results in a constitutively activated cAMP pathway [214]. These tumours contribute to higher GH and IGF-1 levels, but they are characterised by a smaller size and good response to SSA treatment in comparison to patients with *AIP* and *GPR101* mutations [84]. Furthermore, *GNAS*-mutated pituitary tumours have shown higher expression of dopamine receptor 2 in comparison to *GNAS* wild-type PAs [42]. They arise in older age than *AIP* affected. No association was observed between *GNAS* mutation and granulation pattern in histopathology

results [215, 216]. In aggressive sporadic somatotroph tumours resistant to SSA, novel therapeutic approaches including possibly RET pathways have been investigated. The use of sorafenib has been suggested as a potential treatment option in these cases [217].

McCune-Albright syndrome – Acromegaly is present in up to 26% of cases with the mean age of onset in the second decade of life. GH excess in McCune-Albright syndrome is significantly more frequent in males (75%). Concomitant hyperprolactinemia is present in 71–92% of patients. Hyperplasia is the most common pituitary manifestation [175].

Carney complex – Acromegaly is the main pituitary manifestation of Carney complex, usually preceded by other characteristic features, and can occur in up to 12% of cases, mostly in the third decade of life. Asymptomatic elevation of GH and IGF-1 is observed in over 75% of cases [161]. Somatotroph adenomas related to Careny complex are often multifocal with no predominance among sexes.

MEN 4 syndrome – In the absence of *MEN1* mutation in patients presenting MEN1-like features with somatotrophinoma, genetic testing for *CDKN1B* could be considered (to date only four cases of somatotroph adenomas have been described).

SDHx – The prevalence of somatotroph adenoma in *SDHx* mutation is very rare. A careful family history of pheochromocytoma and paraganglioma should be performed.

NF1 – Acromegaly and gigantism have been observed in about 10% of children with neurofibromatosis type 1 and optic pathway glioma without visible pituitary lesion [191]. GH excess should be considered as a relative common endocrine manifestation and frequent auxological evaluation should be performed.

DNMT3A – Acromegaly has also been described in other rare genetic disorders such as Tatton-Brown Rahman syndrome, caused by *DNMT3A* mutation [218], but the link between this gene and somatotroph adenoma has not been found.

4.5.2 Corticotroph Adenoma

Corticotrophinoma is defined as a tumour that presents immunostaining for ACTH and/or shows expression of the transcription factor T-PIT.

ACTH-producing tumours are usually microadenomas and lead to chronic hypercortisolaemia [219]. In children and adolescents, corticotrophinoma is the most common cause of Cushing syndrome (75–80% in comparison to 49–71% in adults) [220, 221]. The overall prevalence of Cushing disease (CD) is estimated to be 0.7 to 2.4 per million per year [219]. 20% of corticotrophinomas appear silently without biochemical and clinical manifestations. They tend to be macroadenomas and do not present local invasion but have a greater propensity for apoplexy [222]. The first choice of treatment is resection of corticotropinomas. The second-line approach must be individualised to each patient; available options are repeated neurosurgery, radiotherapy, medical therapy and bilateral adrenalectomy [223].

FIPA – No patients have been identified with Cushing's disease with unequivocal pathogenic *AIP* variant. Among non-AIP FIPA families only 2.9% of patients have Cushing disease [115].

MEN1 – Corticotrophinoma appears in 5–10% of all pituitary tumours related to *MEN1* mutation. Rarely ACTH- producing adenomas can manifest as a first symptom of MEN1 [224]. In MEN1, due to adrenal Cushing's syndrome, differential diagnosis of hypercortisolaemia should be performed. In clinical management, calcium should be measured due to high penetrance of hyperparathyroidism among MEN1 patients.

DICER1 syndrome – Pituitary blastoma was first described in 2008 in a 13-month-old girl with ACTH-dependent Cushing disease and diabetes insipidus. Blastomas are primitive, malignant neoplasms with the possibility to metastasise, but for pituitary blastomas true metastasis has not been observed to date [195, 196]. The relationship between pituitary blastoma and *DICER1* gene mutation was first noted in 2011 in a child presenting a pituitary blastoma with a positive familial history of *DICER1* mutation. The histopathological appearance is typically characterised by rosettes, Rathke-like epithelial cells and gland-like cells with large secretory epithelium. Pituitary blastomas are a very rare manifestation of DICER1 syndrome with a low penetrance (<1% of cases), but represents a pathognomonic symptom [196]. Clinically, severe Cushing's disease with ophthalmoplegia in an infant strongly suggests pituitary blastoma. The mean age of PitB diagnosis is 8 months, with a range from 7 to 24 months. Almost half of the affected children die within months after diagnosis. Other systemic manifestations of DICER1 syndrome are pleuropulmonary blastoma, cystic nephroma, pineoblastoma, ovarian Sertoli–Leydig cell tumours and multinodular goitre [197, 225–227].

Germline *USP8* mutation. Recently a patient with heterozygous ubiquitin-specific protease 8 (*USP8*) germline mutation (c.2155 T > C, p. S719P) [228] has been described. Clinical presentation of the 16-year-old female with recurrent CD included developmental delay, dysmorphic features, ichthyosiform hyperkeratosis, chronic lung and kidney disease and cardiomyopathy. Further studies are required to investigate potential specific effects of USP8 in other organs. The CD with additional clinical symptoms might be a new genetic syndrome.

Somatic *USP8* mutation is often identified in sporadic corticotroph adenomas. Two research groups identified somatic *USP8* point mutation in a short segment of the *USP8* gene in corticotrophinomas [52, 229]. USP8 removes ubiquitin from internalised epidermal growth factor receptor (EGFR), therefore protecting it from degradation. As a result of this gain-of-function mutation, EGFR recycling to the cell surface and stimulation of POMC production is increased. Normally, 14-3-3 protein regulates USP8 activity via binding to certain domain of the USB8 protein resulting in less cleavage and reduced activity. Mutations in the 14-3-3 binding area lead to increased USB8 cleavage and activity. *USP8* mutations are specific to corticotrophinomas. Corticotrophinomas with *USP8* mutation are common (36–63%), more frequent in women, smaller, and patients usually manifest 10 years earlier than wild-type corticotrophinomas in which the mean age of the diagnosis is 34 years [229]. They seem to have a higher recurrence rate and urinary free cortisol

level at the diagnosis. The prevalence of paediatric ACTH-producing adenomas harbouring *USP8* mutation is 30%, but recurrence of the disease is lower [230]. Identification of *USP8* mutation might also have an influence on the choice of medical treatment. Affected cells show a higher expression of SSTR type 5 and O6-methylguanine-DNA methyltransferase (MGMT) level, which suggests pasireotide as a first choice therapy [42, 231]. Additionally, EGFR inhibitors have already been studied in 2011 as a novel potential treatment option for Cushing's disease, before identification of somatic *USP8* mutation in corticotrophinomas. Recently in wild-type *USP8* corticotrophinomas [62] genetic testing showed new mutations: *USP48* (21.6% of cases) and *BRAF* (18.8% of individuals) mutation. Similar to USP8, they stimulate cells to produce POMC and are unique for ACTH-secreting pituitary tumours [229].

The *CABLES 1* gene, located on chromosome 18q11.2, encodes a protein that regulates cell cycle progression and acts as a tumour suppressor [232]. It is expressed in the nucleus, prevents cells regulators such as DKN1A (P21), CDK5R1 (P35) and TP63 [130] from degradation, and interacts with TP53 and TP73 at the initiation of apoptosis. In 2016 somatic *CABLES1* mutation was found in a few patients (four from 146) with sporadic Cushing's disease, but not in familial cases. All cases were female with macroadenoma – three presented extrasellar extension, and in three cases a second surgery was required to achieve biochemical control [130, 232]. Moreover, histopathology testing showed high Ki67 index. Further studies are needed to assess the prevalence of *CABLES1* mutation among corticotrophinomas and characterise in detail their function in pituitary adenomas.

Carney complex – While the vast majority of patients with Carney complex develop adrenal Cushing's syndrome, now two cases have been described with pituitary Cushing's disease [165, 166]. The diagnosis can be challenging**.**

MEN2 syndrome – Corticotroph adenomas associated with MEN2 syndrome have been found in four cases (three patients with MEN2A and one paediatric patient with MEN2B) [150–153]. Whether these cases represent phenocopies or there is indeed a relationship between the RET gene and pituitary adenoma is unclear.

MEN4 syndrome – Corticotroph adenomas related to *CDKN1B* coding for p27 protein has been found in only two adult cases [145]. Recently the common *CDKN1B* rs2066827 polymorphism has been considered to play a role in corticotroph adenoma tumorigenesis [233], but these data need to be confirmed independently.

4.5.3 Lactotroph Adenoma

Lactotroph adenoma is a pituitary tumour, derived from PIT-1 lineage and secretes prolactin. Prolactin can be produced together with GH in mixed somatotroph-lactotroph or mammosomatotroph adenoma. Prolactinomas express positive immunostaining for the oestrogen receptor-α (ERα), which could be considered as a prognostic factor. Lower ERα expression is associated with higher tumour

proliferation and it is more frequently observed in male patients [234]. Historically, prolactinoma was reported the most common tumour type of pituitary adenoma but recently, due to better availability of brain imaging techniques, detection of non-functional pituitary tumours increased. NFPAs are currently considered as the most prevalent. In most cases, lactotroph adenomas manifest after the age of 12 years as a microadenomas with the prevalence 40:100000, and the peak of diagnosis is between 20 and 50 years old. Females are more frequently and earlier diagnosed than males, with a ratio 10:1 [235, 236]. This imbalance between sexes might be due to reported complaints like menstrual irregularities. In male patients, lactotroph adenomas demonstrate more aggressive behaviour like larger tumour size, higher prolactin level, rapid growth, invasion into surrounding tissue and resistance to treatment. This difference between sexes might be due to galanin expression, oestrogen, vasoactive intestinal peptide (VIP) receptors or recently described somatic *SF3B1*^{R625H} mutation [61]. The first line of treatment for prolactinomas is dopamine agonists. Surgery should be considered in cases unresponsive to pharmacological treatment. Genetic screening for *AIP* and *MEN1* mutation should be considered.

FIPA – Lactotroph adenomas and somatotroph-lactotroph adenomas are the second most common PAs related to *AIP* mutation among FIPA, after somatotrophinomas [79, 124, 237, 238]. Patients harbouring *AIP* mutation are younger, with male predominance, higher prolactin level, greater tumour size and extrasellar extension in comparison to prolactinoma cases without genetic background. Resistance to dopamine agonist therapy is often reported. Feature and multiple neurosurgeries are required to achieve disease control [125], but there are too little data to determine the treatment response of *AIP* positive prolactinomas [79, 115, 124]. Prospective diagnosed *AIP*-related lactotroph adenomas among FIPA were similar to sporadic cases. They were microprolactinomas with no suprasellar extension or local invasion [79, 124]. *AIP* negative families have less number of affected members (in over 80% only two pituitary cases in comparison to three or more in *AIP* related FIPA) and microadenomas as well as macroadenomas have been observed [239].

MEN1 syndrome – Prolactinomas are the most common clinically presenting pituitary tumours in MEN1 patients and represent 50–60% of all pituitary adenomas. Clinical screening of MEN1 families revealed a similar prevalence of NFPA, although many of these are small lesions with no need of intervention [140]. MEN1-related prolactinomas are usually macroadenomas in children, with screening suggested to be started at the age of 5 years. Genetic testing for *MEN1* and *AIP* mutation should be considered for young patients with prolactinoma at the same time.

3 Pa – Paragangliomas and phaeochromocytomas can be associated with pituitary adenomas in families with *SDH* or *MAX* mutations with a very low penetrance, or rarely with MEN1 syndrome [178, 179, 184, 185, 240]. Prolactinomas and somatotrophinomas are the most frequent pituitary tumour types, often presenting as macroadenomas. Whether pituitary imaging and biochemical testing should be part of the paraganglioma surveillance in *SDH* or *MAX* mutation-positive families is currently under investigation.

Somatic *SF3B1*R625H mutations – Using whole genome sequencing, recently a novel hotspot somatic mutation of splicing factor 3 subunit B1 (*SF3B1)* has been identified in 20% of prolactinomas [61]. The mutation is unique for prolactin-secreting pituitary tumours among PAs. Gender preference in affected prolactinomas has been observed with a male predominance of 24% v. 10.7% in females. The *SF3B1* variant impacts on aberrant splicing of one of the oestrogen-related receptor family members, oestrogen related receptor gamma. This promotes prolactin hypersecretion via a stronger affinity for PIT-1 and therefore greater transcriptional activation of prolactin. Additionally, this mutation affects cell proliferation and decreases apoptosis of lactotroph cells. Patients harbouring $SF3B1^{R625H}$ mutation presented higher level of prolactin and significantly poorer treatment response in comparison to the wild-type group, whereas age at disease onset, tumour size and tumour invasion were not significantly different [61].

Prolactin receptor (PRLR) mutation – Previously this gene mutation was described in familial hyperprolactinaemia [241, 242]. Moreover, in animal investigations, PRLR knock-out mice develop lactotroph adenoma [243]. Recent studies have suggested that PRLR gene may play a role in sporadic prolactinomas in humans as well [244]; however, in another study, in a group of patients with sporadic lactotrophinomas, PRLR mutation has not been found [245].

4.5.4 Thyrotrope Adenoma

Thyrotrophinomas are rare and represent only 0.5–3% of all pituitary adenomas [246]. The incidence is around 0.26 per million per year, but in recent years a progressive increase in the recognition of thyrotroph adenomas has been observed, probably due to better imaging methods and heightened awareness among physicians [247]. The mean age at diagnosis is 45 years; there is no difference in sex ratio. Occurrence among paediatric patients is limited only to single case reports. Immunohistochemistry shows strong expression of nuclear staining for the acidophilic lineage transcription factor PIT-1 and GATA-2. In up to 55% of cases they can co-secrete other hormones, mostly GH or prolactin. At the time of diagnosis, TSHomas are more often invasive macroadenomas [110, 246]. In the diagnostic process, thyroid hormone resistance, euthyroid hyperthyroxinaemia, or other drug therapy intake (i.a. amiodaron) should be considered. The genetic background of the majority of thyrotroph adenomas remains undiscovered. TSHoma related to *AIP* mutation has been described in one case [125], while a metastatic TSHoma has been described in a 19-year-old MEN1 patient [248].

Some studies suggest that the mutation of thyroid receptor beta (TRβ), which is responsible for thyroid hormone resistance, may cause predisposition to pituitary tumorigenesis; two cases with this mutation have been described in a patient harbouring TSHoma.

The first-line treatment for thyrotrophinomas is surgical resection. In patients with surgical failure, SSA have been found to be effective in normalising TSH secretion in 90% of cases [249].

4.5.5 Non-functioning Pituitary Adenoma/(Gonadotroph Adenoma and Null Cell Pituitary Adenoma)

Non-functioning pituitary adenomas (NFPAs) are lesions without clinically obvious excess secretion of a hormone. They represent up to 43% of all pituitary adenomas [21], with the manifestation usually in the fourth or fifth decade of life; among children, NFPAs are responsible for only 4–6% of pituitary tumours [19, 20]. Recently, some studies reported an increase of non-functioning pituitary adenoma as a result of better quality and better access to imagine techniques and are considered as the most prevalent [21].

The vast majority of NFPAs are gonadotroph adenomas with very few showing no expression of any hormones or their transcription factors, especially at the RNA level. Silent ACTH, GH, prolactin or TSH adenomas representing a significant minority of clinically non-functioning adenomas have also been described. Null cell adenomas are diagnosed if they do not stain for transcription factors for either of the linages (PIT-1, TPIT, SF1) nor for any of adenohypophyseal hormones [41, 109]. Surgery is the preferred first-line treatment, as most are diagnosed as macroadenomas. Cabergoline treatment has been shown to have stabilising effect in a subset of NFPAs [250].

AIP mutation – Among *AIP*-related pituitary adenomas, NFPA occur in about 10% of cases, but 60% of these are diagnosed as part of follow-up of *AIP* carriers,

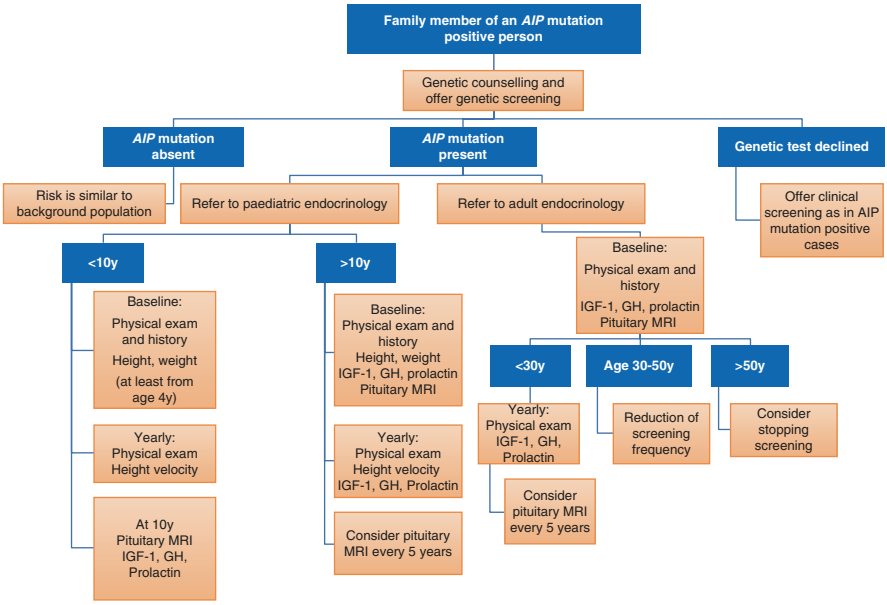

Fig. 4.7 Suggested follow-up of family members of an *AIP* mutation carrier (adapted from Williams et al. 2014 [209])

similar to MEN1 cases, in mutation carriers and it is difficult to distinguish them from incidentalomas identified in patients without *AIP* mutation.

MEN1 syndrome – NFPA are the third most common pituitary adenoma in MEN1 patients. Most of them are macroadenomas, although e-clinical screening has allowed to find many small lesions (42%) with an indolent clinical course [140].

4.6 Follow-Up and Prognosis

Patients with confirmed pituitary adenoma with genetic background should be treated and followed according to current international guidelines for their tumour type and systemic disorder. To enable early detection and therefore a better prognosis of pituitary adenomas, family cascade screening should be performed [79, 125, 135, 209]. The following flowcharts present a suggested follow-up of *AIP* (Fig. 4.7 adapted from Williams et al. 2014 [209]) and *MEN1* (Fig. 4.8 from Thakker et al. 2014 [131]) mutation carrier.

Most pituitary adenomas related to genetic mutation are associated with more aggressive behaviour, i.e. extrasellar invasion, local invasiveness, greater tumour size and higher risk of pituitary apoplexy. Histopathological features may show elevated staining for Ki67 and reduced expression of SSTR. They result in a lower response rate to medical treatment with SSA and often a multimodal approach is required to achieve disease control.

4.7 Further Considerations on Genetic Testing

Genetics is becoming an increasingly important aspect of patient care. Genetic testing for a monogenic disease may result, in general, in three possible scenarios: (1) A pathogenic variant is identified. In this case, the mutation explains the disease and the patient can have further testing, if necessary, to identify other manifestations of the disease and can be treated and follow-up according to available guidelines. Furthermore, cascade testing of the family members can be initiated. (2) The genetic testing can be negative, in this case other genetic causes could be searched for. (3) The third possibility, and this is often the case, a variant of uncertain significance is identified in a particular gene known to be associated with the disease. In this case the disease cannot be attributed to this gene, and family testing is not recommended; however, patients can be later notified if the status of the particular variant has changed based on other patients identified, emerging experimental or genetic data. The genetic landscape is further complicated by the incomplete and variable penetrance of some of the genes. Whereas *GPR101, PRKAR1, MEN1* and *NF1* mutations result in almost 100% penetrance, only 20–30% of *AIP*-carrier patients present clinical signs of the disease and pituitary specific penetrance is less than 100% in most of the genes, except *GPR101*, with very low penetrance in *DICER1* and *SDHx*. Identifying factors that play a role in gene penetrance would improve follow-up of asymptomatic patients and genetic counselling.

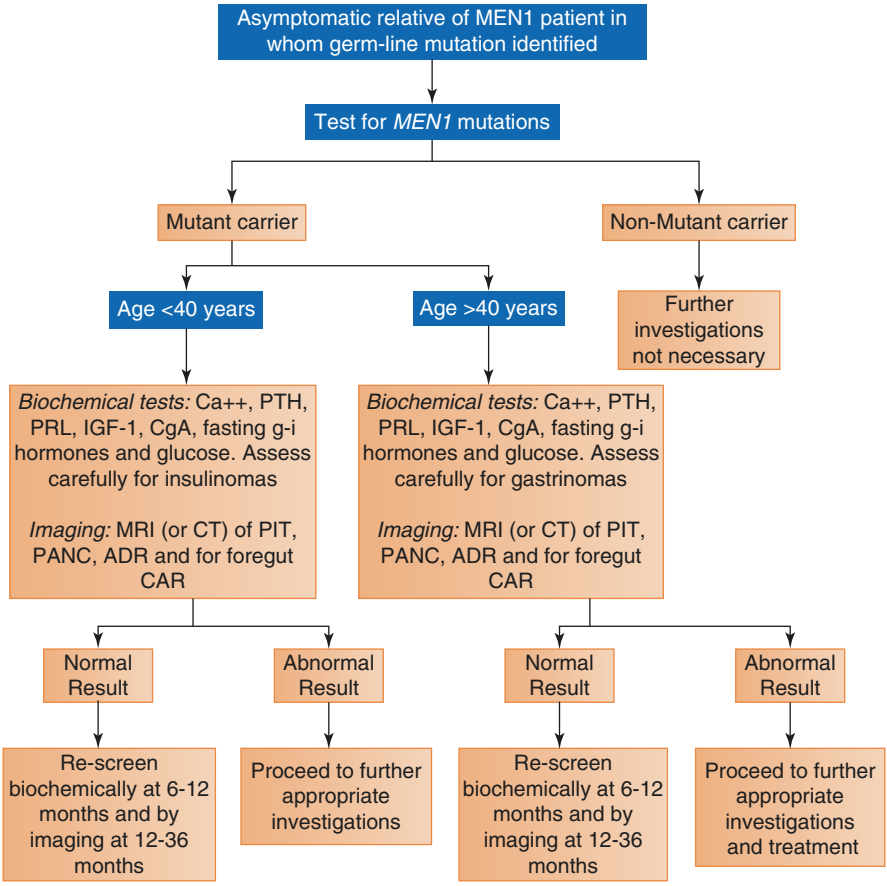

Fig. 4.8 Suggested follow-up of *MEN1* mutation carriers (from Thakker et al. 2014 [131]). *PIT* pituitary, *PANC* pancreas, *ADR* adrenal, *CAR* carcinoid, *PTH* parathyroid hormone, *PRL* prolactin, *IGF-1* insulin-growth-factor-1, *CgA* chromogranin A, and g–i, and gastro-intestinal gut-hormones

Another challenging aspect is false-negative genetic results. In case of strong clinical suspicion of genetic background without a genetic mutation confirmed in single analysis, physicians should use alternative molecular techniques, e.g. MLPA analysis should be performed in case of negative Sanger sequencing in tumour suppressor genes. Intronic mutations causing crypti splice sites and pseudoexons can be identified with cDNA sequencing. Some of these problems can be overcome with next-generation sequencing techniques. Moreover, due to somatic mosaicism, tissue testing could provide answer, such as in *GNAS* in McCune-Albright syndrome, *GRP101* in XLAG [251], and more recently in *MEN1* [66]. When inherited disease with complete penetrance occurs in more than one offspring, germline mosaicism of asymptomatic parents could be considered. Additionally, negative result for the most common genetic causes of a disease could initiate search for an alternative cause, such as cell cycle proteins in *MEN*-negative MEN1 syndrome patients.

4.8 Highlighted Definitions

XLAG – In infant-onset of somatotrophinoma, X-linked acrogigantism diagnosis should be considered. This recently described disease is caused by X26.3 microduplication located at the locus of the *GRP101* gene and leads to overexpression of an orphan G protein-coupled receptor (GPCR) in pituitary tissue.

FIPA – A familial/genetic condition associated with a pituitary adenoma and no other features of a syndrome known to be associated with pituitary adenomas.

MEN1 – An autosomal dominant genetic disorder caused by inactivating mutation in *MEN1* gene. Characteristic tumours are parathyroid adenoma (with prevalence of 90%), pancreatic neuroendocrine tumour, and pituitary adenoma, with other organs often affected such as skin fibromas and adrenal tumours.

McCune-Albright syndrome – A systemic disorder characterised by the occurrence of three classic features: polycystic fibrous dysplasia, precocious puberty, and café au lait spots, caused by an activating mutation of *GNAS* gene at an early postzygotic stage and leading to somatic mosaicism.

3 Pa – Association of pituitary adenoma with paraganglioma/pheochromocytoma ("the three P association") is low penetrance condition, caused by germline mutation of the *SDHx, MAX, MEN1*, and has been also described in cases with *RET* and *VHL* gene.

DICER1 – A systemic disorder associated with a very early onset of ACTH-dependent Cushing's disease and pleuropulmonary blastoma, cystic nephroma, pineoblastoma, ovarian Sertoli–Leydig cell tumours and multinodular goitre. Pituitary blastoma is a very rare manifestation of DICER1 syndrome with a low penetrance (<1% of cases) but represents a pathognomonic symptom.

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Acromegaly

5

Luigi Maione and Philippe Chanson

5.1 Introduction

Acromegaly is a typical rare multisystemic disease associated with progressive enlargement of some parts of the body, featuring somatic disfigurement mainly involving the face and extremities. The archetypal body changes and gigantism are renowned features (Figs. 5.1 and 5.2). Apart from body changes and disproportion, a number of multiorgan comorbidities are frequently associated with acromegaly. Most clinical repercussions originate from growth hormone (GH) and insulin-like growth factor-1 (IGF-I)-dependent organ overgrowth. In most cases, GH excess is due to a pituitary adenoma. Acromegaly itself and the related comorbidities might lead to premature death if not adequately treated.

5.2 Epidemiology

The prevalence of acromegaly has recently been estimated to be approximately 28 to 137 cases per million inhabitants [3, 4], challenging the historical figure of 40 to 70 cases per million [5]. A prevalence of approximately 1000 per million inhabitants was found in a German study based on screening with IGF-I measurement in

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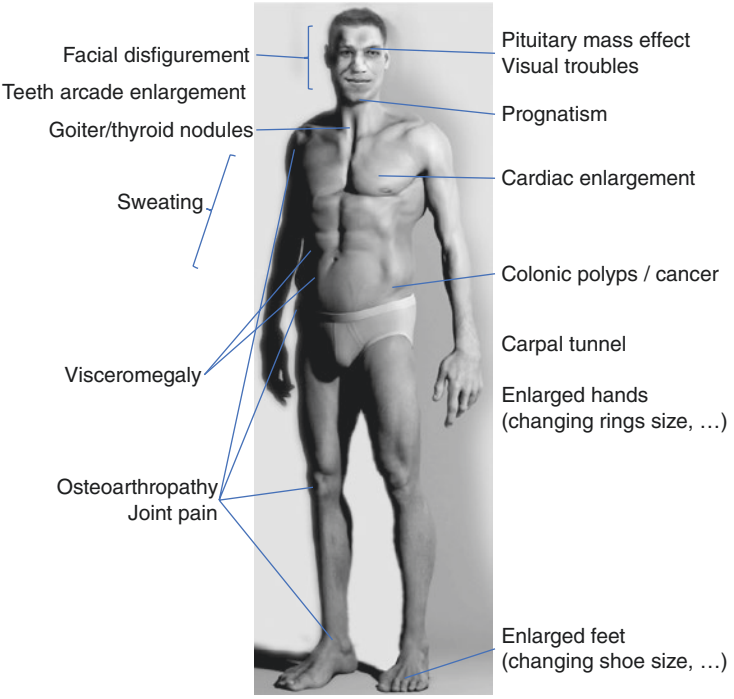

Fig. 5.1 Schematic representation of signs and symptoms of acromegaly. Adapted from [1], with permission

Fig. 5.2 Disease evolution in a woman with acromegaly. Series of photographs taken over time showing the progressive changes in facial appearance. It is possible to presume that the first signs appeared between 1988 and 1990, 22 years before the diagnosis. In 1990, the patient had to enlarge her ring she bought 2 years before because it was too narrow (adapted from [2], with permission)

the general population [6]. Raappana estimated the annual incidence of acromegaly at 3.4 cases per million in Finland [7]. A recent survey analyzing all the studies providing epidemiological data refined the incidence to a range between 0.2 and 1.1 cases/100,000 [8].

Age at diagnosis typically falls within the fourth decade of life, following a quasi-Gaussian curve [9, 10]. However, although more rarely, acromegaly might be found in children and in the elderly.

The sex ratio has been found to be more or less constant across studies. A female to male ratio of 1.26 has been calculated by analyzing data concerning more than 16,000 patients across national registries [11]. Age at diagnosis is typically earlier in males than in females, and a clearly distinguishable sex-related dimorphic Gaussian curve is observed [9, 12, 13].

Owing to the insidious clinical onset and slow progression, acromegaly is often diagnosed late. Older series, in the 1980s, suggested a mean diagnostic latency of 3–10 years after onset, at an average age of approximately 40 years [8, 14–17].

Studies focusing on disease latency seem to show that diagnostic delay appears to be more or less constant throughout decades, without any improvement over time with an earlier diagnosis [9, 18].

5.3 Pathogenesis

In more than 95% of cases, acromegaly is secondary to GH hypersecretion by a benign monoclonal pituitary adenoma that develops from somatotroph cells [19–22]. Pituitary somatotroph adenomas are mostly isolated (sporadic). Rarely, they may develop in the frame of a genetic predisposing disease (Fig. 5.3a).

5.3.1 Somatotroph Pituitary Adenoma

Pure somatotroph pituitary adenomas (60%) are constituted by eosinophilic cells containing either densely or sparsely granulated (secretory granules) cell elements after immunolabeling [19]. In some adenomas, immunostaining reveals colocalization of free alpha-subunits [24]. Silent somatotroph adenomas do not determine clinical acromegaly. Diagnosis is almost exclusively made by tumor immunostaining. Nonetheless, some patients bearing these tumors may have supranormal circulating GH levels without overt clinical signs [25].

Most human somatotroph adenomas seem to be associated with the clonal expansion [26] of cells carrying a specific somatic mutation. However, as in other types of pituitary adenoma, it has proven difficult to isolate a single causative factor explaining pituitary tumorigenesis [27–29]. Mutations in stimulating G-protein [30, 295] have been identified in 35–55% of somatotroph adenomas, according to some series [30–32]. *gsp* mutations are able to inhibit GTPase activity and to lead to constitutive adenyl-cyclase activation [33]. Cell cycle disruption also seems to play an important role, as demonstrated in MEN1 or in patients with *CDKN1B* mutations (coding the

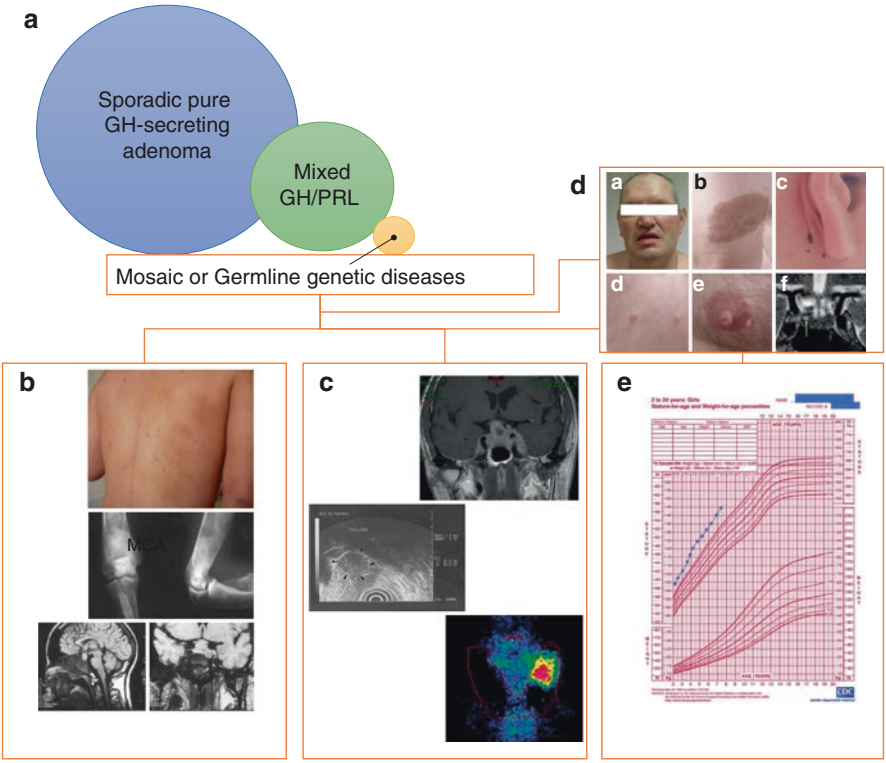

Fig. 5.3 Prevalence of somatotroph tumor forms and associated clinical genetic diseases. Panel **a**: Schematic representing the relative prevalence of sporadic pure or mixed somatotroph adenomas and associated mosaic/germline genetic diseases. Panel **b**: Characteristic clinical features of a patient with McCune Albright syndrome. Please note the typical café-au lait skin spots and X-rays and MRI hallmarks illustrating fibrous bone dysplasia. Panel **c**: Characteristic clinical features in a patient with acromegaly with multiple endocrine neoplasia type-1. Please note the pituitary tumor on a MRI T1W coronal post-gadolinium view, a pancreatic tumor revealed by echo-endoscopy, and parathyroid hyperplasia/adenoma found on ^{99m}Tc -^{123}I subtraction parathyroid scintigraphy. Panel **d**: Characteristics of a patient with Carney complex, from [23], with permission. Panel **e**: Growth curve of a patient affected by X-LAG acro-gigantism

cyclin-dependent kinase inhibitor p27KIP1, a key regulator of the cell cycle) [29]. A number of other genes have been implicated in somatotroph tumorigenesis [29]. The function of the disrupted proteins deriving from mutated genes spans from oncogene/tumor suppression to cyclin/cell proliferation inhibition. The general belief is that a preexisting mutation within somatotrophs should be a predisposing factor for further cell proliferation and GH secretion. Premature senescence likely explains the persistence of a benign phenotype and the rarity of progression to carcinoma. Epigenetic mechanisms may also contribute to cell proliferation by silencing genes such as *CDKN2A*, encoding p16, a cell proliferation inhibitor. The sequence of events leading to somatotroph cell clonal expansion seems to be multifactorial [34, 35].

Cytogenetic studies show that somatotroph pituitary adenomas display substantial intertumor and intratumor DNA copy-number heterogeneity. Intriguingly, somatic *GNAS*-mutated adenomas have low copy number variations, whereas a higher heterogeneity is observed in *GNAS*-intact tumors [36].

5.3.2 Mixed Somatotroph Adenoma

The most frequent mixed adenomas coexpress GH and prolactin (PRL), accounting for 25% of cases. Histopathologists often distinguish between true mixed adenomas, containing either somatotroph or lactotroph cell types, and lactosomatotroph stem cells consisting of more mature monomorphic cells coexpressing GH and PRL [19]. Mixed GH- and TSH-secreting adenomas are rarer and are associated with acromegaly associated with hyperthyroidism by inappropriate secretion of thyroid-stimulating hormone [37, 38]. ACTH cosecretion in somatotroph adenomas is extremely rare.

5.3.3 Genetic Syndromes Associated with Acromegaly

Various genetic syndromes and diseases include acromegaly in a wider spectrum of clinical features. Despite representing a rare cause, the burden of related morbidities requires not neglecting them (Fig. 5.3a–e). This paragraph summarizes the main features of these diseases along with some peculiarities in acromegaly manifestation. In general terms, a germinal genetic disorder should be suspected in patients with early-onset acromegaly bearing other organ involvement. These diseases have also been reviewed in recent articles and book chapters [20, 39–42].

McCune-Albright syndrome (MAS) is a rare genetic disease associated with multiple fibrous bone dysplastic lesions, precocious puberty, "café-au-lait" spots, and multiorgan and soft-tissue tumors (Fig. 5.3b). Pathological features are related to postzygotic somatic mutations leading to constitutive activation of the Gs protein alpha subunit [43–45]. It is of note that this gene is also responsible for most sporadic pure GH adenomas, underlying the importance of stimulating G-protein in the somatotroph environment. In the case of diffuse germinal mutations (MAS), acromegaly is found in approximately 20% of patients [43, 44, 46]. A peculiarity of this form is a relative resistance to somatostatin analogs (approximately 30% of responders). The therapeutic approach is also markedly influenced by the coexistence of skull base dysplasia, making any neurosurgical approach challenging [45].

Acromegaly can also be associated with hyperparathyroidism, neuroendocrine tumors (e.g., gastrinoma, insulinoma, or a nonfunctional pancreatic tumor), adrenal and other endocrine or nonendocrine tumors in the frame of **multiple endocrine neoplasia (MEN) type 1**, which is related to menin (*MEN1*) germline mutations (Fig. 5.3c) [47, 48]. Pituitary adenomas are not enriched in the somatotroph lineage in MEN1 patients. Few cases of GHRH-secreting neuroendocrine tumors have been reported in patients with MEN1 (see the "Extrapituitary acromegaly" paragraph) [49, 50].

Mutations in the *CDKN1B* gene are responsible for a rarer and newer MEN syndrome, **multiple endocrine neoplasia type 4** (MEN4, initially known as MEN-X), which combines hyperparathyroidism, pituitary adenomas (including acromegaly), and other endocrine or nonendocrine tumors [51, 52]. Twenty cases have been published to date. Hyperparathyroidism seems to be the most prevalent disease. Nonetheless, acromegaly by a somatotroph adenoma is found in up to 20% of patients harboring a deleterious *CDKN1B* mutation. Furthermore, contrary to MEN1, pituitary adenomas in MEN4 seem to be more enriched in somatotroph adenomas.

When acromegaly is associated with bilateral pigmented micronodular adrenal hyperplasia (causing ACTH-independent hypercortisolism) or with typical cutaneous pigmentations or cardiac myxomas, the patient should be screened for the **Carney complex** (Fig. 5.3D). This genetic disease is related to a germline mutation of the regulatory subunit of protein kinase A (*PRKAR1A*), whose signaling cascade is also located downstream of the stimulating G-protein [53, 54]. A simplified schematic of the cyclic AMP-dependent signaling pathway with relevant targets for somatotroph tumorigenesis is provided in Fig. 5.4.

Acromegaly is also one of the features described in **familial isolated pituitary adenoma**, partly related to *AIP* germline mutations (aryl hydrocarbon receptor interacting protein) [55–57]. These mutations can also, albeit rarely, be found in some apparently sporadic cases of acromegaly, particularly in young patients [58–62].

GPR101 was the latest gene to be discovered in association with a very early-onset form of **X-linked gigantism** or **X-LAG syndrome** [63]. Affected patients develop large or giant adenomas at a very young age. The pathophysiology is related to Xq26.3 microduplication involving the orphan G-protein-coupled receptor GPR101. However, germline GPR101 mutations are very rare in patients with sporadic pituitary adenomas, particularly in patients with gigantism or acromegaly [64]. Little is known about the pathophysiology and sequence of events leading to somatotroph tumorigenesis (Fig. 5.3E).

5.3.4 GH-Secreting Carcinomas

Somatotroph carcinomas are exceptional (fewer than 20 published cases). The presence of distant metastases is required to support the diagnosis of malignancy [39].

5.3.5 Extrapituitary Acromegaly

Extrapituitary acromegaly refers to growth hormone releasing hormone (GHRH) or GH hypersecretion other than from a pituitary adenoma [39].

GHRH hypersecretion could originate either from hypothalamic tumors, such as gangliocytomas, hamartomas, choristomas, and gliomas, or from the periphery. More often, GHRH hypersecretion comes from ectopic sources. The GHRH peptide

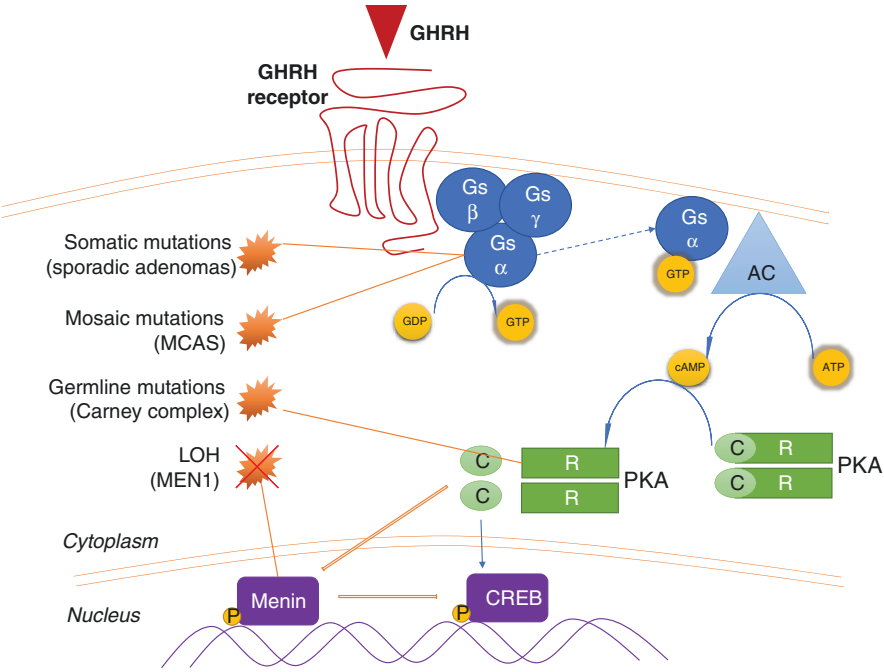

Fig. 5.4 The cyclic AMP-dependent signaling pathway in pituitary somatotroph cells as a model to understand different disease forms. GHRH induces a conformational change in the class II G protein-coupled receptor GHRHR. The Gs-α subunit exchanges GDP for GTP, which activates adenylyl cyclase (AC), converting ATP to cAMP. Elevated cAMP levels activate protein kinase A (PKA). PKA consists of a tetramer of two homo- or heterodimer regulatory subunits (R) and two catalytic subunits (C) responsible for the phosphorylation of several enzymes and transcription factors downstream [e.g., cAMP-response element-binding protein (CREB)]. *MCAS* McCune-Albright syndrome, *LOH* loss of heterozygosity, *MEN1* multiple endocrine neoplasia type-1, *α, β, γ* Gs-protein subunits, *AC* adenyl cyclase, *cAMP* cyclic AMP, *CREB* cyclic AMP response element-binding protein, a transcription factor, *PKA* protein kinase A, *R* the regulatory subunits of protein kinase A, *C* the catalytic subunit of protein kinase A

was indeed originally identified and cloned from large pancreatic tumors [65]. GHRH is expressed by several tissues. However, very large amounts are needed to induce pituitary somatotroph hyperplasia and clinical acromegaly. GHRH hypersecretion may derive from pancreatic cell tumors, small-cell lung cancers, bronchial and other-site carcinoids, adrenal adenomas, and pheochromocytomas. The delivery of high GHRH concentrations stimulates normal pituitary somatotrophs to become hyperplastic and to hypersecrete GH to produce acromegaly [50, 66, 67]. The diagnosis is established by measuring plasma GHRH and by identifying the source (a GHRH-staining neuroendocrine tumor) [68]. The prognosis largely depends on the characteristics of the underlying tumor [50].

GH can also be directly secreted by an ectopic somatotroph adenoma (located near the *sella turcica*, for example, in the sphenoidal sinus, petrous temporal bone,

nasopharyngeal cavity) or, in exceptional cases, by a peripheral tumor (pancreatic islet tumor or lymphoma) [69, 70].

5.4 Clinical Presentation

Acromegaly is generally suspected based on clinical signs and symptoms, which are important to recognize (Fig. 5.1) [2, 16, 17, 71–74].

5.4.1 The Dysmorphic Syndrome

In typical forms, patients present broadened extremities (hands and feet), widened, thickened and stubby fingers, and soft tissue thickening. When specifically asked, affected patients describe enlarged rings over the last years or the need to change shoe size. The facial aspect is somehow characteristic and includes a widened and thickened nose, prominent cheekbones, bulged forehead, thick lips, and marked facial lines (Figs. 5.1 and 5.2). The forehead and the overlying skin are thickened, sometimes leading to frontal bossing. The lower face is also affected, with several degrees of prognathism, maxillary widening, teeth separation, and, more rarely, jaw occlusion impairment. A useful step is to analyze comparatively ancient photographs. This could show a slow, insidious demarcation of the acrofacial syndrome spreading over several years (Fig. 5.2). Because of this slow progression, relatives and physicians may be unaware, and acromegaly may be diagnosed very late. Typically, after variable latency, the diagnosis is raised by a physician who has not seen the patient before [16, 17, 75]. A recent multicenter survey investigating medical practices of more than 3000 patients with acromegaly across several European countries reported that most diagnoses were reportedly made by an endocrinologist (45%), followed by general practitioner (17.5%), internist (13.2%), orthopedist (3.6%), neurologist (3.3%), ophthalmologist (2.3%), and the patient him or herself or one of their relatives (2.3%) [10]. It is therefore not uncommon that a patient could make the diagnosis him or herself by searching the Internet. In most cases, diagnosis is made owing to changes in the face or extremities; in some other cases, acromegaly is diagnosed not for its signs and symptoms but during the biochemical exploration of a pituitary adenoma.

5.4.2 Symptoms

Acromegaly can cause a broad variety of symptoms [16].

5.4.2.1 Skin Changes

Nearly 70% of patients have sweaty and oily skin. Skin thickening is due to glycosaminoglycan deposition and to increased collagen production by connective tissue. These changes may lead to hyperhidrosis and malodorous sweating. Facial

wrinkles, nasolabial folds, and heel pads are increased in thickness, and body hair may become coarsened. Skin tags are frequent and may be a marker of colonic polyps. Raynaud's disease is present in one-third of cases. In some cases, patients describe night-time malodorous sweating.

5.4.2.2 Bone Changes

In response to both GH and IGF-I, new periosteal bone formation leads to an increase in skeletal growth, especially at the level of the mandible (prognathism). Jaw thickening, tooth separation, frontal bossing, malocclusion, and nasal bone hypertrophy are the standard facial bony deformities in acromegaly.

Radiography shows a thickening of the cranial vault and protuberances, frontal internal hyperostosis, and condensation of the *sella turcica* walls with clinoid hypertrophy. Hypertrophy of the sinuses, especially the frontal sinuses, is also clearly visible. This, along with laryngeal hypertrophy, may explain why the voice tends to become deeper and acquires a sonorous resonance [76].

These changes are not only due to soft tissue hypertrophy and excessive growth of bone and cartilage but also to bone deformation. Indeed, radiographic findings are abnormal in half of these patients, showing distal tufting of the phalanges, widening of the base of the phalanges with osteophyte formation, enthesopathy (mineralization of ligamentous insertions), widening of the cortical bone diaphyses, and widening of joint spaces due to cartilage hypertrophy. Deformations can also affect the rest of the skeleton, and dorsal kyphosis with distortion of the rib cage may be observed in severe chronic forms, leading to the paradigmatic "punchinello" aspect, especially when GH hypersecretion begins prior to epiphysis closure.

Bony deformations also affect the spine, with upper dorsal kyphosis and compensatory lumbar hyperlordosis. Vertebral enlargement, widened intervertebral spaces, and osteophyte formation were also observed. The thorax is deformed by protuberance of the lower portion of the sternum and by elongation and divergence of the ribs due to overgrowth of the chondrocostal joints.

Imaging studies show diaphyseal cortical thickening of the long bones and widened joint spaces, sometimes with osteophytes.

Concerning mineral changes, bone remodeling is increased in acromegaly [77, 78]. Cortical bone thickens (as measured by the metacarpal index and histomorphometric parameters) and its porosity is diminished. Trabecular bone mass may be decreased, normal or increased. Measurement of spinal bone mass can give contradictory results, probably because acromegaly is often associated with other endocrine disorders interfering with bone mass. In general, bone mass is normal in the lumbar spine in patients with isolated acromegaly but could be decreased in patients having other related endocrinopathies impacting bone metabolism, such as hypogonadism or hyperparathyroidism. Despite similar bone mineral density values, a lower lumbar spine assessed by the trabecular bone score (TBS) technique was demonstrated in patients with acromegaly compared to controls, especially in hypogonadal patients and women [79]. In a study [80] independent of BMD, the prevalence of vertebral fractures was found to be higher in patients with acromegaly (57.5% vs 22.6%). Fractures were associated with higher serum IGF-I values, a

longer duration of active disease, and a longer history of untreated hypogonadism. This higher prevalence of vertebral fractures persists despite biochemical control of acromegaly [81].

5.4.2.3 Rheumatologic Comorbidity

Peripheral Osteoarthritis

The topic has been reviewed in detail in [82, 83].

Peripheral joint symptoms are very frequent. Arthralgia and myalgia occur in 30–70% of patients. Among the sites, large joints such as the knees, shoulders, hands, wrists, and hips seem to be more affected. Acromegalic osteoarthritis develops within an average of 10 years after diagnosis.

Osteoarthritis develops in two stages. In the initial stage, the growth of joint cartilage and periarticular ligaments is stimulated, leading to enlargement and congestion of the interarticular space that limits mobility and induces joint pain; in the second stage, more degenerative changes of the joint geometry are observed: intra-articular microtrauma and exuberant repair reactions induce scars and subchondral resorption and osteophytotic development, leading to progressive joint deterioration. The pain is thus mainly mechanical, degenerative, and noninflammatory and often persists after treatment of acromegaly. However, rarely, some patients may present symptoms and signs of pseudoinflammatory osteoarthritis, which are dramatically relieved by the treatment of acromegaly. Joint mobility (especially of the shoulders) can be limited in the later stages of the disease. Joint effusion is rare, and synovial aspiration shows a generally degenerative picture with no evidence of inflammation; it may also reveal calcium microcrystals (associated chrondrocalcinosis).

Physical examination of the joints often provides little information. The abnormalities are generally minor despite subjective functional discomfort. The shoulders and hips may show a loss of mobility and function. In contrast, some patients have joint hyperlaxity. There was no correlation between the presence or severity of osteoarthropathy and the age of onset of acromegaly or the mean GH or IGF-I concentration at baseline or during follow-up. Osteoarthritis appears to be more frequent after 45 years of age.

Radiological studies show a widening of the joint spaces, reflecting hypertrophy of the hyaline cartilage, as well as the presence of osteophytes, bone proliferation at the attachment sites of tendons and ligaments, periarticular calcium deposition, and exostosis of the bone. The joint space subsequently diminishes due to destructive arthritis. Sonography shows a thickening of the cartilage in the shoulder, wrist, and knee joints, which is improved with treatment for acromegaly.

Osteoarthritis inexorably progresses in advanced stages of the disease. It is not influenced by successful treatment of acromegaly, with the exception of diffuse articular symptoms and some sites of pain [84]. Acromegalic osteoarthritis considerably impairs patients' quality of life [85–87].

Spinal Involvement

The estimated prevalence of spinal involvement is approximately 40–50% [88]. Backache is more frequent at the level of the lumbar spine than the cervical or dorsal segments. The pain is mainly mechanical in nature, but inflammatory features can occur in later stages (16%). Spinal involvement may be accompanied by nerve compression. Occasionally, bilateral intermittent claudication reveals lumbar spinal stenosis. Pain may also be related to an increased prevalence of vertebral fractures despite normal BMD [80, 89].

Radiological examination shows typical features, including ossification of the anterior and lateral surfaces of the vertebral bodies, contributing to enlargement of their anteroposterior diameter, as well as a biconcave vertebral aspect and scalloping of the vertebral bodies (exaggerated concavity of the posterior vertebral wall). The mechanism of these morphometric changes is poorly understood but may involve hypertrophy of the intraspinal soft tissues (ligamentous hypertrophy, epidural lipomatosis) or bone. In more severe cases, ossification of the anterior surface of the vertebral bodies can bridge the disk space and give an aspect of diffuse idiopathic skeletal hyperostosis. An increased number of vertebral fractures with wedge deformity and thoracic kyphosis is also more prevalent in patients with acromegaly than in the general population [90].

5.4.2.4 Neuropathies

Symptomatic carpal tunnel syndrome is frequent. Nerve conduction studies have shown that the vast majority of patients with acromegaly have subclinical abnormalities of nerve conduction. Magnetic resonance imaging (MRI) shows a higher amplitude and intensity of the median nerve signal in patients with symptomatic carpal tunnel syndrome compared to asymptomatic patients [91]. The mechanism appears to involve median nerve edema more than extrinsic compression due to an excess of connective tissue, bone or synovial hypertrophy or an increase in extracellular fluid within the carpal tunnel itself with Schwann cell demyelination. Nerve edema, which can also easily be evaluated with ultrasonography [92], improves when GH and IGF-I levels fall, suggesting that hormonal control is a key prerequisite for improving these patients' neurological status. Sometimes, however, carpal tunnel syndrome may persist.

Ulnar nerve neuropathy at the cubital tunnel is also frequent in patients with acromegaly [93] and improves with treatment of acromegaly.

Apart from mechanical/compressive effects on nerves, autonomic nervous system dysfunction is present in patients with acromegaly, as shown by assessing the heart rate variability indices (mean sinus heart rate, RR intervals) and reverses after effective treatment [94].

5.4.2.5 Psychologic Consequences

Self-esteem may diminish along with progressive facial and bodily disfigurement. Patients with acromegaly further exhibit impairment in body image distortion, disruption in interpersonal relations, and social withdrawal anxiety [95].

Patients reported more negative illness perceptions than patients with acute illness but more positive illness perceptions than patients with other chronic diseases [96].

Nonetheless, direct unstructured interviews reveal an association between the diagnostic delay and the doctor–patient encounter and the experience of this disease, which is often described as catastrophic, both before and after the diagnosis [97].

It is unclear whether reported depression, mood swings, and apathy result solely from these physical changes or whether they are intrinsic high GH exposure central effects.

Acromegaly carries a significant lifelong burden for the affected patient. When evaluating health-related quality of life by means of dedicated questionnaires (such as the ACROQoL), it is clear that values barely normalize despite disease remission or cure [98]. All the biological, environmental, and biopsychosocial aspects of this burden have been extensively covered in a recent review by Biermasz [99].

5.4.2.6 Cardiovascular Manifestations

Arterial Hypertension

Hypertension occurs in 20–50% of patients. Its prevalence increases with time after the onset of acromegaly, as well as with GH level and age. Several concomitant factors are likely to play a role in the pathogenesis of hypertension in acromegaly. The chronic expansion of extracellular fluid volume (hypervolemia) leads to fluid retention, with plasma volume being 10–40% above normal. At the kidney level, increased renal sodium reabsorption at the distal tubule level is generally observed [100]. Body fluid expansion is related to enhanced epithelial sodium channel (ENaC) activity [1, 101, 102]. Hypertension can also result from endothelial dysfunction [103]. Neither renin-angiotensin aldosterone nor the sympathetic system appears to be involved in the pathogenesis of hypertension in this setting. Other contributors to the onset and maintenance of hypertension in acromegaly are also the increase in peripheral vascular resistance, insulin resistance and diabetes, and the development of obstructive sleep apnea [104–106].

Cardiomyopathy

Cardiac disorders are a consistent feature. Many lines of evidence, especially from experimental studies, point to the existence of specific cardiac disorders in acromegaly that are completely independent of coronary involvement (currently found in only a minority of patients) or valve disorders, diabetes, or hypertension [107, 108].

The first step of acromegaly-related cardiomyopathy mainly consists of myocardial hypertrophy of the interventricular septum and left ventricular posterior wall. This condition is initially asymptomatic, at least at rest. The assessment of initial cardiomyopathy is generally performed by means of cardiac ultrasound examination or magnetic resonance imaging (MRI).

Generally, left ventricle parameters are normal (concentric hypertrophy). Myocardial hypertrophy can occur in the absence of hypertension and even in young patients (<30 years), reflecting the impact of GH excess itself on the myocardium. Its prevalence is likely to be overestimated by echocardiography compared to MRI [109]. Hypertension further aggravates cardiac hypertrophy. Echocardiography and isotope studies show altered diastolic function (abnormal left and right ventricle filling) related to abnormal relaxation: parietal stiffness is, at least in part, probably linked to edematous infiltration of the ventricular wall [110] and perhaps also to a certain degree of fibrosis. Clinical symptoms such as dyspnea during exercise may be observed in patients who are asymptomatic at rest. Systolic function is normal if assessed by conventional methods. However, novel techniques such as two- or three-dimensional speckle-tracking echocardiography reveal, even at an earlier stage, an increased frequency of subclinical systolic impairment in active acromegaly [111, 112]. At later stages, hyperkinetic syndrome (increased cardiac index) is frequent.

Arrhythmias and/or conduction disorders may occur at any stage of acromegalic cardiomyopathy [113]. Their prevalence has long been underestimated in these patients. Ventricular premature complexes have been shown to frequently occur in patients with acromegaly. In one study, systematic 24 h Holter ECG recordings showed complex ventricular arrhythmias in 48% of patients compared to only 12% of controls [114]. Most of these arrhythmias are subclinical and persist despite successful treatment of acromegaly. Myocardial remodeling, hypertrophy, and fibrosis are all likely to play a role in their onset. However, recent studies did not confirm the high prevalence of dysrhythmias [115].

Congestive heart failure can occur if the cardiac disorders progress (if GH hypersecretion persists and, probably, if other risk factors such as diabetes, hypertension, and sleep apnea are also present). Functional signs first appear on effort before becoming permanent. At this stage, echocardiography shows variable degrees of cavity dilation. Fortunately, these severe forms are now far less frequent (prevalence 3%) [116].

A number of cardiovascular parameters improve during effective treatment of acromegaly, even if some changes appear to be irreversible in certain patients. In general, younger patients and patients with a relatively short history of acromegaly show better "recovery" (from diastolic disorders, myocardial hypertrophy, or systolic dysfunction) [71, 117]. In contrast, when dilated congestive heart failure occurs, cardiac function (especially systolic function) may show a short-term improvement [100], allowing some patients to survive or to avoid heart transplantation, but the longer-term prognosis is worse than that of patients with heart failure due to other causes (5-year mortality rate 37%) [116].

There is controversy surrounding the cardiovascular (ischemic) risk carried by patients with acromegaly [118]. An increased prevalence of hypertension, a history of diabetes mellitus and decreased levels of high-density lipoprotein, low-density lipoprotein, and total cholesterol were found in patients with acromegaly, leading to significantly higher Framingham risk scores than in controls [119]. Biomarkers of cardiovascular disease were also found to be altered in another study [120].

However, carotid atherosclerosis and carotid internal media thickening are not more extensive in patients with acromegaly than in nonacromegalic subjects [121, 122]. Importantly, no increase in the prevalence of coronary artery disease (assessed by different means, such as cardiovascular events, calcium scores, or myocardial scintigraphy) is found in patients with newly diagnosed acromegaly compared to the general population [123–127]. The reason for this apparent discordance between observed and expected coronary events is currently unclear. It has been suggested that the known atherogenic effects of hypertension, insulin resistance, and diabetes induced by GH excess are counterbalanced by some other cardioprotective factors, such as decreased endothelial and systemic inflammation [128–130].

Cardiac Valve Disease

Cardiac valve disorders are highly prevalent in patients with acromegaly and can, along with other cardiac abnormalities, also contribute to the onset or aggravation of heart disease in patients with acromegaly [131]. The risk of valve disease increases with time since onset [132]. Acromegaly-related cardiac valve abnormalities, which may be related to fibrotic changes, seem to persist after effective treatment of acromegaly [133]. Furthermore, no cabergoline-induced cardiac valve remodeling was observed.

5.4.2.7 Metabolic Complications

Physiologically, GH increases blood glucose levels, exerts a lipolytic effect, and promotes triglyceride hydrolysis into free fatty acids and glycerol.

GH excess leads to insulin resistance at the level of the liver or in the periphery, leading to fasting and stimulated hyperinsulinemia. The prevalence of type-2 diabetes mellitus (T2DM) in acromegalic patients is more or less constant across studies and ranges from 20% to 56%, depending on the series [71]. The weighted mean T2DM prevalence in individuals with acromegaly is approximately 27% when comparing data from 14 national registries [11]. As long as the compensatory increase in insulin secretion by pancreatic ß cells counterbalances the reduction in insulin sensitivity, glucose tolerance remains normal. Impaired glucose tolerance occurs when insulin secretion is altered, followed by the onset of diabetes [134].

Acromegaly is associated with a decrease in fat mass (both visceral and subcutaneous) but an increase in intermuscular fat mass (which may contribute to insulin resistance) and lean body mass [135, 136]. A recent study found an increase in exercise-induced myokine irisin circulating levels in patients with acromegaly [137]. This increase was independent of the disease status. The consequences on either glucose metabolism or thermogenesis of these findings still need to be demonstrated.

Alterations in lipid metabolism are reported in 30–40% of patients with acromegaly. In uncontrolled disease, a typical lipid profile is found, characterized by increased levels of lipoprotein(a) and triglycerides and decreased levels of HDL cholesterol [138]. The course of lipid parameters (and other cardiovascular risk factors) may vary with the treatment modality after therapeutic control of acromegaly [139].

Hypercalciuria is frequent in patients with acromegaly and may be associated with an increased incidence of nephrolithiasis. It is related to an IGF-I-mediated and PTH-independent increase in calcitriol synthesis, which is responsible for both absorptive hypercalciuria and increased fasting plasma calcium linked to enhanced distal tubular calcium resorption [1, 140]. An increased prevalence of hyperparathyroidism is also observed in patients with acromegaly, either in the context of multiple endocrine neoplasia (see the above "5.3.3 Genetic Syndromes Associated with Acromegaly" section) or independently (phenocopy) as a usual sporadic hyperparathyroidism.

5.4.2.8 Respiratory Complications

Sleep apnea affects 60–80% of all patients with acromegaly at the diagnosis of acromegaly. Men seem to be affected more than women [141]. Sleep apnea is more likely to be sought in patients who snore (reported by 78% of patients with acromegaly) and in those with daytime sleepiness (51%) or morning fatigue and morning headache (16%). Sleep apnea may be a contributory factor in hypertension, cardiovascular disease, and even cognitive decline. In most cases, apnea is obstructive, but one-third of patients have central apnea. Obstructive apnea is linked to anatomical changes due to mandibular and maxillary growth, soft-tissue thickening (especially of the palate and uvula), and changes in the angles of the different bone segments, leading to hypercollapsibility of the posterior and lateral hypopharyngeal walls. Hypertrophy of the tongue also plays a role [142], as does hypertrophy of the submaxillary glands.

Changes in respiratory function are frequent but less well documented. Anatomical modifications of thoracic bones and cartilage (leading to profound changes in the geometry of the rib cage) and mechanical changes in thoracic elasticity and the inspiratory muscles can lead to ventilatory disorders. Respiratory muscle strength is also abnormal. Altered mechanical and energetic properties of some upper airway dilator muscles have recently been demonstrated [143]. The inspiratory time is shorter, and the breathing frequency may increase.

Patients with acromegaly often have an increase in their total lung capacity (81% of men and 56% of women), owing to an increase in alveolar volume. An obstruction is found in 20–30% of patients (small airway or upper airway narrowing). Subclinical hypoxemia may be present. No ventilation-perfusion mismatching has been demonstrated.

The apnea-hypopnea index improves during effective treatment of acromegaly, along with the obstructive apnea index and oximetry values [141, 142, 144]. However, while apnea can disappear in some patients whose acromegaly is cured, it may persist or even worsen (likely due at least in part to associated obesity [145]) in others who thus require nocturnal positive end expiratory pressure. The reevaluation of sleep apnea is thus useful even if patients are cured or well controlled after acromegaly treatment.

Vocal changes have been described in patients with acromegaly. A deepening of the voice and a low fundamental frequency are observed in the population with acromegaly [76]. Modifications of the laryngeal cords and muscles, as well as upper respiratory tract thickening, may be responsible for these findings. However, the

clinical consequences and the phonetic handicap related to these changes are not currently known.

5.4.2.9 Pituitary and Sellar Mass Effects

Headache is a very common symptom. In contrast with nonfunctioning adenoma, headache may be present even in patients bearing a microadenoma, thus reflecting a multifactorial genesis other than a direct adenoma-related compressive/expansive effect. In large tumors (macroadenomas or giant adenomas), low visual acuity and visual field defects may be observed in cases of suprasellar progression. Compression of the normal pituitary may also lead to anterior pituitary deficiency, which must be explored clinically and biochemically. Diabetes insipidus is never associated with acromegaly, except after neurosurgery or in the context of pituitary apoplexy [146].

5.4.2.10 Neoplasia and Acromegaly

Through the GH- and IGF-I-related promotion of cellular proliferation and differentiation, neoplasm and cancer risks have always been a major issue when dealing with acromegaly. In vitro and in vivo studies have shown a direct effect of GH or IGF-I in mediating cell proliferation. Pharmacological blockade of these targets in some cases allowed tumor inhibition in cell and animal models [147]. Despite these data on molecular biology, the link between GH excess and cancer risk in acromegaly is still unclear [147]. Although cancer-related mortality varies across studies, it seems that an excess of cancer and related mortality is present in patients with acromegaly with uncontrolled disease [148]. An overall cancer prevalence of 10% (any type, any site) is found in national registries collecting real-life data [11]. There is also some controversy regarding the incidence of each individual cancer type in patients with acromegaly.

Figures of colorectal cancer relative risk compared with the general population, initially widely overestimated at 10–20, are probably only 2–3 as per novel estimates [149–153]. There are various potential biological mechanisms that could explain the increased risk of colonic cancer in acromegaly: direct effects of GH and/or IGF-I; hyperinsulinemia; increases in IGFBP-3, IGF-II, and IGFBP-2 levels; altered bile acid secretions and local immune response; increased large bowel length; and obesity [150, 153]. Some authors claim that epigenetic alterations predispose patients with acromegaly to cancer development [154]. As colonic cancer may be the consequence of colonic polyp degeneration, many studies have examined the prevalence of colon polyps in patients with acromegaly. Prospective studies show that up to 45% of patients with acromegaly have colonic polyps, which are adenomatous in 24% of cases [155] and can arise in all parts of the colon. The acromegaly-associated colonic lesions seem to exhibit some peculiarities, such as larger, multiple, and more dysplastic adenomatous polyps than in nonacromegaly patients [150]. There is no clear correlation between GH or IGF-I concentrations and the incidence of colonic polyps. Colonoscopy guidelines for patients with acromegaly are controversial. The British Society of Gastroenterology [156] recommends performing a colonoscopy in patients with acromegaly by the age of 40. A subsequent examination should depend on the findings at the original screening and on the disease activity: screening every 3 years

in patients with a previous adenoma or with elevated IGF-I and every 5–10 years in those without adenomatous/dysplastic polyps or those with only hyperplastic polyps. Some technical difficulties may be encountered in patients with acromegaly because of the increased colon length [153].

Goiter is found in a large proportion of patients with acromegaly. Thyroid nodules have been found in nearly 60–70% of patients [157]. Multinodular goiter is autonomous in 10–20% of patients, sometimes causing patent thyrotoxicosis. Although thyroid nodules are in most cases benign, the risk of thyroid cancer has been found to be higher than that in the general population (odds ratio, OR = 7.9, relative risk, RR = 7.6), with a prevalence of nearly 4%. These findings were confirmed by a recent Finnish study [158]. Nevertheless, contrary to colorectal cancer, most studies about thyroid cancer contain recruitment biases, and the real incidence of thyroid cancer in acromegaly is still a matter of debate [153, 157, 159]. As is the case for colonic cancer, a relative overestimation of thyroid cancer may arise because of increased physician awareness for these tumors, as well as the large use of ultrasonography during the screening of comorbidities in acromegaly.

Neoplasms of the breast, lung, prostate, skin, soft tissues, brain, bone, and lympho-hematopoietic system, initially described in association with acromegaly, do not seem to be overrepresented in these patients [160]. There is therefore remarkable agreement among all experts and reported guidelines, pointing out that surveillance in relation to these cancer sites should follow the same recommendations as for the general population [161].

It is currently acknowledged that, along with other cancers and neoplasms, the description of cancer occurrence is probably overestimated because of enhanced proactive screening. Modern imaging techniques may detect subclinical lesions and therefore affect the incidence rates. Other benign lesions may be found at a higher prevalence in patients with acromegaly. A higher incidence of meningioma has been found when analyzing encephalic MRI in patients with somatotroph adenomas versus those with other cell-type pituitary adenomas [162].

5.5 Diagnosis of Acromegaly

The diagnosis of acromegaly is suspected on clinical grounds and is confirmed by a typical biochemical profile [2, 74]. Clinical diagnosis is suggested by typical disfigurement due to progressive acral enlargement and modification of the facial appearance. In the case of very low progression or clinical incertitude, it is sometimes useful to assess the evolution by comparing serial photographs over several years (Fig. 5.2). Deep learning approaches are currently going to be tested to assist semiology [163]. It is of note that the regions of interest of these tools using aprioristic algorithms are primarily the same as those used by clinicians [163].

IGF-I (with reference to the age-adjusted normal range), the main GH-dependent growth factor, is the screening test recommended for acromegaly, with the diagnosis being confirmed by a nonsuppressive level of GH after an oral glucose load, OGTT [161].

5.5.1 GH and IGF-I

The introduction of international standards has minimized GH variability, which was mainly due to the use of polyclonal or monoclonal antibodies recognizing a mixture of different molecular forms. Manufacturers were recently advised to calibrate their GH assay kits with the international standard (IS) 98/574 [164].

The latest assays allow the limit of quantification to be as low as 0.05 μg/l with an interassay coefficient of variation (CV) of <20% [164, 165].

In most cases, GH levels are elevated, both at baseline and after OGTT [166]. GH levels in the population with acromegaly are inversely correlated with age, in which the youngest patients have the most elevated serum GH concentrations, and with the maximal tumor diameter [10]. Previous recommendations consider a diagnosis of acromegaly if nadir GH levels are above 1 μg/l [74, 167]. However, a few patients with clear clinical signs of acromegaly and high IGF-I levels could have low GH output and can thus suppress GH levels to less than 1 μg/l during the OGTT. Thus, a more stringent criterion of a nadir GH at 0.4 μg/l after OGTT has been proposed [168] and is now increasingly recognized as the recommended threshold if a sensitive GH assay is used. This is in line with recent normative data in healthy subjects underlining the importance of sex, BMI, and the use of contraceptive (estroprogestative) pills in defining the threshold for GH under OGTT [169]. However, it must be emphasized that the last Endocrine Society guidelines continue to recommend the 1 μg/ml threshold rather than the 0.4 μg/l threshold, considering that in the United States, the use of ultrasensitive GH assays is not yet generalized [161]. A paradoxical increase in GH following OGTT is observed in approximately 10–30% of patients with acromegaly [170, 171].

For the IGF-I assay, the IS 02/254 WHO reference standard has recently become available. It is an ~97%-pure recombinant standard recommended for manufacturers [164, 165]. The IGF-I level increases in parallel to the log of the GH concentration and must be determined by using age-adjusted norms because levels fall with age. A multicenter cohort study comparing six IGF-I immunoassays in 911 healthy individuals showed good agreement at lower but not upper levels [172]. This variability, especially in upper levels, which are more interesting when evaluating the biologic control of disease, leads to a marked variability in each individual's IGF-I levels. Concordance between assay values in intraindividual patients with acromegaly was on average good (ranging from moderate to excellent) [173]. These differences in assay performances must be considered when evaluating disease control in subjects with acromegaly [173].

Similar to GH, IGF-I levels in patients with acromegaly are inversely correlated with age and with the maximal tumor diameter [10].

High IGF-I concentrations are also systematically found in other physiological states, such as pregnancy, puberty, and the postpubertal period. The concentration of IGFBP-3, the main IGF carrier protein, is usually increased in patients with acromegaly, but this marker offers little further diagnostic information in differential diagnosis.

GH and/or IGF-I measurements are of limited use for diagnosis (or treatment efficacy assessment) in patients with uncontrolled diabetes mellitus, chronic renal failure or pregnancy, and at the time of puberty.

Estradiol increases either basal GH or nadir GH levels after OGTT. This explains why GH is rarely inhibited by OGTT in women taking estrogen-containing pills. GH nadir concentrations are also significantly higher in lean and normal weight compared to overweight or obese subjects [169].

There are some individuals with a typical clinical picture of acromegaly but normal IGF-I and GH concentrations. This situation could correspond to two different situations: (1) spontaneously resolving real acromegaly, probably through necrosis or apoplexy of a previous GH-secreting pituitary adenoma; facial sequelae and disfigurement could have persisted despite the normalization of the somatotroph axis after spontaneous adenoma shrinkage; and (2) acromegaloid features may also be encountered in other diseases, such as severe insulin resistance, severe hypothyroidism, some forms of lipodystrophy, genodermatoses, or rarer overgrowth disease [174–176]. An extensive review focusing on various causes of pseudoacromegaly has recently been published [177].

Finally, some adenomas excised for mass effect or upon another surgical indication were revealed to derive from the somatotroph lineage only after histopathological examination [178]. In most of these cases, the somatotroph adenoma is silent, and no clinical signs of acromegaly are found. Nonetheless, subtle abnormalities revealing GH/IGF-I hypersecretion may be encountered [25, 179].

5.5.2 Neuroimaging

MRI is the imaging method of choice to detect a pituitary lesion. T1- and T2-weighted coronal and T1-weighted sagittal sections are routinely performed in diencephalic studies; gadolinium contrast usually shows a retardation in lesion enhancement demarcating the remaining hypophyseal tissue.

The majority of patients clearly have a pituitary macroadenoma (lesion above 10 mm). In patients with a sellar macroadenoma, once the diagnosis is established, before initiating treatment for acromegaly, patients must undergo a thorough work-up focusing on tumor mass effects (headaches, changes in the visual field and acuity, MRI abnormalities) and anterior pituitary function.

Although the majority of somatotroph adenomas are large tumors, in recent decades, the prevalence of microadenomas has seemingly increased in patients with acromegaly. It has to be known whether this trend depends on an improved clinical skill to detect disease (and therefore smaller lesions) or an intrinsic biological characteristic of somatotroph adenomas.

GH-secreting pituitary adenomas can be hypo-, iso-, or hyperintense on T2-weighted MRI sequences. Some authors suggest that hypointense imaging on T2-weighted MRI predicts a better outcome after somatostatin analog treatment either in terms of biochemical profile or tumoral shrinkage [180].

When MRI is contraindicated, a skull base CT scan may still be used. In patients with macroadenoma, this technique may show the presence of the pituitary mass and various extents of enlargement of the sella turcica.

Novel tools such as ^{11}C-methionine positron emission tomography seem to detect small pituitary remnants, especially those with a high metabolic rate and hypersecretion [181]. This technique seems particularly promising in equivocal MRI images [182]. Nevertheless, the accuracy of this technique has yet to be extensively established, and ^{11}C-methionine PET sequences are not routinely indicated in assessing pituitary imaging. Moreover, this technique is available in very few centers where a cyclotron is available on site due to the very short half-life of the radionuclide.

5.5.3 Pituitary Assessment

Associated prolactin hypersecretion is present in up to 30% of cases and may be either functional, secondary to impairment of hypothalamic dopamine production or compression of the pituitary stalk by the tumor, or due to a mixed adenoma.

In patients with microadenoma, no other pituitary defect or sellar mass effect is expected.

5.5.4 Total Body Imaging

If, despite an overt disease, no image is found on MRI, an ectopic GHRH secretion must be suspected and appropriate imaging requested [50].

Some occult neuroendocrine tumors may require total body scans (CT scans, MRI) or functional imaging (Octreoscan®, F-DOPA, or DOTATOC) [183]. Biopsy may help prove the neuroendocrine nature of these neoplasms. Complete excision of the underlying tumor usually cures disease.

5.6 Management and Follow-Up

Management of acromegaly is multimodal and quite consensual across different American and European guidelines and clinical practices [74, 165, 184–188].

The main aim is to relieve symptoms, normalize or decrease GH/IGF-1 excess, remove or reduce pituitary tumors, and improve long-term morbidity and mortality [185, 189]. Recent epidemiological studies have helped to refine the definitions of "cure" and "good disease control", which are now far more precise: the GH concentration (in a random sample) must return to less than 1 μg/l in the new sensitive assays that are now widely used (if the OGTT is used, the nadir needs to be less than 0.4 μg/l) and the IGF-I level must return to normal according to sex and age [165, 187]. A stepwise therapeutic strategy based on surgery and/or radiotherapy and/or medical treatment allows these goals to be achieved.

5.6.1 Neurosurgery

Surgery is generally the first-line treatment. Tumor excision, usually by the transsphenoidal route, is the most rapid way of reducing GH and IGF-I concentrations in patients with acromegaly. Nevertheless, these levels normalize in only 40–70% of cases after surgery [11, 190–194]. The success rate depends on a range of features, such as the tumor size (microadenomas are more amenable to cure), the preoperative GH concentration (the success rate is higher when GH concentrations are <10 μg/l), and the surgeon's experience. Endoscopic techniques, now used in the majority of expert centers [195], though not improving the success rate, may attenuate local adverse effects [192].

Postoperative outcome in terms of symptom relief and biological disease control is generally assessed 3 months after surgery. When surgery fails to achieve disease control or when surgery is impossible or contraindicated, patients are offered radiotherapy and/or pharmacological treatments.

5.6.2 Radiating Techniques

Radiotherapy techniques have evolved over time, refining the techniques and number of sessions. Radiosurgery is a term used to define high-dose radiation delivery. It better applies to small targets and requires a single or few sittings. Fractionated radiotherapy refers to radiation therapy delivered at smaller doses but with multiple treatments (typically 25–30 sittings during 5–6 weeks). In order to minimize the dose to surrounding tissues, stereotactic localization is now used. Stereotactic radiosurgery (SRS) may use different radiating particles, such as photons (gamma knife, Linac, CyberKnife) or charged ions (protons). Stereotactic fractionated radiotherapy (SFR) is a hybrid form combining stereotactic localization with fractionated therapy administered by 3D-conformal radiation therapy, intensity-modulated radiation therapy, or proton radiation therapy [196].

Radiating techniques have consistently evolved over the last decade, from conventional radiotherapy to three-dimensional (3-D) conformal and stereotactic techniques. Technical improvements have been performed in all aspects of radiation treatment, including better imaging and 3-D planning, patient immobilization, sophisticated imaging systems for accurate patient repositioning and a more precise dose delivery, and reduction in normal surrounding brain structures exposed to high radiation doses [197].

In patients with somatotroph adenomas, normalization of GH/IGF-I levels occurs in approximately 40–60% of patients 5–10 years after treatment, with a 50% decline in GH and IGF-1 preradiation levels in approximately 2 and 5 years, respectively [190, 198, 199].

The choice between SFR and SRS, like for any other pituitary adenoma, in part depends on the size of the tumor and on its contiguity with the optic apparatus [200]. It is of note that the baseline GH concentrations predict treatment outcome

and the time-to-normalization of patients with high (>3–4-fold ULN) IGF-1 levels requiring up to 10 years to achieve biochemical control of disease [199, 201].

SRS provides more focused irradiation. In a French series of over 80 patients, the efficacy of gamma-knife irradiation was close to that of SFR [202]. In a recent meta-analysis, disease control (without complementary medical treatment) was achieved in 48–53% of cases after a mean follow-up of 4 years. The relatively larger figures by SRS are probably explained by the small size of tumors (2.1 ± 1.2 mL) [203]. Apart from disease control, the different SRS techniques give excellent results in controlling tumor growth, with >95% success according to different series [200, 202–204].

A recent review of the literature proved a similar rate of tumor control between stereotactic radiosurgery and SFR for patients with persistent active acromegaly after surgery and/or during medical therapy [205]. Tumors were stable or decreased in 93–100% of patients at 5–10 years, whereas endocrinological remission was achieved in 40–60% of patients at 5 years [205].

On the other hand, radiotherapy leads to variable degrees of anterior pituitary insufficiency in 50–100% of patients after 10–15 years, regardless of the technique. Complications such as radionecrosis and optic neuropathy are very rare. In contrast, the risk of stroke and cerebrovascular events may be increased, sometimes many years after irradiation [206]. When compared to patients not exposed to radiotherapy, stroke incidence appears to be increased from 1.7 to 2.8 times [200, 207]. Along with cortisol deficiency and inadequate hormonal substitution, these findings seem to account for the excess mortality in these patients [208]. The question of whether cerebrovascular risk may be lowered by newer radiating techniques is presently still unanswered.

5.6.3 Medical Treatment

5.6.3.1 Dopamine Agonists (DA)

Cabergoline appears to be the most effective among DA agents [209, 210]. In a meta-analysis of all published studies, IGF-I normalization was achieved in up to 34% of cases [211]. Multivariate analysis showed that the efficacy depended on the initial IGF-I concentration, the treatment duration, and the basal concentration of PRL (and, to a lesser degree, the dose of cabergoline) [211]. As in patients with hyperprolactinemia, cardiac valve disease does not seem to be increased in patients with acromegaly treated with cabergoline in the long term [133, 212].

Because of their dual origin, mixed lactotroph/somatotroph tumors are more likely to respond to DA [213]. Half of the patients with GH/PRL-secreting adenomas normalize their IGF-I levels, and 60% of those with macroadenomas display tumor shrinkage [209].

5.6.3.2 Somatostatin Receptor Ligands (SRLs)

SRLs suppress GH secretion by binding to somatostatin receptor subtypes (sst) sst2 and sst5, which are mainly present on somatotroph adenoma cells [214]. These

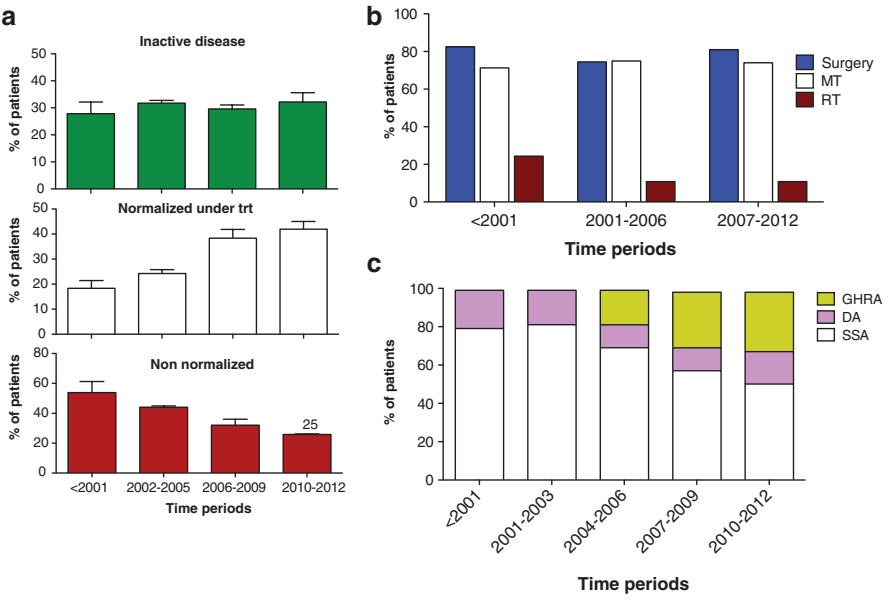

Fig. 5.5 Trends of disease control, treatment approach, and medical therapy over time in acromegaly—the example of the French Registry of Acromegaly. Panel **a**. Evolution of disease status across 4-year follow-up periods in the French acromegaly registry. Histograms indicate the percentages of patients and standard deviations; trt: medical treatment. Panel **b**. Distribution of treatment approaches in different follow-up periods. *MT* medical treatment, *RT* radiotherapy. Data are reported as percentages of patients. Panel **c**. Evolution of drug therapy in medically treated patients: somatostatin analogs (SSA), dopamine agonists (DA), GH receptor antagonist (GHRA). The histograms report the prescribed classes of treatment per patient according to different follow-up periods as the percentage of prescriptions. Note: Pegvisomant was approved for the treatment of acromegaly in France in late 2003. Adapted from [23], with permission

drugs have been demonstrated to exert either antisecretory or antitumoral effects (Fig. 5.5).

The first SRL to be marketed, octreotide (Sandostatin®), can be injected subcutaneously (SC), generally by the patient him/herself, at a dose of 100–200 µg two or three times a day [215]. Sustained-release SRLs (lanreotide and octreotide LAR) progressively followed and had an impact on the market due to their comfort. The former requires deep SC injections every 28 days at variable doses (Somatuline® Autogel® 60, 90, or 120 mg). The latter is administered intramuscularly once a month (Octreotide LAR, Sandostatin® LAR 10–20 or 30 mg). The dose and frequency of injections may be initiated and adjusted depending on the GH/IGF-1 concentration.

These SRLs bear similar efficacy [216] in driving GH concentrations below 2 µg/l (60% to 70% of cases) and in normalizing IGF-1 levels (50–80%) [217, 218]. A recent meta-analysis has emphasized that control rates were highly variable from one study to the other. If clinical design characteristics had no statistically significant impact on efficacy determination, then later year of publication, study duration,

and prior somatostatin analog use were significant efficacy determinants for acromegaly trial outcomes. In that meta-analysis, overall achieved control rates were 56% for mean GH and 55% for IGF-1 normalization [219].

Several long-term studies have shown that the cure rate may improve over time [220–222].

In a handful of good responders, SRL injection frequency may be lengthened or even safely halted with no subsequent increase in GH/IGF-I concentrations [223, 224].

Tumor volume shrinks in a weighted mean of 37–51% of patients [225]. It seems that the reduction in tumor volume is larger when an SRL is used as first-line treatment [226]. When not shrinking, tumor volumes remain at least stable in the vast majority of cases [217].

SRLs may cause gastrointestinal disorders (abdominal bloating, nausea, diarrhea), which are generally transient. SRLs induce the occurrence of gallstones in 10–20% of cases that did not respond to ursodeoxycholic acid [227, 228]. Some practitioners prescribe pancreatic enzymes in SRL-related diarrhea. Changes in glucose metabolism are sometimes observed, including impaired glucose tolerance or even diabetes in patients who are overweight. In other cases, however, glucose tolerance improves following the reduction in insulin resistance due to the lowering of GH concentrations. Overall, according to recent meta-analyses, despite a decrease in fasting plasma insulin levels, no consistent changes in fasting glucose and HbA1c levels have been observed [229, 230].

Pasireotide (SOM230 or Signifor®) is a second-generation SRL compound that binds to sst1, 2, 3, and 5 with high affinity [231], which has been proven to be effective in controlling acromegaly [232–234]. When directly compared to octreotide LAR, pasireotide was able to control a higher proportion of patients (36% versus 21%) [232]. In a crossover study, 15% of noncontrolled patients under maximal octreotide doses responded to pasireotide [235].

Concerning the side effects, glucose metabolism abnormalities (diabetes or glucose intolerance) were far more frequent in patients receiving pasireotide than in those administered conventional SRLs [232, 233]. Gastrointestinal symptoms after pasireotide treatment seem to occur at a similar or slightly increased frequency [233].

This drug may be particularly interesting in patients with partial resistance to first-generation SRLs.

Among the factors believed to influence SRL effectiveness, there is the T2-weighted hypointense signal on MRI [236, 237], the presence of specific somatostatin receptor subtypes, and the aspect of densely granulated cells at histological examination [238].

5.6.3.3 GH-Receptor Antagonists

Pegvisomant (Somavert®) acts peripherally, blocking the effects of GH on its target organs by binding to GH receptors and by preventing their dimerization, GH signal transduction, and downstream activity, including IGF-I production [239]. As pegvisomant inhibits the action of GH but not its secretion, GH concentrations cannot be

used to evaluate treatment efficacy. IGF-I is used as a surrogate marker, together with clinical parameters. Pegvisomant is administered subcutaneously at a daily dose of 10–20 mg (sometimes more), with the dose being adapted to the hormone response (IGF-I normalization). Pegvisomant is highly effective, as IGF-I levels normalize in more than 90% of patients in the initial trials reported [240, 241]. In routine practice, the pegvisomant efficacy rate seems to be as low as 70% of cases, as shown by observational studies [242–247]. This treatment is reserved for patients in whom SRLs fail.

In a series of 304 patients in whom tumor volume was monitored for at least 3 years, an increase in tumor volume occurred in 9 cases within 8 months after commencing pegvisomant. This is likely related to rebound expansion after discontinuation of SRLs and/or to the natural history of aggressively growing pituitary tumors [248]; this latter situation may justify combination with an SRL to reduce tumor volume [249]. Tumor volume must therefore be monitored (by MRI) during this treatment. Available clinical data on pegvisomant concern a relatively small number of patients and relatively short treatment periods. Independent of disease control, pegvisomant improves glucose metabolism [250]. Adverse effects are limited to rare liver enzyme elevations, which are observed in between 2.5% and 3% of patients according to surveillance studies [242, 247]. Liver enzyme elevation generally normalizes either spontaneously or after treatment interruption. Exceptional cases of true hepatitis have been reported [246, 247]. Gilbert disease has been suggested as a risk factor for severe hepatitis [251, 252], but this was not confirmed by a recent Italian study [253].

SRL-pegvisomant combination therapy has also been developed [254]: a slow release formulation of the SRL is given once a month at the highest marketed dose (30 mg octreotide LAR or 120 mg lanreotide Autogel), and pegvisomant is injected once a week at escalating doses until the IGF-I level normalizes. IGF-I normalization was obtained in all patients with a median weekly pegvisomant dose of 60 mg [255]. This decreased dose requirement during combined therapy might be partially explained by an increase of approximately 20% in serum levels of pegvisomant [256]. Biochemical hepatic anomalies were quite frequent (although always transient) with this combination and appeared to be twice as common in patients with acromegaly with diabetes [257]. Compared with octreotide monotherapy, this combination appears to have a greater positive impact on quality of life for a given degree of IGF-I normalization [258]. This has raised the hypothesis of an extrahepatic effect of pegvisomant [259].

Cabergoline-pegvisomant combination therapy has also been proposed. In a multicenter, open-label, prospective clinical trial [260], the combination of cabergoline and low-dose pegvisomant (10 mg/day) was associated with a significant decrease in IGF-I levels compared with cabergoline alone, and 68% of patients achieved normalization. Then, when cabergoline was withdrawn and pegvisomant continued as monotherapy, only 26% of patients maintained normal IGF-I levels. The adjunction of cabergoline may be interesting when pegvisomant alone achieved minimally increased IGF-I levels [261].

5.6.4 Treatment Strategy

By analyzing large caseload series, several studies have evaluated medical practices and the evolution of treatment strategies over time [9, 11, 13]. The advantages, disadvantages, and costs of the different treatment options must be considered [262]. A marked evolution in clinical practice has been observed in recent decades (Fig. 5.5a–c).

An algorithm indicating a putative therapeutic strategy is proposed in Fig. 5.6. A surgical procedure is tried whenever possible. This depends on the availability of an

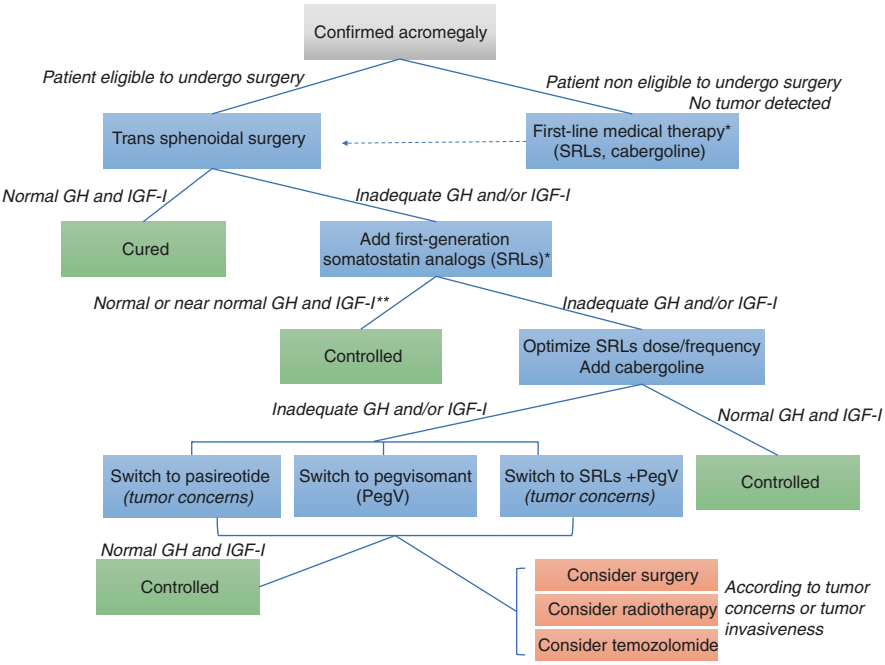

Fig. 5.6 Proposed algorithm of the treatment strategy for acromegaly. After confirmation of acromegaly, the first step is to establish the patient's eligibility for neurosurgery. In the absence of disease remission/cure after surgery, long-acting somatostatin receptor ligands (SRLs) are indicated. SRL doses and frequencies should be adapted and optimized, especially in partial responders (≥50% decrease in growth hormone (GH) and/or insulin-like growth factor 1 (IGF-I)). In the case of mild IGF-I elevation (<2–2.5-fold of the adjusted value for sex and age of the upper limit of normal value (ULN)), the addition of cabergoline can be considered. If disease control is not achieved, patients should be switched to the second-generation SRL pasireotide if there is clinically relevant residual tumor on imaging and/or clinical concern of tumor growth. Patients with impaired glucose tolerance should be switched to the GH antagonist pegvisomant (PegV). Patients with impaired glucose tolerance and tumor concern could be treated with a combination of a first-generation SRL and PegV. Patients who remain uncontrolled despite this second-line medical therapy should be discussed by a multidisciplinary team and considered for a second surgical intervention, a radiating therapy or temozolomide (features of aggressiveness, high Ki-67, tumor progression). *: consider using cabergoline in place of first-generation SRL if IGF-I elevation is <2–2.5-fold the ULN and/or in case of mixed GH/prolactin-secreting tumor; **near-normal IGF-I is considered for IGF-I values <1.3 ULN

experienced neurosurgeon, on the feasibility, on the fact that there are no anesthesiology constraints, and on the patient's choice. Surgery has been chosen in nearly 80% of cases, considering the weighted mean of 19 studies across different countries encompassing more than 16,000 patients with acromegaly [11].

In the case of surgery failure to cure acromegaly, medical treatment with SRLs is preferred as an elective option. SRL therapy is not only indicated after surgical failure but can sometimes be used for first-line treatment, especially when severe comorbidities create a risk of perioperative complications. Thus, when heart failure or respiratory problems are associated with acromegaly [71, 141], it is preferable to prepare the patient for surgery by administering SRLs for a few months first. In some cases, when a large tumor extends outside the *sella* and is not completely extractable by surgery, SRLs can be administered in the hope of controlling GH hypersecretion and tumor growth, thus avoiding the need for surgery [226, 263–267]. First-line SRL treatment before surgery has been chosen in 0–52% of cases, according to different series [11]. In these cases, when clinical conditions improve, neurosurgery could be performed as second-line treatment. According to a meta-analysis, IGF-I more likely normalizes after second-line treatment than after first-line drug therapy [218].

There is some controversy surrounding the ability of preoperative SRL therapy to improve surgical outcome: some studies [268–274] indicate that, in some patients with isolated somatotroph macroadenomas, surgery provides better control of acromegaly when patients are pretreated with an SRL, while other studies showed no difference [275–278]. Yang et al., in their latest meta-analysis, showed that preoperative SRL treatment was able to improve short-term (OR 2.07, 95% CI 1.50–2.87, $p < 0.00001$) but not long-term biochemical control [279].

The overall ability to normalize IGF-I by a first-line full-dose SRL (either octreotide LAR or lanreotide) is approximately 50% in recent series and the latest meta-analyses [280].

In some selected patients, especially in those with moderately increased IGF-I levels (below 2–2.5-fold the upper limit of normal) or in those with elevated concomitant prolactin levels, cabergoline may be tried first. It has indeed been shown that cabergoline is particularly useful in patients with low IGF-I excess [211].

After a first surgical approach, when full-dosed SRL therapy fails to achieve remission, several options may be chosen, mainly according to the patient status, his/her willingness, and the severity of clinical/biochemical disease persistence:

(a) In the case of a large tumor remnant, it may be of utmost interest to repeat surgery. The main aim, in this case, will not be to cure disease but to debulk and decrease the secretory mass before trying medical treatment again [281, 282].
(b) In the case of partial response to SRLs, physicians may choose to further adapt the adjusted doses [283, 284], to combine with other agents such as cabergoline [211], to administer pasireotide, or to initiate pegvisomant. When analyzing current anti-GH/IGF-I medical choices in these cases, a striking similarity is found between Germany and France, two countries where drugs are similarly available [9, 13]. In second-line treatment (after surgery failure), 60% of

patients have been treated with SRLs alone, 10% by a combination of SRLs and DA, 10% by pegvisomant, 8.6% by DA alone, 6.8% by SRLs and pegvisomant, 2% by DA and pegvisomant, and 2.7% by tritherapy (SRLs+DA + pegvisomant). Notably, at the time of the survey, pasireotide was not yet available in these countries.

(c) In the case of full persistence or disease that is still clinically and biochemically severe, pegvisomant should be rapidly tried. Increasing doses may be chosen to improve symptoms and to normalize IGF-I levels. If possible, surgical debulking may also be interesting to propose for reducing the dose of pegvisomant necessary to achieve normal IGF-I.

(d) Radiotherapy is far less chosen as the first or second line. Studies evaluating clinical practices over time show a remarkable reduction in radiotherapy use between <2001 and > 2007 (Fig. 5.5b) [9]. However, radiotherapy may be particularly interesting when aiming at avoiding remnant enlargement and controlling GH secretion. In the case of a small remnant, gamma- or CyberKnife could be proposed. In the case of large tumors, fractionated radiotherapy may be proposed to avoid further tumor enlargement. Apart from clinical and biochemical issues, the cost of these long-term medical treatments should be weighed against the risks of radiotherapy. In any event, medical treatment will be necessary while waiting for the benefits of radiotherapy to emerge.

All these treatments must be reassessed on a yearly basis. After radiotherapy, if medical treatment is necessary while waiting for the effects of irradiation, regular withdrawal is necessary for assessing the persistence of active disease.

5.7 Prognosis

Several targeted studies and meta-analyses have been conducted to explore mortality in populations with acromegaly. The overall body of evidence globally shows an increased mortality rate in patients with acromegaly [11, 285, 286].

According to the earliest series published in the 1980s–1990s, approximately 60% of patients die from cardiovascular disease, 25% from respiratory complications, and 15% from cancer. If untreated, patients with acromegaly have been reported to die approximately 10 years earlier than healthy subjects [5]. Several studies have shown that cerebrovascular disorders are a frequent cause of death, especially among women, but these studies involved patients treated in various ways (craniotomy, radiotherapy), many years ago, and a deleterious effect of these treatments (especially radiotherapy) cannot be ruled out [287, 288]. Two recent meta-analyses [286, 289] showed a standardized mortality ratio (SMR), i.e., the ratio of observed mortality in the population with acromegaly to expected mortality in the general population, of 1.72 (IC 95%, 1.62–1.83). However, recent reports describe a reduction in this trend, with mortality rates that seem no longer higher than the general population [290]. Bolfi et al. compared the mortality rates across 26 studies according to the date of publication of the series [285]. They found that the

mortality in acromegaly was increased from the 17 studies published before 2008, while no difference from the general population was found from the nine studies published after this date (SMR, 1.35; CI, 0.99–1.85). The posttreatment GH concentration is probably the best predictor of survival for all causes of death, independent of the type of complication. Thus, life expectancy outcomes can be stratified according to the posttreatment GH concentration: if GH secretion is controlled (<2 or 2.5 μg/l, or IGF-I normalization), life expectancy merges with that of the matched general population [287, 290, 291].

In the meta-analysis published by Holdaway and colleagues, it has been shown that the prognosis of acromegaly has improved in the last 20 years. This improvement is probably due to better disease control through a multimodal treatment strategy or to better management of comorbidities through improved awareness by physicians. Concerning the former aspect, a multimodal strategy and/or various anti-GH drug combinations witnessed a more aggressive treatment of the disease [9, 13, 292]. Concerning the latter, it is believed that mortality in acromegaly largely depends on the numerous and severe comorbidities. High GH/IGF-I concentrations, arterial hypertension, and cardiomyopathy are factors of poor prognosis, while the duration of symptoms and other factors (diabetes, lipid disorders, and cancer) are less important. Quality of life is also altered in acromegaly and is improved by effective treatment [293].

Finally, it must be stressed that, with the current therapeutic strategy, the vast majority of patients with acromegaly achieve very good control of GH/IGF-I secretion and have no problems related to tumor growth. Up to two-thirds of patients have normal IGF-I at the last visit, and these numbers seem to have increased in the latest series [11].

Adverse effects are infrequent and minor, even in the very long term. The figure is broadly different from the disease management 20 or more years ago, before the advent of somatostatin analogs. In addition, the use of more stringent criteria to define cure, together with aggressive treatment of comorbidities, has significantly improved the outlook of patients with acromegaly [118, 294]. However, even cured or well-controlled patients may have invalidating sequelae, such as joint pain, deformities, and impaired quality of life [145].

5.8 Conclusion

Acromegaly is a rare disease characterized by excessive GH and IGF-I exposure. In addition to the typical dysmorphic syndrome, a number of highly morbid diseases are associated with acromegaly. The cardiovascular, respiratory, rheumatologic and metabolic consequences and an increased oncologic risk represent the real burden of acromegalic disease and determine the prognosis. A detailed work-up of the various organs potentially involved in these complications is therefore recommended. Treatment is aimed at correcting (or preventing) tumor compression of neighboring tissues by excising the culprit lesion and at reducing GH and IGF-I levels to normal values. Selective pituitary sphenoidal surgery is often the

first-line treatment. When surgery fails to correct GH/IGF-I hypersecretion, a multimodal therapeutic strategy is available, including several medical treatment classes (standard and novel somatostatin analogs, dopamine agonists, or the GH receptor antagonist pegvisomant). Radiotherapy is currently proposed as a third-line treatment. The prognosis of acromegaly has improved in recent years: adequate hormonal control is achieved in most cases, providing a life expectancy increasingly similar to that of the general population. It remains to be shown whether the criteria used to define control of the disease in terms of mortality also apply to optimal management of comorbidities.

5.9 Learning Points

- Acromegaly, even if clinicaly manifest is diagnosed after a long delay which makes the cure of the disease more difficult and aggravates comorbidities.
- Preoperative GH level (high GH output) is an important predictor of remission in acromegaly.
- Persistent elevation of biochemical markers (GH and IGF1) following surgery indicates uncontrolled disease.
- Medical therapy nowadays is an important part of the treatment of patients with acromegaly.
- Age, size of the tumor, GH levels on presentation, histopathological type, and the somatostatin receptor status of the tumor in acromegaly may predict response to medical therapy.
- Despite the availability of multiple treatment modalities (surgery, radiotherapy, somatostatin analogs first and second generation, GH receptor antagonists, etc.), acromegaly sometimes is a challenging condition to treat.

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Prolactinoma

6

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6.1 Introduction

Prolactin-secreting tumors of the pituitary gland are called prolactinomas. It is the most common secretory tumor of the pituitary gland accounting for up to 40% of total pituitary adenomas [1] and 44–67% of functioning pituitary adenomas [2]. Prolactinomas may cause a wide variety of symptoms either due to mass effect of the tumor or due to hypersecretion of prolactin. Based on the size of the tumor, prolactinomas can be classified as microprolactinoma (smaller than 10 mm), macroprolactinoma (10 mm or larger), or giant prolactinoma (larger than 4 cm). Hyperprolactinemia is the hormonal hallmark of prolactinomas; however, the etiological diagnosis of prolactin levels above the reference range may be challenging since hyperprolactinemia is not always due to prolactinoma and other physiological or nonphysiological causes like pregnancy, drugs, and pituitary stalk effect due to locally expanding masses or infiltrative disorders should be considered in the differential diagnosis [3]. Moreover, macroprolactinemia, a laboratory finding due to the formation and the measurement of immunoglobulin–prolactin complexes, should be also ruled out [4].

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Most pituitary tumors occur sporadically. Prolactinomas arise from the monoclonal expansion of pituitary lactotrophs, which have undergone somatic mutation. Pituitary tumor-transforming gene overexpression and mutation of a receptor of fibroblast growth factor 4 have been found in pituitary adenoma mainly prolactinoma [5]. Some patients with prolactinomas are associated with a genetic disorder called multiple endocrine neoplasia type I (MEN-1). MEN-1 is an inherited condition most often characterized by the occurrence, or the predisposition of occurrence, of neuroendocrine tumors of the pancreas, parathyroid, and pituitary gland. Other syndromes or genetic predispositions have been identified as factors possibly leading to the development of a prolactinoma. For example, linkage to aryl hydrocarbon-interacting protein (AIP) gene mutation has been identified in some families with prolactinoma and childhood-onset pituitary adenomas [6].

In terms of anatomical behavior, a subset of prolactinomas, in particular macroprolactinomas, tend to grow with time and may require aggressive treatment to prevent complications. The growth rate varies with the individual and cannot be reliably predicted, depending on a number of factors. Careful monitoring of the clinical signs and symptoms coupled with pituitary gland imaging and with serial measurements of serum prolactin levels (i.e., to detect any major change in tumor behavior) remains the cornerstones of the follow-up for prolactinoma patients [7].

Dopamine agonists are the first-line treatment of most prolactinomas [8]. Surgical treatment, or radiotherapy, is reserved for patients who fail to respond to the medical treatment [9].

Patients with microprolactinoma generally have an excellent prognosis. In up to 95% of microprolactinoma patients, these pituitary tumors do not enlarge over a 4- to 6-year follow-up period [10]. These patients generally do well for extended periods on suppressive therapy with dopamine agonists, and tumor shrinkage is often achieved medically [10].

6.2 Epidemiology

The exact frequency with which prolactinomas occur in the general population is not established. However, a study of 81,449 inhabitants of an area of Oxfordshire, UK, determined the incidence of pituitary adenomas to be 77.6 cases per 100,000 population, with the majority of cases (57%, or 44.4 persons per 100,000 population) being prolactinoma [11].

Prolactinomas may account for up to 40% of all clinically pituitary adenomas, with a peak prevalence in women aged 25 to 34 years [1, 12]. Microprolactinoma is diagnosed more commonly in a female with a female-to-male ratio of 1:20, whereas macroprolactinoma is equally common in both genders [13]. Giant prolactinoma is a rare tumor that occurs mainly in males (about 85%) with an estimated frequency of 0.5–4.4% of all pituitary tumors in that patient population [14].

6.3 Pathophysiology

Prolactin secretion is under dual control from the hypothalamus, where dopamine serves as an inhibitory signal preventing prolactin secretion, while thyrotropin-releasing hormone somehow stimulates prolactin production and release [15]. Increased prolactin secretion can occur from a prolactin-producing adenoma or inflammation (hypophysitis). Also, conditions that result in impaired dopamine delivery or enhanced thyrotropin-releasing hormone signaling or both may result in increased prolactin release.

Some medications result in increased prolactin secretion through their anti-dopaminergic properties. Chest wall injury and breast stimulation serve as peripheral triggers of autonomic control, which impinge on central neurogenic pathways that attenuate dopamine release into the hypophyseal portal circulation. In some conditions, such as renal or hepatic insufficiency, prolactin is cleared less rapidly from the systemic circulation, which results in increased blood levels of prolactin.

Most cases of prolactinomas are sporadic, and no specific trigger for the development of the prolactin-secreting adenoma is identifiable. When compared to their sporadic counterpart, familial cases of prolactinoma are rare [16]. Most adenomas that secrete prolactin and cause hyperprolactinemia are comprised solely of lactotroph cells. However, combined prolactin and growth hormone (GH) secretion has been reported in 5% of all pituitary tumors, as these are comprised of both lactotroph and somatotroph cells, a co-secretion status, which is usually diagnosed simultaneously [17]. The combination of GH, PRL, and the alpha-subunit of the glycoprotein hormones is also possible, whereas other hormone combinations within a single tumor are extremely rare [18].

6.4 Clinical Presentation

The clinical manifestations of hyperprolactinemia are relatively few and usually easy to recognize. The clinical presentation of a prolactin-secreting adenoma may depend on the direct effect of high prolactin levels, hypogonadotropic hypogonadism occurring through the inhibitory effect of high prolactin concentrations on the hypothalamic gonadotropin-releasing hormone (GnRH) axis, or due to the mass effect of the prolactinoma.

In females, prolactinomas are most often smaller than in males, and therefore, the endocrine symptoms are more common than the mass-related effects, especially in premenopausal women. The classic symptoms of prolactinoma in women include oligomenorrhea or amenorrhea, galactorrhea, and infertility. Recent studies in female patients with hyperprolactinemia have found the prevalence of menstrual disorders as high as 85–90%, but galactorrhea is much less frequent 45–50% [19, 20]. The symptoms of hypogonadism due to hyperprolactinemia in premenopausal women correlate with the magnitude of the hyperprolactinemia. Very high prolactin levels may determine hypogonadism, ultimately leading to low estradiol levels. Low estrogens induce a series of issues, which may manifest as

amenorrhea, hot flushes, and vaginal dryness. A moderate degree of hyperprolactinemia can nevertheless cause amenorrhea or oligomenorrhea, while a mild degree of hyperprolactinemia may just affect progesterone secretion only and therefore determine a short luteal phase of the menstrual cycle [21]. In postmenopausal women, the clinical presentation is different due to the already established physiological hypogonadism. In this setting, prolactinomas most likely manifest with mass effect symptoms and the hormonal effects are very limited, if present at all (i.e., galactorrhea).

Males present with a macroprolactinoma more frequently than females [22], and this may impact on the clinical manifestations of the disease. Larger tumor size in males is not primarily related to diagnostic delay, but rather to gender-related differences in tumor behavior. Retrospective analyses reported higher Ki67 (marker that reflects tumor cell proliferation and growth) in surgically resected macroadenomas from males compared to similar tumors from females [23, 24]. In general, half of the men with prolactinoma present with symptoms due to mass effect of the pituitary adenoma and the other half present with symptoms of hypogonadism including loss of libido, erectile dysfunction, infertility, gynecomastia, osteopenia, or more rarely with galactorrhea [25]. The level of hyperprolactinemia roughly correlates with the symptoms [26]. Erectile dysfunction, on the other hand, appears to be mostly caused by mechanisms not directly related to hypogonadism, as it is more likely corrected by prolactin control via dopamine agonist administration rather than by testosterone replacement [27].

The mass effects of a prolactinoma depend on the size, the location, and the anatomical extension of the adenoma. Among the possible symptoms, which are common to all pituitary adenomas, headache is a well-recognized hallmark of pituitary tumors and may be disabling [28]. The reported incidence of headache in adenomatous pituitary disease ranges with tumor type from 33% to 72% and was reported to be particularly high in prolactinomas [28]. It was previously thought that headache in pituitary adenomas is related to the size of the adenoma via dural stretch due to expansion of the lesion within the sella turcica. These compressive effects in turn stimulate the afferent fibers innervating the dura mater causing pain [29]. Also, an adenoma invading the cavernous sinus can explain the occurrence of headache since the cavernous sinus contains the ophthalmic branch of the trigeminal nerve and internal carotid artery. However, there are cases of noninvasive microprolactinoma manifesting with a severe headache that quickly resolve with the administration of dopamine agonist, suggesting that pituitary tumor-associated headache may derive also from locally effective neurotransmitters or neuromodulators rather than from anatomical reasons only [28]. Various types of headaches can occur to patients with prolactinoma, including chronic and episodic migraine [30], short-lasting unilateral neuralgiform headache attacks with conjunctival injection and tearing [31], cluster headache [32], and trigeminal neuralgia [33]. Visual field deficits, blurred vision, and decreased visual acuity are most likely to occur in patients with a large prolactinoma, and these clinical manifestations are due to the direct mass effect of these lesions. The typical visual field defect, bitemporal hemianopia, is due to the anatomical compression of the optic chiasm, which contains the crossing nasal fibers of

each optic nerve [34]. Nevertheless, the visual field defect depends on the relation between the optic chiasm and the adenoma itself. If the tumor is anterior to the optic chiasm or if the patient has an anatomical post-fixed chiasm, conditions such as central scotoma, arcuate scotoma, and monocular visual constriction can be noted [35].

Cranial nerve palsies are rare but occur especially with invasive tumors, including aggressive prolactinomas, or with pituitary apoplexy. Pituitary apoplexy although representing a more common complication of nonfunctioning pituitary adenomas than prolactinomas can happen in prolactinomas as well, especially in pregnant women who over-respond to dopamine or as an adverse effect of thyrotropin-releasing hormone (TRH) stimulation test. In its most dramatic presentation, pituitary apoplexy causes sudden onset of severe headache, diplopia due to pressure on the oculomotor nerves, and hypopituitarism with a possible life-threatening outcome [36].

Hydrocephalus may rarely occur through several anatomical mechanisms; for example, the adenoma may grow upward and compress the anterior horn of the lateral ventricle. The most common directional growth is upward and backward invading the suprasellar area at an early stage, and large tumors may invaginate the floor of the third ventricle possibly obstructing the aqueduct of Sylvius. Also, the cisterna chiasmatica and the cisterna interpeduncularis can be encroached or obliterated and, as a consequence, may determine the occurrence of hydrocephalus [37]. Very rare though, possible manifestations of prolactinomas with extrasellar extension may also include impaired hearing, unilateral hemiparesis, temporal lobe epilepsy, or even dementia due to frontal lobe extension [38].

6.5 Diagnosis

The hallmark of prolactinoma is hyperprolactinemia. Assay-specific normal values are gender-dependent with a higher normal range in women. In general, a prolactin level below 25 μg/L (530 mIU/L) is usually considered to be normal [39]. However, differences among laboratories and diagnostic kits exist and should be always carefully considered when comparing prolactin values. Diagnosis of hyperprolactinemia is determined by the measurement of basal prolactin, which roughly correlates with prolactinoma size. Ideally, serum sample should be obtained limiting as much stress as possible, while venipuncture is performed. Stimulatory or inhibitory dynamic tests of prolactin secretion following administration of TRH (stimulates), domperidone (stimulates), L-dopa (inhibits), or nomifensine (inhibits) are not superior to basal prolactin for the diagnosis of hyperprolactinemia and, moreover, may be risky for the patient [40]. Thus, no dynamic test is recommended for the diagnosis of hyperprolactinemia. Basal prolactin concentrations below 200 μg/L cannot discriminate prolactinoma-related hyperprolactinemia from physiological, pharmacological, or other secondary causes of hyperprolactinemia (Table 6.1). Grossly, higher prolactin levels tend to suggest an adenomatous prolactin hypersecretion, though this consideration cannot be taken as a definite rule.

Table 6.1 Possible causes of non-prolactinoma-dependent hyperprolactinemia

Physiological	Hypothalamic–pituitary stalk damage	Pituitary diseases or dysfunctions	Systemic conditions	Pharmacological causes with (with examples)
Coitus Exercise Lactation Pregnancy Sleep Venepuncture	Infiltrative diseases Radiations Rathke's cyst Traumas with pituitary stalk section, including local surgery Renal cell carcinoma Gonadoblastoma Ovarian teratoma Perivascular epithelioid cell tumors	Acromegaly Idiopathic Local surgery Lymphocytic hypophysitis Macroadenoma with pituitary stalk compression Multiple hormones secreting adenoma	Chest wall trauma Surgery HZV Chronic renal failure Liver cirrhosis Cranial radiation Epilepsy PCOS	*Anticonvulsants* (valproate) *Antipsychotics* Typical (prochlorperazine) Atypical (risperidone) *Antidepressants* Tricyclic antidepressants (amitriptyline) Selective serotonin reuptake inhibitors (sertraline) Monoamine oxidase inhibitors (pargyline) *Anti-H2 receptors* (cimetidine) *Antihypertensives* (verapamil, labetalol) *Prokinetic agents* (metoclopramide) *Miscellaneous* Estrogens (17α-ethynylestradiol) Opiates (morphine) Fenfluramine Isoniazid

In the absence of evident secondary causes of hyperprolactinemia, in doubtful situations, or in cases with mass-related symptoms, imaging should be performed. Magnetic resonance imaging (MRI) of the pituitary is the radiological investigation of choice. A targeted MRI examination of the pituitary region includes sagittal (Fig. 6.1), and transverse and coronal (Fig. 6.2) views with accurate field of view. T1 pre- and post-contrast images as well as dynamic contrast-enhanced coronal images are critical for the identification of small microadenomas.

In general, it is recommended to perform MRI in every patient with any degree of hyperprolactinemia if secondary causes are excluded or very unlikely. If MRI is contraindicated, contrast CT with direct coronal scans can be done, although it is less informative due to poorly accurate definition of the surrounding vasculature, the optic chiasm, and the cavernous sinus. CT also has limited accuracy for small microadenomas.

Despite the often-straightforward clinical presentation and diagnostic pathway, there are several pitfalls when suspecting a prolactinoma. For example, patients with very high prolactin levels may display the so-called hook effect [41, 42]. This effect can occur when very high serum prolactin (i.e., above 5000 ng/mL) saturates both the capture and signal antibodies used in immunoradiometric and chemiluminescent assays, preventing the binding in a "sandwich." The result reflects into a

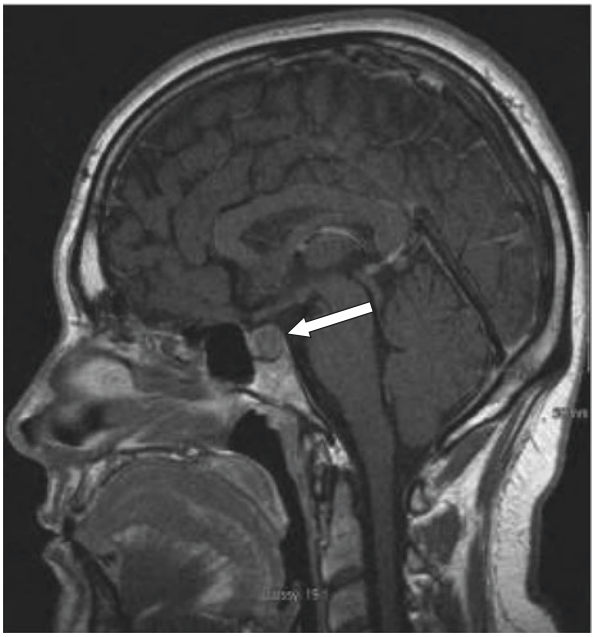

Fig. 6.1 Post-contrast sagittal T1 MRI showing a prolactinoma

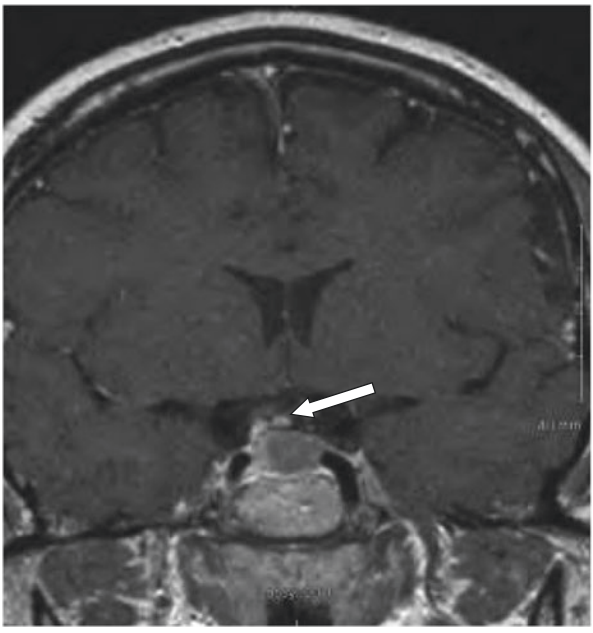

Fig. 6.2 Patchy enhancement of a prolactinoma post-IV contrast at T1W1 MRI. This prolactinoma measures 10.4 × 9 × 5.5 mm

lower than correct prolactin concentration, which is often only mildly or moderately elevated possibly misleading to a diagnosis of nonfunctioning adenoma. This laboratory artifact can be avoided by repeating the assay using a 1:100 dilution of serum [43, 44].

Another laboratory pitfall that may occur in the definition of the cause of hyperprolactinemia is represented by the presence of macroprolactin. Macroprolactin is native prolactin bound to immunoglobulin G and is usually 150–170 kDa in size compared with 23 kDa size of monomeric prolactin [45]. Macroprolactin causes a nonfunctionally active hyperprolactinemia through decreased prolactin clearance. This entity is not of clinical significance per se, but patients can be investigated and misdiagnosed with the obvious consequences of the same as if they were affected by hyperprolactinemia. The macroprolactinemia-induced misdiagnosis could be avoided by pretreating the serum with polyethylene glycol to precipitate the macroprolactin before performing the immunoassay for prolactin in the supernatant. Nowadays, this is standard practice for most laboratories in every patient with high prolactin.

The diagnostic approach in the suspicion of prolactinoma is summarized in Fig. 6.3.

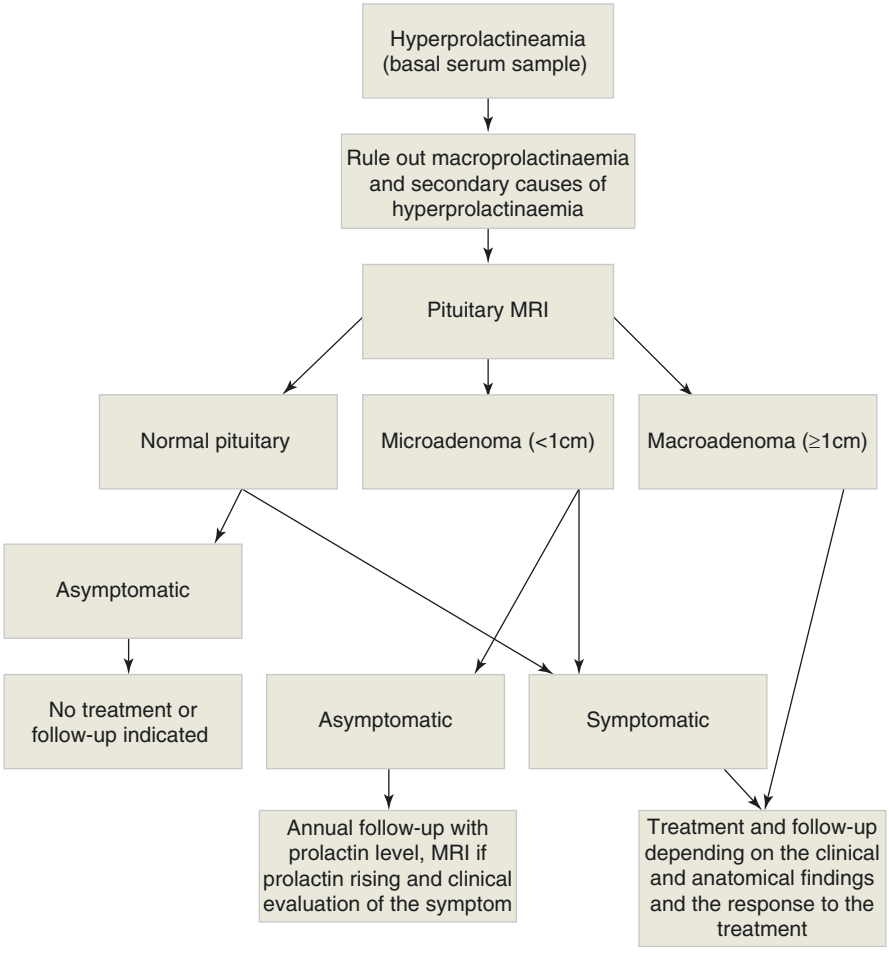

Fig. 6.3 The diagnostic algorithm of hyperprolactinemia

6.6 Management

The objectives of the treatment of prolactinoma are to correct the biochemical consequences of prolactin hypersecretion and to reduce the tumor mass with preservation of normal pituitary function (Table 6.2). Asymptomatic eugonadal women with microprolactinomas do not represent an absolute indication for treatment as it is very unlikely for a microprolactinoma to grow significantly without increased serum prolactin levels [46]. As such, a number of patients with microadenomas verified by imaging may be monitored with serial prolactin measurements. Microprolactinoma with an increase in size mandates treatment as there is a tendency to become a macroadenoma, regardless of the symptoms and the prolactin levels. However, a significant increase in prolactin levels usually, though not always, reflects tumor growth. Women and men who are hypogonadal should be treated as hyperprolactinemia is associated with impairment of fertility and often also sexual functions. In hypogonadal women with microadenoma for which fertility is not a concern, the management options may include estrogen replacement therapy, providing that prolactin levels do not increase substantially and there is no evidence of tumor enlargement [47], or dopamine agonist, which remains the preferred therapeutic option anyway.

Macroprolactinomas are more likely to grow and become aggressive [23]. On the top of this, most macroprolactinomas are associated with prolactin elevation significant enough to elicit symptoms that would require treatment.

Dopamine agonists (DAs) are the first-line treatment for prolactinoma as they are proven to resolve the hyperprolactinemic symptoms in most cases and reduce the tumor size [8, 48]. DAs inhibit prolactin synthesis and secretion by binding to and activating dopamine D2 receptors on the pituitary lactotroph cell membrane. Dopamine and its agonists bind to D2 receptors that are functionally linked to membrane channels and G proteins and suppress the high intrinsic secretory activity of the pituitary lactotrophs. In addition to inhibiting PRL release by controlling calcium fluxes, DA activates several interacting intracellular signaling pathways and suppresses PRL gene expression and lactotroph proliferation [49]. It can also induce the apoptosis of lactotroph cells [50]. Several DAs are or have been available on the market upon prescription for the medical treatment of prolactinomas such as bromocriptine, cabergoline, pergolide, and quinagolide. Factors in favor of success of the treatment with a DA include continuous DA treatment for at least 2 years,

Table 6.2 Indications for therapy in patients with prolactinomas

Enlarging microadenoma
Macroadenoma
Infertility
Amenorrhea or oligomenorrhea
Acne and/or hirsutism
Troublesome galactorrhea
Gynecomastia
Testosterone deficiency

MRI images of the pituitary gland

low-normal prolactin levels with the lowest dose of DA, and a reduction in tumor size of at least 50% with no evidence of cavernous sinus invasion. These patients require long-term follow-up with prolactin measurements every 3 months for the first year and annually thereafter, and review of the pituitary gland by MRI, yearly initially, or if prolactin increases above normal [51]. Among the DA, the use of cabergoline rather than bromocriptine seems to be characterized by higher chances of therapeutic success.

Bromocriptine was the first DA introduced to treat prolactinomas. Bromocriptine is an ergot derivative with D1 and D2 receptor agonist properties, has a short half-life of 3–4 h, and should be taken two or three times per day. Bromocriptine normalized serum prolactin in 78% and 72% of patients with microprolactinomas and macroprolactinomas, respectively [52]. It has been found to be able to restore ovulation in about 80% of women [53]. The majority of macroprolactinomas treated with bromocriptine show some degree of reduction in size as assessed by MRI [54].

Cabergoline is an ergot derivative, which is more selective for D2 receptors and has a long in vivo half-life, which permits once or twice weekly administration. The long duration of action stems from its slow elimination from the pituitary tissue due to its high-affinity binding to the dopamine receptors in the pituitary. It is highly effective in normalizing serum prolactin levels, reducing tumor mass, and restoring the gonadal function. Roughly, cabergoline treatment may normalize prolactin levels in 92% of patients with idiopathic hyperprolactinemia or microprolactinomas, 77% of patients with macroprolactinomas, and 86% of the overall study population [55]. The tumor size reduction rate has been estimated ranging between 50% and 100% [56]. Overall, cabergoline is superior to bromocriptine as it has greater affinity and selectivity for the pituitary dopamine D2 receptors and longer duration of action, presents a better tolerability profile, and achieves a higher rate of shrinkage of the prolactin-secreting adenoma [57]. Cabergoline is the first-choice drug for the management of patients with prolactinoma who require medical treatment.

Pergolide is a long-acting ergot derivative acting as an agonist of D1 and D2 receptors, and also 5-HT receptors. It has comparable efficacy and tolerance with bromocriptine, including tumor size reduction [58]. However, also because of concern regarding valvular abnormalities in patients with Parkinson disease taking high doses of pergolide, this drug is not used for the treatment of prolactinomas anymore.

Quinagolide is a non-ergot derivative DA with selective D2 receptor activity and relatively long duration of action. Its efficacy in normalizing prolactin levels and reducing tumor size is at least as good as that of bromocriptine and pergolide [56]. Furthermore, approximately 40% of patients resistant to bromocriptine respond to quinagolide [59]. This medication is not available in the USA, but available in some EU countries.

The principal side effects of DA are nausea, postural hypotension, and mental fogginess [60]. Less common side effects include nasal stuffiness, depression, Raynaud phenomenon, alcohol intolerance, and constipation. Nausea appears to be more common with bromocriptine than cabergoline. Symptoms of psychosis or exacerbation of pre-existing psychosis and impulse control disorders, such as pathological gambling, hypersexuality, or compulsive shopping or eating, have also been

shown to be associated with both bromocriptine and cabergoline. Ergot derivative DAs (cabergoline, bromocriptine, and pergolide) are potentially associated with an increased risk of dose-dependent valvular heart diseases [61]. However, no real concern for prolactinoma patients treated with a DA has been raised so far in the routine clinical practice [62] and the occurrence of DA-related valvular disease seems to basically occur in patients with Parkinson disease receiving a high cumulative dose of DA [62].

A subset of individuals with prolactinomas does not respond satisfactorily to DA. Prolactinomas exhibit varying degrees of responsiveness to DAs, ranging from the complete response at one end of the spectrum to total resistance at the other end. DA resistance is defined as failure to normalize prolactin levels and failure to decrease tumor size by 50% [63]. The factors determining the sensitivity of a prolactinoma to DA treatment are multiple, with the D2 receptor expression and sensitivity at the levels of the prolactinoma cells playing a key role. The estimated prevalence of DA resistance is approximately 24% for bromocriptine and 11% for cabergoline [56]. The management of drug-resistant prolactinomas includes several options. Any bromocriptine-resistant patient should be switched to cabergoline, which will likely normalize prolactin in 80% of patients. As long as adverse effects do not develop, dose escalation of cabergoline is reasonable, with the expectation that subsequent dose reduction will be possible, if and when appropriate. Echocardiographic monitoring is advised in patients taking high cabergoline dose because of the potential association with cardiac valvular fibrosis. Of note, complete resistance to cabergoline is infrequent [64]. Doses of cabergoline of up to 2.0 mg/week are usually effective in controlling prolactin secretion and reducing tumor size in most prolactinomas [65]. The clinical presentation, management, and outcome of patients that are not well controlled by the commonly used dose of DA-resistant patients are still largely undefined [65]. DA resistance is rarely encountered in microprolactinomas and is more frequent in cases of macroprolactinomas (3–6%). Often, it is characteristic of invasive tumors and associated with male gender [66]. Molecular mechanisms implicated in DA resistance likely encompass a diverse set of alterations, and genetic heterogeneity found among sparsely available surgical specimens complicates the assessment of individual factors responsible for aggressive behavior and drug resistance of prolactinomas [67].

Historically, surgical resection was the preferred treatment of prolactinoma, like for the other pituitary adenomas, before discovering the high efficacy of the DA in lowering prolactin levels, reducing prolactinoma size, and restoring gonadal function [44, 56]. Since DAs are so highly effective, recognized indications for surgery of prolactinoma have become rare, usually targeting specific situations where medical treatment is failing. Nowadays, surgical tumor resection is indeed indicated in the prolactinoma patients with severe intolerance or resistance to DA, in patients with acute complications such as apoplexy or cerebrospinal fluid leak, or in young patients with a likely resectable adenoma and who do not wish to take long-term medical treatment [44, 56, 68]. In the last decade, a number of studies have also raised the concern of an increased risk for cardiac valve disease with the prolonged use of DA with partial serotoninergic activity [61, 69]. However, this does not

appear being a real issue in patients with prolactinomas, while it may be a serious concern in patients exposed to higher DA cumulative dose, like those affected by Parkinson's disease. Also, emerging evidence has been provided for a link between chronic DA therapy and impulsive behavior disturbances such as gambling, hypersexuality, or bulimia [70, 71]. Transsphenoidal approach represents the standard of surgical care for the prolactinomas requiring surgery, in particular for the overwhelming majority of macroprolactinomas. Craniotomy is reserved only for tumors requiring neurosurgery and that are inaccessible via the transsphenoidal approach. On the top of the invasiveness of the tumor, surgical outcomes are highly dependent upon the expertise and experience of the neurosurgeon, as well as the tumor size.

Radiotherapy, i.e., external beam radiation therapy or stereotactic radiosurgery, is only rarely used in patients with prolactinomas and should be reserved for those tumors that do not respond to DAs, recur or progress after surgery, are highly aggressive and/or malignant, or in cases where surgery cannot be an option [72]. Prolactinoma patients who recur after prior treatment with radiotherapy may also be potentially salvaged with radiosurgery. The most frequent long-term morbidity of radiotherapy is radiation-induced hypopituitarism, like for every kind of pituitary adenoma undergoing radiation [73].

Experimental data have demonstrated that different somatostatin receptor (SSTR) subtypes are expressed at various levels in prolactinomas, SSTR5 being the most important in the regulation of prolactin secretion [74, 75]. The most used somatostatin analogs, octreotide and lanreotide [76], which are the cornerstone of the medical treatment for the control of GH secretion in acromegaly, bind efficiently to SSTR2 and with lower affinity to SSTR5. Somatostatin suppresses in vitro prolactin secretion from prolactinoma cultures [77]. However, somatostatin and octreotide minimally alter serum prolactin levels in prolactinoma patients [78]. It appears that only SSTR5-selective agonists (BIM-23052, BIM-23268) can efficiently suppress prolactin release from adenoma cells [75]. SSTR5-specific agonists might potentially be effective in the treatment of PRL-secreting pituitary adenomas and could be tested in vivo in patients with dopamine agonist-resistant prolactinomas [75]. A few years ago, hybrid molecules such as dopastatin, a somatostatin, and dopamine receptor agonist with high binding affinity for SSTRs and D2DR have been developed. These molecules, when tested in culture studies from mixed GH/PRL-secreting tumors partially responsive to the agonists taken individually, showed a greater efficacy in suppressing GH or PRL secretion [79]. Unfortunately, this therapeutic potential did not translate into a clinical effect due to in vivo pharmacological issues.

As a newcomer in the scenario of the medical treatment of hyperprolactinemia and prolactinomas, phytotherapy should be taken into consideration, and some phytotherapic agents have lately made their appearance among the potentially available therapeutic tools. For example, Vitex agnus-castus extracts, traditionally used for the treatment of menstrual disorders and premenstrual syndrome, have been used also for the treatment of hyperprolactinemia [80]. Good tolerability and substantial lack of significant side effects and drug interactions are the advantages of a trial of Vitex agnus-castus in patients willing to try an alternative approach for the

management of their prolactinoma. However, no univocal evidence-based efficacy of Vitex agnus-castus extracts has been demonstrated yet.

6.7 Peculiar Aspects

6.7.1 Prolactinoma in Pregnancy

Prolactin physiologically increases during pregnancy and lactation, and therefore, it does not reliably reflect an increase in tumor size in pregnant prolactinoma patients and it cannot be useful for clinical assessment during pregnancy and lactation. Prolactinoma cells express estrogen receptors [81], and as a result of the increased estrogen level during pregnancy [44] (and eventually of cessation of previous DA treatment), there can be a substantial increase in the volume of the prolactinoma, with a progressive increase in serum prolactin due to lactotroph cell hyperplasia [82]. The main concern relates to the possible prolactinoma enlargement during pregnancy. The risk of tumor enlargement during pregnancy largely depends on adenoma size. Data in the literature indicate that although the average adenoma enlargement is only 3% for microprolactinomas, it can be as high as 32% for macroprolactinomas that were not previously operated on [56, 83].

In patients at risk of an increase in the size of their prolactinoma, definitive treatment by transsphenoidal surgery should be considered and ideally performed before attempting the pregnancy [84]. The treatment of pregnant women must be tailored to the individual patient, though it is generally advised against medical treatment throughout pregnancy and lactation in women with a microprolactinoma and in most women with macroprolactinoma not affecting the optic chiasm and presenting without any sign of invasiveness. On the other hand, in women with an invasive prolactinoma or with a macroprolactinoma abutting the optic chiasm or the cavernous sinus, continuation of the DA treatment may be preferred [83]. In the context of the very low risk of microprolactinoma enlargement during pregnancy, there is considerable evidence supporting the discontinuation of DA treatment once pregnancy is confirmed. In such a case, the patient should be told that the risk of enlargement of the adenoma during her pregnancy is indeed very small and medical treatment would not be required during her pregnancy. However, patients should be advised to report for urgent assessment in case of new symptoms such as severe headache or visual disturbances [44]. The patient should undergo baseline formal visual field testing at the time of diagnosis and should be followed clinically every 2–3 months during pregnancy. In case the patient becomes symptomatic with visual disturbance or progressive headaches, an MRI should be performed to assess changes in tumor size. Otherwise, no MRI follow-up is usually recommended during pregnancy. If substantial growth of the prolactinoma is clinically and radiologically evident, a suitable treatment option should be considered. The most straightforward option is represented by the DA treatment restoration. Traditionally, bromocriptine was the preferred option since there was a larger experience on the use of this molecule in such a specific setting

[44, 85]. However, in the last two decades, cabergoline has become the first-choice DA because of its higher therapeutic ratio. Experience with both drugs shows no increase in spontaneous abortions, preterm deliveries, multiple births, or congenital malformations, compared to what is expected in the normal population [84, 86]. Some selected pregnant women with macroprolactinoma may require continuation of the DA throughout the pregnancy [44]. If an enlarging prolactinoma does not respond to DA therapy within 2–3 weeks from the restoration of the medical treatment, transsphenoidal surgery, preferably in the second trimester, should be considered. If the pregnancy is approaching the time of delivery, partum induction would be a reasonable option [87].

Treatment discontinuation is recommended at the time of delivery in women with microprolactinoma or non-compressive macroprolactinoma. For microprolactinomas, the risk of symptomatic tumor enlargement during pregnancy is very low (2–3%). It is higher for macroprolactinomas (20–30%) and careful follow-up is advised, including MRI without contrast injection if symptoms or visual disturbances develop. If a symptomatic tumor enlargement does occur, reinitiation of the dopamine agonist (BRC or CAB) is indicated rather than surgery. Breastfeeding has no harmful effect on tumor growth and DA treatment, if still needed, may be postponed as long as breastfeeding is desired. Finally, about 40% of women with a microprolactinoma or an intermediate size macroprolactinoma may be in prolonged remission after one or more pregnancies [86].

6.7.2 Giant Prolactinoma

Giant prolactinoma is defined as a prolactin-secreting adenoma with a diameter of 40 mm, at least. They usually present with massive extrasellar extension, baseline prolactin concentration of 1000 ng/mL or more, and no concomitant GH or ACTH secretion. They are much more frequent in young to middle-aged men than in women, with a male-to-female ratio of about 9:1 and a mean age around 40 years [38, 88, 89]. A giant prolactinomas have a challenging management as the therapeutic goals differ from the one of a smaller macroprolactinoma where normalization of prolactin levels, restoration of eugonadism, and reduction in tumor size are achievable targets. In giant prolactinomas, the priority of the treatment is often to obtain a rapid alleviation of the neurological symptoms, or of the risk of the same, and other complications due to encroachment upon or invasion of the prolactinoma into surrounding nervous and vascular structures. Furthermore, in longstanding tumors the reduction in the size may not reverse visual field defects or hypopituitarism. In this case, a reasonable goal may be represented just by the prevention of further growth of the prolactinoma and the replacement of the pituitary hormones, which eventually became insufficient, if indicated. DAs are the recommended initial modality of treatment of giant prolactinomas and surgery should be reserved for those patients who demonstrate inadequate responses to medical therapy [38, 90]. Even if surgery may be

necessary at some point with a debulking purpose, it may be curative only in exceptionally rare cases and reinitiating dopamine agonist is usually required for the treatment of persistent hyperprolactinemia, though possibly with higher chances of therapeutic response.

6.7.3 Malignant Prolactinomas

Malignant prolactinomas are very rare tumors, defined by the occurrence of distant cerebrospinal, meningeal, and/or systemic metastases [2]. They differ from invasive pituitary tumors, which remain contiguous with the primary tumor site. Malignant prolactinomas may occur at any age, even in children [91], but mostly develop in the fifth or sixth decade of life [92]. Their exact incidence is not precisely known, but overall, pituitary carcinomas account for only 0.1–0.2% of all pituitary tumors, and prolactinomas correspond to approximately one-third of these.

There are currently no reliable pathological markers, whereby the malignant potential of a prolactinoma can be predicted [2]. The diagnosis of an aggressive pituitary tumor should be considered in patients with a radiologically invasive tumor and/or with an unusually rapid tumor growth rate, or in those patients with a clinically relevant tumor growth despite optimal standard treatment (surgery, radiotherapy, and conventional medical treatment). MRI should be used for quantification of tumor dimensions, invasion, and growth [93]. Dopamine agonists are recommended standard medical treatment with maximally tolerated doses in order to control tumor growth [93]. Unfortunately, resistance to DA occurs very frequently in such clinical scenario, and once cerebrospinal or systemic metastases become obvious, the potentially effective therapeutic options are limited, and the treatment is mostly palliative. Chemotherapy including procarbazine, vincristine, cisplatinum, and etoposide has been used with little effect [94]. Temozolomide monotherapy should be used as first-line chemotherapy for aggressive pituitary tumors and pituitary carcinomas, following documented tumor growth [93, 95]. Temozolomide has been shown to reduce prolactin levels and control tumor growth if tumor specimens do not express methylguanine-DNA methyltransferase (MGMT) [96]. Surgery may be useful in debulking the lesion and relieving local compressive effects, if required. Surgery should be performed by a neurosurgeon with extensive experience in pituitary surgery [93]. The expected survival of patients with a diagnosis of malignant prolactinoma, which is made at the time of the detection of metastatic disease, is approximately 1 year [94].

6.7.4 Inherited Prolactinomas

Prolactinoma is most frequently a sporadic disease. However, it may occur also as part of an inherited syndrome. Roughly, inherited prolactinomas behave more aggressively and are larger than their sporadic counterpart. Prolactinomas occur in

15–25% of patients with MEN-1 and represent the most frequent pituitary adenoma type observed in the setting of this syndrome [92, 97]. The general treatment strategy for prolactinomas in patients with MEN-1 does not differ from that for sporadic prolactinomas, but more intensive pharmacologic therapy or the use of multiple therapeutic modalities may be required to achieve a fully satisfactory outcome [98].

Prolactinomas in MEN-4, an endocrine tumor syndrome more recently described, is caused by germline mutations in putative tumor suppressor gene CDKN1B and is characterized by primary hyperparathyroidism followed by pituitary adenoma [99].

Prolactinomas may also occur—although rarely—in Carney complex, well-characterized autosomal dominant disorder presents with a clinical picture of, or predisposition to, several types of tumors arising from a number of sites, including the pituitary, thyroid, testes, and ovaries [100].

AIP- related isolated familial pituitary adenoma is defined as the presence of an AIP germline pathogenic variant in an individual with a pituitary adenoma [101]. The most commonly occurring pituitary adenomas in this disorder are GH-secreting adenomas, followed by prolactinomas, mixed GH and prolactin co-secreting adenomas, and nonfunctioning pituitary adenomas [101].

As a matter of good practice, the presence of pituitary adenomas in the family of a patient with prolactinoma, like any other pituitary adenoma, should always be explored by anamnesis and in case of positive findings a more specific and targeted investigation of the possible familial issue should be carefully carried out.

6.8 Prognosis

The majority of patients with microprolactinomas have an excellent prognosis. These patients can be managed medically also for extended periods, if required, though often can be medically cured in a few years' time [12]. Macroprolactinomas, on the other hand, can grow over time and require more aggressive treatment, with lower success rate in curative terms, and definitely require a more careful follow-up than their sub-centimeter counterpart. The growth rate of macroprolactinomas is unpredictable, and thus, the patient must be closely followed up. The decision to taper medical therapy requires sound judgment because the tumor can grow in size if without treatment or after DA withdrawal [102].

Once prolactin levels have reached normal levels, or in some cases near-normal, they can be monitored every 3–6 months for the first year and then every 6–12 months thereafter. Macroadenoma tumor size can be monitored by serial yearly MRI scans, and once maximal size reduction has been documented and deemed stabilizing, further scans may not be necessary as long as prolactin levels are being monitored. Whether a follow-up MRI scan is necessary in patient with microadenomas is debatable, if prolactin levels are regularly monitored. It is extremely rare for a tumor to increase in size without evidence of a significant increase in prolactin levels. Visual field testing should be repeated until they normalize or remain stable and then do not need to be repeated [8].

In the PROLEARS study, patients with a microprolactinoma had no increased mortality, which is reassuring as this is the most common type of pituitary adenoma associated with hyperprolactinemia, while patients with hyperprolactinemia due to a macroprolactinoma had increased mortality [103]. There are insufficient data to explore the possible reasons for such increased risk of death, although premature mortality has been generally reported in patients with a pituitary macroadenoma. For example, other hormonal deficiencies as a result of hypopituitarism or excessive steroid replacement in the past may contribute to adverse health outcomes [104].

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Cushing's Disease

7

Anna Aulinas and Susan M. Webb

7.1 Introduction

Cushing's syndrome (CS) constitutes a group of signs and symptoms due to extended and inappropriately high exposure to excess glucocorticoids (GCs). Iatrogenic corticosteroid administration is the most common cause of CS, and pituitary corticotroph adenoma (Cushing's disease; CD) is the most frequent cause of endogenous excessive GC secretion. CD is a rare and severe disease, and chronic exposure to high GC levels has been associated with increased multisystemic morbidity and mortality. Therefore, an early diagnosis and treatment are mandatory to avoid long-term complications. Although most of the related comorbidities improve after initial therapy of hypercortisolism, many of them are not completely reversible and a lifelong follow-up is necessary to control comorbidities and rule out potential recurrences. Therefore, prompt identification and treatment of hypercortisolism and its comorbidities are important to reduce mortality. This chapter addresses epidemiology, diagnoses, treatment, outcomes, and follow-up of Cushing's disease.

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7.2 Epidemiology

Cushing's syndrome is considered a rare disorder with an estimated incidence between 0.5 and 3.0/10^6 people/year; however, accurate data on prevalence and its incidence in the general population are scarce [1]. Only countries with national registries or databases provide information on its incidence and prevalence (i.e., incidence ranged from 1.2 to 1.7/10^6 inhabitants/year in Denmark [2]; standardized incidence rate (SIR) of 0.8/10^5/year in Iceland [3]; and SIR of 0.07/10^5/year in Malta [4]). The most frequent cause of endogenous hypercortisolism is an ACTH-producing pituitary adenoma, namely Cushing's disease (CD). The incidence according to an 11-year period population-based study in Denmark is 1.2–1.7/10^6/year for CD, followed by an incidence of 0.6/10^6/year for adrenal adenomas [2]. Other types of CS are extremely rare. Some data indicate a high proportion of subclinical CS in risk populations such as patients with uncontrolled type 2 diabetes or early-onset osteoporosis [5]. CS is more frequent in females with an estimated female:male ratio of 3:1 and median age at diagnosis of around 40 years [1, 6]. CS is associated with increased mortality (SMR of 3.7 (2.3–5.3)); mortality rates are increased during the first year after diagnosis and in those patients with persistent hypercortisolism after initial therapy [2]. Although mortality risk decreases with remission of hypercortisolism, it still remains increased compared to general population, with circulatory diseases being the most important cause of mortality [7, 8].

7.3 Pathogenesis

Table 7.1 summarizes the different causes of CS. In adult population, the most frequent cause of endogenous CS is an adrenocorticotropic (ACTH)-dependent CS; a corticotrope pituitary adenoma (CD) is the cause of about 70% of ACTH-dependent CS, followed by an ectopic (extrapituitary) ACTH-secreting tumor [1]. CD is characterized by an excessive production of ACTH due to an ACTH-producing pituitary adenoma (90% of which are microadenomas), which induces high levels of cortisol and androgens, often accompanied by diffuse adrenal hyperplasia [9].

The molecular pathophysiology of CD is not completely understood. However, the identification of some molecular genetic abnormalities has provided new insight into the pathogenesis of pituitary ACTH-producing adenomas. Most pituitary tumors are sporadic as a consequence of monoclonal expansion of a mutated cell. CD rarely occurs, although cases have been described, in the context of germline mutations like multiple endocrine neoplasia (MEN) type 1, aryl hydrocarbon receptor-interacting protein (AIP), and cyclin-dependent kinase inhibitor (CDKN1B) genes in patients with MEN1 or MEN4 features and in patients with succinate dehydrogenase subunit mutations [10]. In contrast, somatic mutations of MEN1 or AIP have not been found in sporadic pituitary adenomas or CD. Instead, very rare mutations of the GC receptor gene or proteins related to GC8 receptor functions have been found in CD [11]. Inactivation of the ubiquitin-specific peptidase 8 gene (USP8), present in 35–62% of the corticotroph adenomas, is responsible for the

Table 7.1 Causes and prevalence of endogenous Cushing's syndrome

Etiology	Prevalence	Features
ACTH-dependent	**70–80%**	
Cushing's disease (Corticotroph adenoma)	60–70%	Age peak around 40 years. F:M ratio 3:1. 50% tumors not visible on MRI. Very rare corticotroph hyperplasia
Ectopic source (ACTH, very rare CRH)	10–15%	Age peak benign 30–40 years. Malignant 50–60 years. Slightly more prevalent in males. Most frequent sources: Small cell lung carcinoma, neuroendocrine tumors of lung, thymus, pancreas
ACTH-independent	**20–30%**	
Adrenal adenoma	10–20%	Age peak 40–50 years. F:M ratio 6:1
Adrenal carcinoma	5–7%	Age peak <10, 50–60 years. F:M ratio 2:1. Mixed cortisol and androgen production is common
Macronodular adrenal hyperplasia (aberrant G-protein-coupled receptors, autocrine ACTH production, sporadic or familiar ARMC5)	<2%	Age peak 50–60 years. F:M ratio 2.5:1. Autocrine or paracrine ACTH might be produced and contribute to cortisol secretion (ACTH-independent classification might be modified in the future). Bilateral involvement
Micronodular adrenal hyperplasia (primary pigmented nodular adrenocortical disease, isolated or familial with Carney complex)	<1%	Age peak 10–30 years. F:M ratio 0.5:1 if <12 years and 2:1 if >12 years. Adrenal size often normal. Bilateral involvement
McCune Albright syndrome	Very rare	Infants (<6 months), F:M ratio 1:1. Internodular adrenal atrophy

[a]*ACTH* adrenocorticotropic hormone, *CRH* corticotropin-releasing hormone, *F:M* female:male, *ARMC5* armadillo repeated containing 5 gene

increased expression of EGFR and ultimately ACTH synthesis. Epidermal growth factor receptor (EGFR) and pituitary-transforming gene (PTTG) can be overexpressed and play a causal role in the development of CD and defects in this pathway may also be a key to therapeutic targets. In animal models, a PTTG inhibitor (R-roscovitine) or an inhibitor of the EGFR inhibitor (gefitinib) inhibited corticotroph tumor growth and features of hypercortisolism [10], nevertheless, the translation of these findings in humans is still unknown.

7.4 Clinical Presentation

Manifestations of hypercortisolism are systemic and symptoms at clinical presentation may be unspecific and highly variable, and the diagnosis of the disease is often delayed up to several months or years. There is no pathognomonic sign or symptom. Table 7.2 summarizes the most frequent clinical presentation according to different series [1]. The most common features at diagnosis are weight gain, hypertension, skin abnormalities, myopathy, and menstrual irregularities. Hypertension and hirsutism present more frequently in ectopic CS than in other causes.

Table 7.2 Clinical features and signs of Cushing's syndrome (CS) and overlapping conditions and prevalence (%)

Features that best discriminate CS (higher specificity)	Features also common in the general population and less discriminatory of CS (lower specificity)
Decreasing growth in children (100%)	Weight gain or obesity (95%) (dorsocervical fat pad, facial fullness)
Facial plethora (90%)	Decreased libido (90%)
Easy bruising and skin atrophy (80%)	Menstrual abnormalities (80%)
Proximal myopathy and muscle weakness (60%)	Hirsutism, acne, or female balding (75%)
Reddish purple striae (>1 cm wide) (60%)	Depression, irritability, impaired memory (70%)
	Early onset hypertension (75%), hyperlipidemia (70%), glucose intolerance (60%), osteoporosis (50%)
	Kidney stones (50%)

Table 7.3 Physiological or pathological conditions associated with hypercortisolism in the absence of Cushing's syndrome (CS) features; however, if there is high clinical suspicion of CS, patients should be screened for hypercortisolism, especially in the pseudo-Cushing group

Unlikely to have clinical features of CS	Some clinical features of CS may be present (pseudo-Cushing)
Physical stress (pain, surgery)	Pregnancy
Malnutrition	Depression, bipolar disorder
Hypothalamic amenorrhea (anorexia nervosa)	Morbid obesity
Intense chronic physical exercise	Alcohol abuse
Cortisol binding globulin excess in serum	Poorly controlled diabetes
	Glucocorticoid resistance

Several signs and symptoms are more suggestive and specific of hypercortisolism due to catabolic effects of cortisol on skeletal muscles, skin, and connective tissue, including skin atrophy, easy bruising with no trauma, proximal muscle weakness, or unexplained osteoporosis. Increased and impaired distribution of fat depots is one of the most precocious signs, with abnormally increased fat in the trunk, face, and neck. Wide reddish-purple striae of more than 1 cm on the abdomen and limbs and proximal muscle weakness are characteristic features seen in CS. Lower bone mineral density and a high prevalence of fractures are common in CS. Hirsutism, menstrual irregularities, obesity, diabetes, and low libido may also be present, but are also frequent in the general population. Psychopathology (emotional lability, cognitive disturbances, anxiety, depression) is very common. Metabolic syndrome features (dyslipidemia, impaired glucose metabolism, hypertension) together with an hypercoagulability state lead to an increased cardiovascular risk.

Usually, progression of symptomatology is slow, except for ectopic ACTH CS due to a paraneoplastic manifestation of a malignant tumor, where clinical presentation is usually rapid with prominent myopathy, edema, hyperpigmentation, and hypokalemia.

Several physiological and pathological situations can produce an overactivity of the hypothalamic–pituitary–adrenal axis (Table 7.3). Hypercortisolism without signs or symptoms of CS might be found in physical stress (surgery, pain), anorexia

nervosa, malnutrition, cortisol-binding globulin elevation, or hypothalamic amenorrhea; hypercortisolism with mild signs/symptoms of CS might be seen in psychiatric disorders (depression, bipolar disorder), alcoholism, pregnancy, morbid obesity, or poor controlled diabetes mellitus.

7.5 Diagnosis

In this first step of diagnosing CS, hypercortisolism needs to be confirmed using screening tools. Once CS is diagnosed, the cause must be identified to determine the specific etiology in order to guide treatment decisions.

7.5.1 Establishing the Diagnosis of Cushing's Syndrome

Screening is recommended in individuals with a high risk of CS, after excluding current or recent use of any type of exogenous GC (oral, inhaled, topic, rectal, parenteral) and high dose of progestogens [12, 13]. In children with decreasing height percentile together with progressive obesity, suspicion should be raised for CS. In addition, in young adults with hypertension or osteopenia, in patients with adrenal incidentaloma, and in women with menstrual irregularities, screening for CS may be indicated, especially if patients have additional features of the syndrome. Some studies have revealed a higher prevalence of "occult CS" in patients with poorly controlled T2DM or early-onset osteoporosis; however, there is no strong evidence to support a routine screening for occult CS in these patients based on the data available currently [14–17].

Specificity and sensitivity of screening tools vary enormously across studies, and it is often necessary to perform several tests before reaching a diagnosis. Specificity is not optimal, and false positives may occur. Moreover, the use of screening tools and their variability vary across countries [18]. Importantly, it is important to recognize the impact of physiological stress on cortisol levels when interpreting results. Recommended screening tests are outlined in Fig. 7.1:

- *24-hour urinary free cortisol* (UFC; at least two measurements) measures the cortisol that is not bound to cortisol-binding globulin (CBG) and thus filtered unchanged by the kidney. Therefore, UFC is not affected by medications or conditions that alter CBG. Concomitant measurement of urine creatinine helps to identify if collection is complete and to interpret collections with excessive volume (false positives with a 24-h diuresis of >5 l/day) [19]. False negatives may be seen in chronic kidney disease, if GFR < 60 ml/min and in cases with mild CS, in whom late-night salivary cortisol (LNSC) might be more useful [13, 20]. Importantly, the use of carbamazepine and fenofibrate or licorice can increase UFC levels.
- *1 mg overnight dexamethasone suppression test* (DST; alternatively, 2 mg/day for 48 h). In healthy individuals, the administration of supraphysiological GC

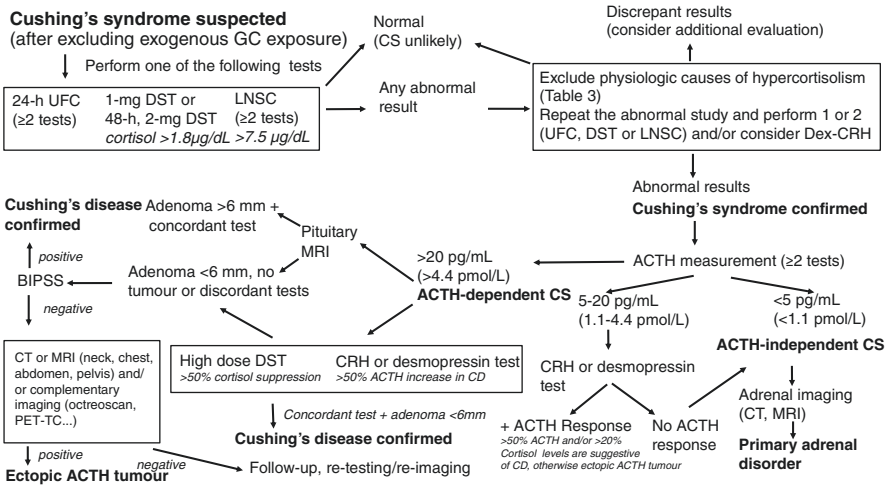

Fig. 7.1 Algorithm for testing patients when Cushing's syndrome (CS) is suspected and for the differential diagnoses of different etiologies once Cushing's syndrome is confirmed. *GC* glucocorticoids, *UFC* urinary free cortisol, *DST* dexamethasone suppression test, *LNSC* late-night salivary cortisol, *Dex-CRH* dexamethasone–corticotropin-releasing hormone test, *ACTH* adrenocorticotropic hormone, *CD* Cushing's disease, *BIPSS* bilateral inferior petrosal sinus sampling, *CT* computed tomography

Table 7.4 Drugs that may interfere with dexamethasone metabolism and alter dexamethasone suppression test (DST) results

Accelerate dexamethasone metabolism by induction of CYP3A4 (causing false-positive results in DST)	Impair dexamethasone metabolism by inhibition of CYP3A4 (causing false-negative results in DST)
Carbamazepine Phenobarbital Phenytoin Primidone Pioglitazone Rifampicin Ethosuximide Rifapentine	Fluoxetine Ritonavir Diltiazem Itraconazole Cimetidine Aprepitant/fosaprepitant

doses suppresses ACTH and cortisol secretion, while in CD patients there is a failure of this suppression when low doses of dexamethasone are given. Variable absorption, use of concomitant drugs, and renal or liver failure may influence the results of DST. Some drugs enhance hepatic enzymatic clearance of dexamethasone, reducing plasma dexamethasone concentration, and ultimately causing false-positive results of the DST, as well as alcohol abuse (2 weeks of alcohol abstinence is recommended to reduce false-positive results). On the contrary, other agents impair dexamethasone metabolism, increasing plasma dexamethasone levels and causing false-positive results in DST (Table 7.4). Some authors advocate for simultaneous measurement of cortisol and dexamethasone concentrations to ensure appropriate plasma dexamethasone levels (>3.3 nmol/l (>0.13 μg/dl)); however, dexamethasone measurement is not available in some countries [21]. The recommended serum cortisol cutoff for suppression is 50 nmol/l (1.8 μg/dl) to maximize the sensitivity as a screening tool.

– *Late-night salivary cortisol* (LNSC; two measurements) is a useful tool to assess the loss of circadian rhythmicity (the absence of LNSC nadir), a consistent abnormality in patients with CS. Salivary cortisol concentrations are in equilibrium with serum active free cortisol, there is a good correlation in cortisol concentrations between both specimens, and salivary cortisol levels are not affected by the rate of saliva production [22]. It is important to note that circadian rhythm might be altered in shift workers, smokers, patients with depression, or in those who are critically ill. Also, false-positive results are commonly seen in patients with obesity or diabetes [23].

To optimize sensitivity of screening tests, it is recommended to use the upper limit of the reference range for UFC and LNSC and a fasting cortisol lower than 50 nmol/L (1.8 μg/dl) following DST. Two abnormal screening tests are enough to confirm hypercortisolism in individuals with high pretest probability of having CS. For individuals with low pretest probability of having CS, or in those patients in whom cyclical CS is suspected, it is recommended to perform additional testing. In these cases, serial LNSC might be useful to follow the progression. Postponing additional testing to allow progression of biochemical and clinical features might be useful in some cases. Subjects with abnormal results in these tests should be referred to an endocrinologist for further testing [13]; performance and interpretation of subsequent testing require considerable expertise. Assay accuracy and normal ranges differ widely, so it is essential to interpret results in the context of the assay used and with appropriate normal ranges.

It is important to differentiate between endogenous pathological hypercortisolism (CS) and pseudo-Cushing states (alcoholism, depression, anorexia nervosa, obesity). Pseudo-Cushing seems to be mediated via increased hypothalamic secretion of CRH, instead of CRH suppression observed in CS. Commonly, pseudo-Cushing patients have biochemical hypercortisolism but minimum features of CS and no presence of tumor (Table 7.3).

UFC is recommended rather than DST in pregnancy, as well as in situations that can increase/decrease CBG (oral contraceptive pill use, critically ill, or nephrotic patients) or when concomitant use of antiepileptic drugs occurs, as these medications may enhance dexamethasone clearance. However, during pregnancy, only UFC values greater than 3 times the upper limit of normal can be considered for further testing for CS, since UFC excretion physiologically increases up to threefold during pregnancy. In contrast, DST rather than UFC is recommended in severe renal failure and adrenal incidentaloma for initial testing. If cyclical CS is suspected, LNSC or UFC rather than DST is recommended [13].

If further evaluation to rule out a possible non-Cushing's hypercortisolism is required, a *dexamethasone-suppressed CRH stimulation test* might be useful in specific situations. This test involves a 48-h 2 mg/d DST followed by the administration of CRH (1 μg/kg iv) 2 hours after the last dose of dexamethasone is administered and cortisol measured 15 min later. However, the optimal cutoff for diagnosis needs further clarification and CRH is not available in some countries. The hypercortisolism of pseudo-Cushing's states is thought to be mediated through increased

hypothalamic secretion of CRH in the setting of an HPA axis that is otherwise appropriately restrained by negative feedback from cortisol. In contrast, the hypercortisolism of true CS suppresses hypothalamic CRH secretion and is less responsive to the negative feedback of exogenous CS. Therefore, in comparison with true CS, patients with pseudo-CS states show a greater suppression of cortisol production by exogenous GC and a diminished response to CRH injection [24]. Measurement of midnight serum cortisol while an individual is asleep (>50 nmol/l) provides high sensitivity for the diagnosis of CS, and it is useful to exclude CS when cortisol <50 nmol/l, but this test requires inpatient admission and this approach may not be possible in some clinical practice settings.

7.5.2 Establishing the Etiology of CS

The first step to localize the source of hypercortisolism is the measurement of *plasma ACTH* (at least twice), although most of the commercially available ACTH assays are not always accurate, especially in the low range. To avoid falsely low results, samples should be collected in an ice bath and processed immediately. Measurement of ACTH can be done at any time of the day because normal circadian rhythm is lost; however, peak values are higher in the morning. In a patient with endogenous hypercortisolism, ACTH concentrations <1.1 pmol/l (< 5 pg/ml) suggest an ACTH-independent origin (an adrenal cause), while ACTH concentrations >4.4 pmol/l (20 pg/ml) suggest an ACTH-dependent origin (mostly pituitary or less frequently an ectopic source). Intermediate values require further evaluation, with a CRH stimulation test that might unmask ACTH responsiveness, or else repeating test over time to either confirm or rule out the diagnosis. The concept that underlies the use of CRH stimulation in CS is that pituitary tumors (CD) usually respond to CRH with an increase in ACTH and cortisol levels, while ectopic ACTH-secreting tumors usually do not. A mean cortisol increase at 30 and 45 minutes of >20% and a mean ACTH increase at 15 and 30 min of >35% over their respective mean baseline values are thought to be consistent with CD [25, 26]. An increase greater than 50% in ACTH levels and/or 20% in cortisol levels following 100 μg of intravenous CRH administration is highly suggestive of CD (Fig. 7.1) [27].

No single best approach to test patients with ACTH-dependent CS exists. The evaluation of CS requires a systematic approach. After biochemical testing has confirmed a diagnosis of ACTH-dependent hypercortisolism, the source of ACTH excess has to be determined. The choice of diagnostic tests used to distinguish a pituitary vs ectopic source varies by institution and depends on the availability of IPSS, cost considerations, patient preference, and availability of CRH and sensitive MRI technology. The most accurate method to discriminate between pituitary and non-pituitary sources of ACTH is to evaluate the central-to-peripheral ACTH gradient via bilateral inferior petrosal sinus sampling (BIPSS). Corticotroph tumors have a clear setup in the petrosal samples, while ectopic ACTH-producing tumors do not. BIPSS is considered the gold standard test to differentiate between a pituitary and an ectopic source of ACTH (sensitivity and specificity of 95%), and it is recommended for

patients with pituitary lesions <6 mm or in those with discordant noninvasive tests [28, 29]. BIPSS is the best way to document a central-to-peripheral ACTH gradient in the blood draining the tumor. Despite the high sensitivity and specificity of BIPSS, false-positive results can occur in ectopic ACTH production with cyclical or mild hypercortisolism without suppression of normal corticotropes, and in CRH-producing tumors. It is recommended to perform BIPSS only in cases with documented hypercortisolism (at least twofold increase in UFC in the 6–8 weeks prior to BIPSS) to ensure that normal corticotroph cells are suppressed. Inability to cannulate veins or abnormal venous drainage might cause false-positive results. Concomitant measurement of prolactin can confirm successful catheterization. CRH is not available in some countries, and desmopressin administration has been shown to offer similar results to CRH stimulation in some studies. BIPSS has limited value in identifying intrapituitary localization of the tumor. If MRI is negative, the gradient of BIPSS might be helpful to choose the side for the initial surgical approach; however, if tumor is not found, the other side of the pituitary gland should be explored. Alternatively, bilateral internal jugular venous sampling has been proposed, since it is more simple and safe and it does not require specialized expertise; however, sensitivity is lower than that of BIPSS [30, 31].

Noninvasive tests to assess the etiology of CD:

– *High-dose dexamethasone suppression test (HDDST), CRH, or desmopressin stimulation* tests are noninvasive tests that might contribute to localization of the source of CS when tumor is not seen or is very small on an MRI, or when BIPSS is not available. The HDDST relies on the concept that pituitary corticotroph tumor cells retain sensitivity (albeit impaired) to glucocorticoids, but tumor cells in ectopic ACTH secretion (EAS) do not. HDDST (2 mg every 6 h for 48 h) is used to distinguish between pituitary CD and EAS. Accuracy of these tests is inferior to that of BIPSS. In general, CD adenomas maintain sensitivity to CRH or desmopressin stimulation and are resistant to negative feedback regulation by GC (HDDST), while malignant tumors (ectopic ACTH source) do not. However, some benign carcinoid tumors may respond equally to what is observed in CD. Similarly, some ectopic ACTH-secreting tumors might express vasopressin receptors and respond to desmopressin like CD tumors; in these cases, desmopressin test is not helpful in differentiating the source of ACTH. Actually, it is not recommended to use the desmopressin test routinely until more data validating the test become available [13]. Discordant results of these tests are reported in up to 60% of patients, BIPSS being the most recommended one.

Additional testing might be helpful to delineate the source of ACTH, as well as to evaluate comorbidities of CS. Chromogranin A, 5-hydroxy-indoleacetic acid, calcitonin, and gastrin might point to ectopic ACTH tumors. Metabolic alkalosis and hypokalemia as a result of severe hypercortisolism (UFC > 1500 μg/24 h) are common in ectopic ACTH sources, but it can be also present in 10% of CD. Dehydroepiandrosterone sulfate is normal or increased in ACTH-dependent causes, but is decreased in cases of adrenal origin [32].

7.5.3 Localization of the ACTH Source

Once ACTH-dependent CS is confirmed and noninvasive tests or BIPSS suggest a pituitary origin, pituitary imaging is mandatory. T1 gadolinium contrast MRI identifies pituitary tumors in around 50% of patients with CD. Importantly, roughly 10% of healthy individuals have incidental pituitary lesions up to 6 mm; therefore, a lesion smaller than 6 mm does not always identify CD as a cause of CS (Fig. 7.1). Sensitivity of MRI can be improved up to 80% using more advanced techniques (spoiled gradient recalled acquisition or dynamic MRI sequences) [33]. A cranial CT scan might be requested by the surgeon since it provides better information on bone structures. It has recently been reported that pituitary adenomas not visible via MRI may be detectable by CRH-stimulated 18-F-fluoro-deoxy-glucose PET imaging [34].

7.6 Management

CD requires a multidisciplinary and individualized management strategy. The main goals of therapy are to reverse clinical features, normalize cortisol levels, minimalize morbidity, and reach long-term remission without recurrences. The best treatment options involve a multidisciplinary team, including an endocrinologist experienced in the management of CD. Surgery is considered the first therapeutic option. When surgery is not possible or non-curative, the choice for second-line therapy might depend on several factors, i.e., patient preferences, urgency to treat, location and size of remnant tumor, drug interactions and side effects, and cost and availability of medical therapies.

7.6.1 Surgical Treatment

Selective pituitary adenomectomy using transsphenoidal surgery (TSS; microscopic or endoscopic techniques) by an experienced neurosurgeon remains the first-line treatment for CD. If surgery is successful (pathological confirmation of the adenoma or biochemical demonstration of remission after resection), the patient is cured. Ideally, the whole pituitary tumor is removed and normal pituitary tissue is left. However, pituitary deficiencies as a consequence of surgery might be present after initial therapy and an interval of hypoadrenalism is common since normal corticotroph cells have been suppressed by longstanding hypercortisolism. Reasons for surgery failure include lack of experience of the surgeon, diffuse corticotroph hyperplasia (very rare), invasive tumor that cannot be resected, or adenomas arising in unusual sites (parasellar, pituitary stalk, neurohypophysis). On the contrary, a well-defined, noninvasive (to the cavernous sinus), and well-visualized tumor on MRI, histological confirmation of an ACTH-secreting tumor, low postoperative cortisol levels, and long-lasting adrenal insufficiency are favorable prognostic factors [35].

If surgery is not successful the first time, repeated TSS, medical therapy, and radiotherapy are potential second-line therapies. As a final resource, surgical or medical adrenalectomy can be performed if hypercortisolism is still uncontrolled. The choice of a second-line treatment must be discussed with the patient. Repeated surgery as soon as active and persistent hypercortisolism is confirmed might be undertaken especially if the pituitary tumor is visualized; nevertheless, overall successful rates are lower than for the first surgery and carries a high risk of pituitary insufficiencies [36].

Surgical complications are more likely to occur in macroadenomas or extensive pituitary exploration. Anterior pituitary deficiencies are observed in about 20 to 25% of the cases, as well as transient central diabetes insipidus. Permanent diabetes insipidus is less frequent. Symptomatic hyponatremia might occur between 1 and 10 days after surgery, with a maximum antidiuresis around day 5, in about 8% of the patients [37]. It is recommended to measure serum sodium several times during the first days after surgery. Other complications include venous thrombosis, hemorrhage, and infections [38]. Since a hypercoagulability state exists in CS, perioperative prophylaxis a few days prior to surgery is recommended [39]. Within 2 weeks of surgery, measurement of free T4 and prolactin may help to identify hypopituitarism, when compared to preoperative values.

7.6.2 Radiotherapy

Conventional fractionated photon beam radiotherapy (1.7–2 Gy daily for a total dose of 45 Gy over 6 weeks) or *stereotactic radiosurgery* (single-dose radiation, including gamma knife, linear accelerator, and proton beam) control hypercortisolemia in up to 80% of patients within 3–5 years [40], but results vary across series [41]. Radiotherapy is indicated in persistent CD after surgery and when local invasion precludes a surgical cure. Radiosurgery may provide a faster biochemical control and less risk of radiation damage to the surrounding structures than conventional radiation therapy, but there are no direct comparative studies. Since medical therapy is effective in normalizing cortisol, it is recommended before administering radiotherapy, since these agents will be required while waiting for the effects of radiation. Long-term follow-up is mandatory to detect both relapse and pituitary insufficiencies. The risk of a second neoplasia (most frequently meningiomas) is estimated in 2% of the patients at 20 years, but further studies are required to confirm these data [42]. Optic neuropathy and other cranial neuropathies may also occur (2%). Measuring UFC without concomitant glucocorticoid supplementation at 6–12 months after radiation therapy is necessary to assess if adrenal insufficiency (AI) has developed. In addition, patients should be counseled on symptoms of AI and to alert their physician if they develop symptoms so that more frequent testing may be undertaken. Diurnal rhythm is not necessarily achieved following radiotherapy, so LNSC is not a good tool to assess remission.

7.6.3 Bilateral Adrenalectomy

In general, bilateral adrenalectomy is considered the last treatment option: It provides definitive and immediate control of hypercortisolism, resulting in permanent hypocortisolism that requires lifelong GC and mineralocorticoid replacement therapy and careful education of the patient. Furthermore, regular pituitary MRI and assessment of hyperpigmentation and ACTH levels are mandatory because of the risk of developing Nelson's syndrome (corticotroph tumor progression) described in up to 25% of patients [43]. If remission is not achieved after the second surgery, the decision of pituitary radiotherapy or bilateral adrenalectomy must be individualized and requires consideration of the pituitary status after the second surgery. Another factor that needs to be taken into account includes patient tolerance to medical therapy while awaiting the effects of radiotherapy. Finally, women who wish to maintain fertility without the need for ovulation induction may opt for adrenalectomy instead of pituitary radiotherapy [44].

7.6.4 Medical Therapy

Medical therapy is indicated in preoperative patients with severe hypercortisolism and associated clinical manifestations, as well as in patients awaiting a response after radiotherapy, and/or when palliative treatment is needed. Hypoadrenalism may occur when treating with these agents; therefore, the possibility of adrenal insufficiency must be addressed if suspected and interrupting the medication may be indicated. Some of these agents affect CYP3A4 leading to significant drug–drug interactions; thus, reviewing all other medications taken by a patient is necessary before starting the treatment. Follow-up for individuals on medical therapy should include UFC and clinical features, aiming for normalization of both [45]. Medical treatments include steroidogenesis inhibitors, agents that modulate ACTH secretion or GC receptor antagonists (Table 7.5).

7.6.4.1 Steroidogenesis Inhibitors

Steroidogenesis inhibitors are effective at blocking the hypercortisolemia despite the fact that they do not treat the underlying tumor. The most commonly used steroidogenesis inhibitors include metyrapone, ketoconazole, mitotane, and etomidate.

– Metyrapone inhibits 11-β hydroxylase (conversion of 11-deoxycortisol to cortisol) and inhibits aldosterone biosynthesis with accumulation of aldosterone precursors that have a weak mineralocorticoid activity. It has a rapid onset of action, and it controls hypercortisolism in 50–75% of the patients with CS [46]. The risk of hirsutism and acne development due to accumulation of androgenic precursors may be metyrapone less desirable as initial therapy in females. Monitoring for edema, hypokalemia, and hypertension is recommended due to the accumulation of mineralocorticoid precursors.

Table 7.5 Medical therapy for Cushing's syndrome

Drug	Dose[a]	Adverse events (AE) and concerns/considerations
Steroidogenesis inhibitors		
Ketoconazole	400–1600 mg/day every 6–12 h orally Quick onset of action	AE: Gastrointestinal, hepatotoxicity, male hypogonadism and gynecomastia Requires acid for biological activity (avoid use of proton-pump inhibitors) Approved by the European medicines agency (EMA) for the treatment of CS
Metyrapone	500–4500 mg/day every 6–8 h orally Quick onset of action	AE: Gastrointestinal, hirsutism, hypertension, hypokalemia Accessibility variable across countries. Approved by the EMA for the treatment of CS
Mitotane	3–5 g/day orally. Starting dose 250 mg/day. Slow onset of action	AE: Gastrointestinal, gynecomastia, hepatotoxicity, neurotoxicity, teratogenic, hypercholesterolemia. Accumulates in body fat. Approved for the treatment of adrenal cancer
Etomidate	0.1–0.3 mg/kg/h intravenously (bolus and titrate). Quick onset of action	AE: Gastrointestinal, myoclonus and pain at the injection site Requires monitoring in intensive care unit
Osilodrostat (LCI699)	4–100 mg/day orally	AE: Gastrointestinal, headache, dizziness, arthralgia, hypokalemia Under investigation. Not approved yet
Pituitary tumor-directed drugs		
Pasireotide	0.6–0.9 mg twice daily 10–20 mg/4 weeks intramuscular	AE: Diarrhea, cholelithiasis, hyperglycemia, transient increase LFTs Most successful when UFC < two-fold normal. Approved for patients with CD who are not surgical candidates or have failed surgery
Cabergoline	1–7 mg/week orally	AE: Asthenia, dizziness, gastrointestinal, headache, potential risk of cardiac valvulopathy
Retinoic acid	10–80 mg/day orally	AE: Arthralgia, dryness of mucosae, headache, gastrointestinal Under investigation, not approved yet
Glucocorticoid receptor antagonist		
Mifepristone (RU486)	300–1200 mg/day orally Difficult to titrate (no biomarker)	AE: Clinical adrenal insufficiency, endometrial hyperplasia, hypertension, edema, hypokalemia. Approved for patients with CD and diabetes mellitus or glucose intolerance who are not surgical candidates or have failed surgery

[a]The lowest dose is recommended initially, unless UFC > 5xULN, starting dose must be doubled

– Ketoconazole is an imidazole derivative that inhibits steroidogenesis by blocking side-chain cleavage, and to a lesser degree 17,20-desmolase, and 11-β hydroxylase enzymes. It has a rapid onset of action and an acidic environment is required to maximize the absorption; therefore, concomitant use of proton-pump inhibitors is contraindicated. Efficacy reported varies across series; on average, it normalizes UFC levels in 60% of the patients with CD [47]. Liver function should be monitored; mild elevation of liver enzymes, up to threefold, is not a contraindication. Hepatotoxicity is usually mild and resolves after drug withdrawal. The

possibility of development of hypogonadism in men may favor the use of metyrapone as initial medical therapy in men. Recently, ketoconazole was withdrawn from the market due to hepatic dyscrasia for the treatment of fungal infections by the European Medicines Agency; however, it is indicated to control hypercortisolism.

Combination therapy with both ketoconazole and metyrapone can be used to control severe hypercortisolemia.

- Mitotane has a specific adrenolytic action and acts by inhibition of CYP11A1, P450 side-chain cleavage. Mitotane provides a long-term suppression of hypercortisolism in patients with CD. However, its onset of action is slow (weeks or months) and potential adverse events may be serious (gastrointestinal and neurological); careful monitoring of drug levels is required and only available in few centers [48]. However, monitoring circulating levels of mitotane is made available by the manufacturer in many countries. Mitotane increases CBG and plasma total cortisol levels; thus, biochemical monitoring relies on UFC or salivary cortisol. Hypoadrenalism is common and requires concomitant supplementation with higher doses of hydrocortisone than in other causes of hypoadrenalism, because mitotane activates CYP3A4 and increases hydrocortisone clearance.
- Etomidate is an intravenous anesthetic and an imidazole derivative used when rapid control of cortisol levels is required, and oral agents cannot be taken. Etomidate may be useful in emergency settings with unmanageable symptoms (respiratory failure, psychosis). Monitoring cortisol levels every 4–6 h is required to titrate the infusion rate.
- Osilodrostat (LCI699), an oral 11 β-hydroxylase inhibitor, has recently been authorized for its use in Cushing's syndrome by the European Medicine Agency (January 2020). Osilodrostat was effective at lowering the levels of cortisol in at least 80% of the patients compared to placebo, and it was in general well-tolerated and more convenient since it can be given orally twice a day [49].
- Levoketoconazole (COR-003), a ketoconazole stereoisomer, is under investigation. Preliminary results show promising results in the treatment of CD patients; however, it is still being evaluated in multicenter trials [50].

7.6.4.2 Another Modality for Treating CS

Another modality for treating CS includes the class of medications that are *GC receptor antagonists*. One such drug is mifepristone (RU486), which is both a GC receptor antagonist and an anti-progestin. Few experiences with CD are reported, and the assessment of its efficacy without a biochemical marker makes its use challenging and restricts the ability to assess overtreatment or undertreatment. Cortisol levels remain unchanged or increased while on mifepristone and are not useful to guide efficacy of the therapy. Therefore, dose adjustment is based on clinical

symptoms, which have been shown to improve in a significant number of patients (diabetes in 60%, hypertension in 40%, and at least one of the following clinical parameters: weight, depression, quality of life (QoL), or clinical appearance in 87%) [51]. Mifepristone is a second-line therapy in CS relegated for patients with diabetes who failed surgery or those with CS who are not surgical candidates and have persistent disease. CORT125134 is a new selective GC receptor antagonist under investigation.

7.6.4.3 Tumor-Directed Therapeutic Agents

Tumor-directed therapeutic agents act directly on corticotroph cells, inhibiting ACTH production

- Dopamine D2 receptor agonists (bromocriptine, cabergoline) may be effective in a subset of CD patients. Usually, higher doses of cabergoline than the ones used for hyperprolactinemia are required (2–3 mg/week). Efficacy is limited; small studies showed normalization of UFC in 30–40% of patients [52]. Clinical trials are needed to evaluate combination therapy with cabergoline.
- Pasireotide (SOM230) is a somatostatin receptor (SST) agonist with higher and broader affinity for SST 1, 2, 3, and 5 than octreotide or lanreotide. It has been demonstrated to be useful as a tumor-directed medical therapy for CD, since corticotroph tumors have a high expression of SST5. It is approved for patients who have failed surgery or for those with contraindications to surgery. The main side effects of pasireotide include gastrointestinal upset and hyperglycemia. Long-acting pasireotide (monthly administration) has been developed, with a similar safety profile and efficacy to that of twice-daily formulations, normalizing UFC in about 40% of CD patients [53].

Potential new drugs are under investigation as a tumor-directed targets, such as retinoic acid, silibinin, and roscovitine, whose efficacy and safety are still unclear [54].

7.6.5 Perioperative Management

GC replacement before surgery for Cushing's syndrome is not necessary unless the patient has been treated with adrenal enzyme inhibitors. Regimens differ across centers, and no study comparisons between one approach and the other have been done. A typical regimen is the administration of GC during the 24 h prior to transsphenoidal surgery [55].

Almost all patients require GC replacement after transsphenoidal surgery for an ACTH-producing adenoma until the HPA axis is recovered [55]. Some patients may have a "GC withdrawal syndrome" despite the use of physiologic GC replacement therapy, and they should be warned that this is expected after "successful surgery." Occasionally transient supraphysiological doses of GC are needed [56].

7.7 Follow-Up

Initial remission rates are achieved in 80–90% of the patients with microadenomas and 60% of patients with macroadenomas with an experienced neurosurgeon. However, 20% of the patients present late recurrences even 10–15 years after initial therapy [2, 6]. Therefore, lifelong follow-up is mandatory for CD.

Measurement of morning serum cortisol during the first week after surgery is recommended to assess remission by withholding treatment with GCs at least 24 hours [44]. Hydrocortisone (<20 mg) 2–3 times daily is the preferred regimen of GC supplementation, since it has a shorter half-life and supraphysiological doses should be avoided to allow quicker recovery of HPA axis. Usually, GCs are maintained for a few days before withdrawal, being careful about the potential development of adrenal insufficiency. It is recommended to measure morning serum cortisol every 2–3 months, followed by an ACTH stimulation test if cortisol levels are >200 nmol/l (7.3 μg/dl). Alternatively, insulin-induced hypoglycemia can be done to assess recovery of the HPA axis within 6 weeks of surgery [45]. GC can be stopped if morning serum cortisol or cortisol response to ACTH stimulation test is >500 nmol/l (>18 μg/dl). If baseline cortisol is still <138 nmol/l (5 μg/dl), GC replacement should be continued while retesting the patient every 3–6 months. Recovery of the HPA axis may take up to 1 year after TSS. Once HPA axis function is recovered, assessment for possible recurrences should be performed annually or sooner if patients have clinical symptoms. Interestingly, LNSC seems to be one of the earliest biochemically detectable alterations of recurrence, preceding elevations of UFC [57].

There is no consensus on the criteria for "cure" after initial surgery for CD. Persistent elevations of UFC after the immediate postoperative period are indicative of lack of remission. While levels of cortisol at either end of the spectrum are more clear-cut, there are intermediate levels that may present a diagnostic dilemma. It is well accepted that serum cortisol levels <50 nmol/l (<1.8 μg/dl) or UFC levels below the normal range (<55 nmol/24 h, ideally <28 nmol/24 h) define remission and are associated with low recurrence rate of 10% at 10 years and high remission rates at long-term follow-up of 85–100%. On the contrary, persistent cortisol >138 nmol/l (>5 μg/dl) for up to 6 weeks requires further evaluation [58]. In patients with intermediate postoperative values of serum plasma cortisol (between 50 and 138 nmol/l), management and follow-up should be individualized; a postoperative cortisol cutoff <138 nmol/l (5 μg/dl) is associated with long-term remission rates of between 65 and 80%. Sometimes cortisol levels decline gradually over the months following surgery, indicating a progressive necrosis of the remaining tumor cells. Therefore, it is important to confirm that levels reached a nadir, before additional testing and therapy are prescribed in patients with persisting hypercortisolism immediately after surgery [59]. Young individuals (<25 years) and patients with

macroadenomas are at a higher risk of recurrences [60]. Some patients might present a gradual decline of cortisol levels over the months following surgery, so follow-up is mandatory.

The true long-term recurrence rate is uncertain because of different criteria of remission and inadequate follow-up. The median interval to recurrence is about 40 months but varies across series and duration of follow-up. Postoperative serum cortisol levels or UFC might provide prognostic information. Long-term monitoring should be done annually for several years using LNSC, DST, and/or UFC or at any time that patients experience a return of symptomatology, to rule out recurrences. Documentation of recovery of a diurnal cortisol pattern might be helpful to support a remission state.

Testing for GH deficiency is advised at least 12 months after remission, because hypercortisolism affects GH axis [61] and a postoperative MRI scan is recommended beyond 3 months after surgery [45].

7.8 Prognosis

Although biochemical remission is associated with significant clinical improvement, some symptoms and comorbidities may not completely normalize [7]. Therefore, it is mandatory to monitor and provide additional treatment for cortisol-dependent comorbidities throughout the life of a CS survivor (Fig. 7.2).

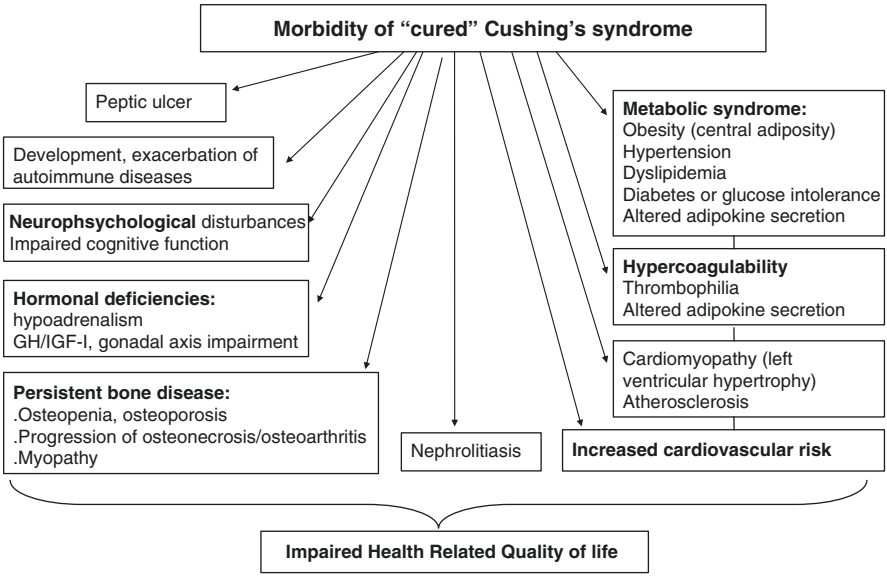

Fig. 7.2 Main clinical manifestation after remission of Cushing's syndrome

7.8.1 Mortality

Higher mortality, mainly due to cardiovascular and infectious diseases, is observed within the first year after diagnosis and initial therapy, and in those patients whose initial cure was not obtained. Before the development of steroidogenesis inhibitors and surgical techniques, prognosis was poor with a mean survival of 4 years [62]. In patients who have persistent mild hypercortisolism despite therapy, SMR is increased 3.8- to 5.0-fold compared with the general population [2, 8]. Although more recent studies show that mortality rates have been dramatically reduced after successful normalization of cortisol, mortality is still increased up to 2–3 times in most of the series, while others reported similar SMR to that of age-matched populations [60, 63, 64].

7.8.2 Cardiovascular Risk and Metabolic Syndrome

Cardiovascular risk and metabolic syndrome may remain abnormal after years of cortisol normalization. Hypertension, glucose, and lipid metabolism improve after normalization of hypercortisolism, but a higher risk of diabetes, dyslipidemia, and hypertension remains years after remission compared to a healthy population or patients with nonfunctioning pituitary adenomas [7, 65]. Moreover, persistence of central obesity contributes to insulin resistance and an increased inflammatory and hypercoagulable state with an unfavorable adipokine profile (increased levels of TNF-alpha, interleukin-6, and leptin) contributing to persistence of increased cardiovascular risk. An increased risk of myocardial infarction, greater prevalence of coronary calcifications, left ventricular dysfunction, and cerebrovascular disease have also been reported in survivors of CS [7, 63–66].

7.8.3 Psychopathology and Cognition

Even years after CD is cured, patients have an increased prevalence of psychopathology (anxiety disorders, major depression, maladaptive personality) compared to healthy individuals or patients treated for other types of pituitary diseases [7]. The use of screening tools in clinical practice to identify psychopathology promptly is recommended [67]. Cognitive impairment (short-term memory and attention deficits, as well as working memory and impaired decision making) is a common complaint after hypercortisolism [68]. Chronic hypercortisolism exposure causes structural changes in cerebral areas; specifically, structural brain abnormalities (more severe white matter lesions commonly associated with increased cardiovascular risk, smaller gray matter volumes in the anterior cingulate cortex, greater cerebellar volumes, hippocampal dysfunction) were found in patients in long-term remission of their CS compared to matched controls [69–72]. Fluoxetine has been suggested as a neuroprotectant and antidepressant for these patients, but no

prospective studies are available yet [73]. Data on long-term recovery regarding cognitive impairment are scarce.

7.8.4 Bone Metabolism

Factors involved in higher risk of osteoporosis in CD are multiple: direct effects of GC on bone cells, GC-induced hypogonadism, hypopituitarism secondary to surgery, hydrocortisone replacement therapy, or reduced bone strain due to muscle atrophy [74]. Approximately 40% of CS patients present with fractures, particularly vertebral fractures. Although there is a decrease in bone mineral density (BMD) and increased fracture risk during hypercortisolism and before diagnosis, studies report BMD normalization after several years of cure [75, 76]. Meanwhile, in severe osteopenia, alendronate might be useful to induce a more rapid improvement of BMD or to prevent further bone loss in persistent postsurgical hypercortisolism [77]. Time of excess GC exposure and duration of GC replacement after surgery were found to be predictors of low BMD in CS. A detailed fracture assessment is recommended, evaluating BMD at the spine and hip, adequate calcium and vitamin D supplementation, and individualized long-term follow-up accordingly.

7.8.5 Quality of Life

Because of all comorbidities associated with CS despite cure, health-related quality of life is likely to be affected long term. Perceived QoL is impaired especially in patients with CD [7, 78], even independently of the disease control or adequately replaced hypopituitarism [2], compared to healthy individuals or other pituitary tumors. Although QoL improves in patients in remission, long-term residual impairment in physical and social functioning is commonly reported. Altogether, it has a significant impact, with social and economic consequences, since inability to return to work or sick days is common [73, 79].

7.8.6 Other Cortisol-Related Comorbidities

CS patients have a significant increased risk of venous thromboembolic events and impaired coagulation profile (activation of coagulation cascades and impaired fibrinolysis) compared to the general population, especially in the postoperative period [80]. Muscle myopathy may persist long-term after cure; aerobic and resistance exercises might be effective in attenuating GC-induced muscle atrophy. Increased rate of nephrolithiasis in both active and cured CD compared to healthy controls has been reported, but the pathogenic mechanisms in CS are not yet elucidated.

Importantly, resolution of hypercortisolism is associated with new onset or exacerbation of pre-existing autoimmune or inflammatory diseases (psoriasis, rheumatoid arthritis, ulcerative colitis, etc.), likely due to the suppressive effects of hypercortisolism on the immune system [81]. The most common autoimmune disease is autoimmune thyroiditis; therefore, patients with positive antibodies should be followed after remission of hypercortisolism to identify precociously the onset of a hypothyroidism.

7.9 Summary and Conclusions

CD is a rare disorder characterized by chronic exposure to excess glucocorticoids leading to multisystemic comorbidities and increased mortality. Although clinical presentation is often nonspecific and hypercortisolism might not be suspected, a prompt diagnosis is mandatory to reduce long-term comorbidities due to hypercortisolism. Pituitary surgery can be curative in most of the cases but not all. Radiotherapy and medical therapy can be used as additional therapeutic options. Fortunately, new medical therapies are under development with promising results. A multidisciplinary and individualized approach is essential to choose the best treatment approach, minimize complications, and manage cortisol-related comorbidities, since some of them persist even after hormonal normalization. A lifelong surveillance is advisable to identify recurrences and treatment of persistent comorbidities.

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TSH-oma

8

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8.1 Introduction

Thyrotropin (TSH)-secreting pituitary tumor (TSH-oma) is a rare disease. The excess of TSH secretion from tumoral cells, which are unresponsive to the negative feedback of thyroid hormones, leads to hyperstimulation of the thyroid with consequent hypersecretion of T4 and T3 [1–3]. Thus, TSH-omas can be classified as a form of "central hyperthyroidism."

In 1960, the first case of TSH-oma was documented by measuring serum TSH levels with a bioassay [4], and 10 years later, another case was proved by a RIA assay of TSH [5].

TSH-omas were typically diagnosed at the stage of invasive macroadenoma and were considered difficult to cure, but nowadays they are more often diagnosed at an earlier stage. Indeed, in the last years the routine use of ultrasensitive immunometric assays for TSH as first-line test of thyroid function has allowed to detect

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hyperthyroid patients with unsuppressed TSH secretion in the presence of high free thyroid hormone, improving the diagnostic workup. The recent introduction of the so-called TSH with reflex free T4 strategy (i.e., FT4 measurement only in the presence of abnormal TSH) fails to recognize both central hypo- and hyperthyroidism, thus leading to TSH-omas misdiagnosis.

Thyroid function tests that value characteristic of TSH-oma patients are also recorded in patients with resistance to thyroid hormones (RTHs), and signs and symptoms of hyperthyroidism are frequently found in the so-called pituitary RTH (PRTH), characterized by a more severe resistance to thyroid hormone action at the pituitary level than at the peripheral tissue level [6–8].

These rare entities represent a diagnostic and therapeutic challenge, because failure in distinguishing between the two disorders may result in improper treatment, while the correct identification of a TSH-oma may prevent the occurrence of both neurological and endocrinological complications, thus leading to a better cure rate.

8.2 Epidemiology

TSH-oma is a very rare disorder; it accounts for about 0.5–2% of all pituitary tumors. The prevalence in the general population is 1–2 cases per million [1, 3]. However, an increase in the number of reported cases was registered in the last decade, confirmed by data obtained from the Swedish Pituitary Registry [9]. This recent study demonstrated an increasing incidence of TSH-omas over time, with a national prevalence of 2.8 per one million inhabitants in 2010. The increased incidence of TSH-omas is principally due to a reasonable catch up effect resulting from improved diagnostic tools. The introduction of the ultrasensitive TSH assays in the late 1980s, which detects low TSH, allowed a distinction between primary hyperthyroidism (Graves' disease) and syndrome of inappropriate TSH secretion [1, 3, 6, 8].

TSH-omas has been reported at ages ranging from 8 to 84 years [3, 10, 11], with a peak of onset in the fifth/sixth decade of life. In contrast with other more common thyroid disorders, TSH-omas occur with equal frequency in men and women and occur mainly as sporadic forms; nevertheless, familial cases can be part of multiple endocrine neoplasia type 1 (MEN1) syndrome [12] and familial isolated pituitary adenoma (FIPA) with AIP mutation [13].

8.3 Pathogenesis

TSH-omas can be classified in functional and silent tumors. Classically, TSH-omas present with elevated thyroid hormones and elevated or inappropriately normal TSH levels. Patients with TSH-secreting adenomas present signs and symptoms of hyperthyroidism, whereas silent thyrotropinomas are positive for β-TSH by immunohistochemistry without clinical or biochemical evidence of central hyperthyroidism [14, 15].

The presence of TSH beta subunit, either free or combined, has been confirmed in all tumor cells from every type of thyrotropinoma by immunostaining studies, with few exceptions [1, 3, 16–18].

In 70% of the cases, thyrotropinomas secrete TSH only, often accompanied by unbalanced hypersecretion of alpha-subunit of glycoprotein hormones (alpha-GSU) (Table 8.1). Interestingly, by double gold particle immunostaining, the existence of thyrotropinoma composed of two different cell types, one secreting alpha-GSU alone and another cosecreting alpha-GSU and the entire TSH molecules (mixed TSH/alpha-GSU tumors), has been documented [17]. In particular, the presence of a mixed TSH/alpha-GSU adenoma is suggested by the finding of an extremely high alpha-GSU/TSH molar ratio and/or by the observation of dissociated alpha-GSU and TSH responses to TRH [1, 19].

The remaining 30% of cases are classically considered mixed tumors since they cosecrete TSH and other anterior pituitary hormones (GH, PRL, and LH/FSH) (Table 8.1). In this regard, hypersecretion of GH and/or PRL is the most frequently found, possibly leading to acromegaly and/or amenorrhea/galactorrhea syndrome. The occurrence of these mixed tumors may rely on the expression of common transcription factors, such as Prop-1 and Pit-1, by thyrotroph, somatotroph, and lactotroph cells. On the contrary, no association with ACTH hypersecretion has been documented so far, probably due to the distant origin of corticotroph and thyrotroph lineages. Rare is the occurrence of mixed TSH/FSH/LH tumors [20].

It has to be taken into account that silent TSH-secreting tumors may give positive immunohistochemical results for one or more pituitary hormones without a

Table 8.1 Recorded cases of different types of TSH-secreting tumors

	Number of cases	% of total
Total TSH-secreting tumors	470	---
Pure TSH--secreting tumors	332	70.6
TSH-secreting tumors associated with other pituitary hormones hypersecretion (mixed tumors)	138	29.4
Mixed TSH/GH-secreting tumors	83	17.7
Mixed TSH/PRL-secreting tumors	47	10.0
Mixed TSH/FSH/LH-secreting tumors	8	1.7

correlation with its or their hypersecretion in vivo [17, 21, 22]. In addition, the coexistence of TSH-secreting tumors with Hashimoto's thyroiditis and hypothyroidism has been reported [2, 23].

When diagnosed, most thyrotropinomas have a diameter size >1 cm presenting invasive features in the surrounding structures (i.e., the dura mater and bone) with extrasellar extension in the supra- and/or parasellar direction found in the majority of cases [1, 16, 17, 18, 24, 25]. Interestingly, patients with intact thyroid display a significantly lower occurrence of invasive tumors with respect to those who underwent previous thyroid ablation by surgery or radioiodine [1, 3]. In these cases, the reduction in circulating thyroid hormone levels due to thyroid ablation may be the cause of feedback mechanism alterations, thus supporting tumor growth. Thyrotropinomas with a diameter <1 cm were usually found in less than 15% of the cases [25], but their prevalence is progressively increasing thanks to improved testing of thyroid function and awareness among endocrinologists. Indeed, 30% of thyrotropinomas have been found with a diameter <1 cm in the series recently published [7, 26].

As for majority of pituitary lesions, the molecular mechanisms leading to the formation of thyrotropinoma are not fully understood. TSH-secreting tumors are usually characterized by a very fibrous consistency [27]. This observation has been linked to enhanced basic fibroblast growth factor (bFGF) blood levels and specific transcript in the tissues removed from two patients with invasive mixed PRL/TSH-secreting tumors and displaying by marked fibrosis [28]. This finding suggests a possible autocrine role for bFGF in tumor development. According to X-chromosomal inactivation analysis, thyrotropinomas derive from the clonal expansion of a single cell initially transformed cell as most pituitary tumors [29]. The hypothesis of a transforming event favoring gain of cell proliferation followed by secondary mutations or alterations leading to tumor progression is supposed to be applicable to TSH-omas. Mutations of proto-oncogenes (Rb, MEN1), either oncogenes (Ras, protein kinase C, G-protein subunits, TRH receptor) or pituitary-specific genes, able to confer growth advantage to pituitary cells, have been screened extensively. However, to date, no mutations in these candidate genes have been found. In particular, none of the thyrotropinomas screened presented activating mutations of genes encoding for G-protein subunits, such as αs, αq, α11, or αi2 [30]. On the contrary, GH-secreting tumors frequently present mutations in the oncogene *gsp*. Similarly, no mutations in the gene encoding for TRH receptor have been found in 9 and 3 thyrotropinomas, respectively [30, 31]. Since the transcription factor Pit-1 is a key regulator of cell differentiation and in PRL, GH, and thyrotropin gene expression, Pit-1 gene has been screened for mutations in 14 thyrotropinomas but found to be wild type [1]. However, Pit-1 resulted to be overexpressed in thyrotropinomas, similarly to what observed in GH-omas, although the biological meaning of this finding remains to be clarified [18, 20, 32].

Another candidate gene investigated is located on 11q13 and named MEN1. The MEN1 gene, encoding for menin, is linked to the multiple endocrine neoplasia type 1 (MEN1). About 3–30% sporadic pituitary tumors show loss of heterozygosity (LOH) at 11q13, which has been associated with the transition from the noninvasive

to the invasive phenotype. LOH on 11q13 has been found in 3 out 13 thyrotropinomas tested, but no MEN1 mutations were found [33]. Interestingly, hyperthyroidism due to thyrotropinomas has been reported in five cases within a familial setting of MEN1 syndrome [12].

As far as the loss of tumor suppressor genes is concerned, no loss of p53 was found in one thyrotropinoma studied, and analysis on retinoblastoma gene (Rb) is still lacking in thyrotropinomas. Mutations in the aryl hydrocarbon receptor-interacting protein (AIP) are involved in sporadic pituitary tumors, to note that AIP mutations were found in two patients with TSH-omas [13, 34].

Interestingly, recent whole-exome sequencing analysis is allowed to identify several candidate somatic mutations and variation in copy numbers in 12 sporadic TSH-secreting tumors [35]. Further studies in combination with epigenetic and transcriptomic approaches are needed to reveal the potential of such genetic lesions.

Thyroid hormone receptors (TRs) have been proposed as a potential candidate oncogenes and alterations in the feedback control mechanisms, as factors involved in the thyrotropinomas pathogenesis have been evaluated. Somatic mutations of thyroid hormone receptor beta (TRb) and aberrant alternative splicing of TRb2 mRNA encoding a TRb variant lacking T3-binding activity have been associated with impaired negative feedback on TSH secretion in some thyrotropinomas [36]. An aberrant expression of a thyroid hormone receptor β isoform (TRβ4) may partly cause the inappropriate secretion of TSH in thyrotropinomas [37].

In vitro studies on primary cultures from TSH-secreting tumors showed that these tumors express a large number of functioning receptors [3], although TSH response to TRH is usually lacking in vivo. Similarly, almost all TSH-omas express somatostatin receptors (SSTR) at variable ratio. Higher SSTR expression levels have been found in mixed GH/TSH-secreting tumor [38–40]. The inhibitory response to somatostatin analogs appears to be correctly mediated by SSTR in these tumors with resulted decrease in TSH secretion by neoplastic thyrotrophs [41–44]. Specific polymorphisms and LOH at the somatostatin receptor type 5 (SSTR5) gene locus seem to be associated with an aggressive phenotype and pharmacological resistance to somatostatin analogs, possibly because of absent somatostatin-induced inhibition of thyrotropin secretion [45]. In addition, the presence of dopamine receptors on the tumor thyrotrophs was the rationale for therapeutic trials with dopaminergic agonists, such as bromocriptine [38, 46, 47], with several studies showing a wide heterogeneity of TSH responses to dopaminergic agents, either in primary cultures or in vivo [48–50]. To date, no mutations in dopamine type 2 receptor (DRD2) have been found in TSH-omas [30, 31] and the effects of these inhibitory agents should be re-evaluated in light of the demonstration of the possible occurrence of SSTR5 and DRD2 heterodimerization [51].

Finally, the transformation of a TSH-oma into a carcinoma is an extremely rare event [52–54]. However, very high circulating levels of free alpha-subunit may be predictable of a progression toward a malignant behavior. Moreover, spontaneous and strong decrease in both TSH and alpha-GSU serum concentrations might indicate that the tumor is becoming less differentiated and correlates with invasive and metastatic features. In this regard, it has to be mentioned that, in a mouse model of

TSH-secreting tumor, the activation of phosphatidylinositol 3-kinase pathway favored pituitary growth that may induce transformation of the benign tumor into a carcinoma [55].

8.4 Clinical Presentation

Patients with TSH-omas usually present signs and symptoms of hyperthyroidism (Fig. 8.1). Many patients had been mistakenly diagnosed as having primary hyperthyroidism (Graves' disease), and about one third had inappropriate thyroidectomy and/or radioiodine treatment prior to the correct diagnosis. However, a true coexistence of Graves' disease and TSH-oma has been reported in a few cases [56, 57] and several untreated patients with TSH-oma were described as clinically euthyroid [11, 58]. The prevalence of circulating antithyroid autoantibodies (antithyroglobulin: Tg-Ab, and antithyroid peroxidase: TPO-Ab) is similar to that found in the general population, but some patients develop Graves' disease after pituitary surgery and few others present bilateral exophthalmos due to autoimmune thyroiditis [3, 59, 60] or unilateral exophthalmos due to orbital invasion by the pituitary tumor. It is worth

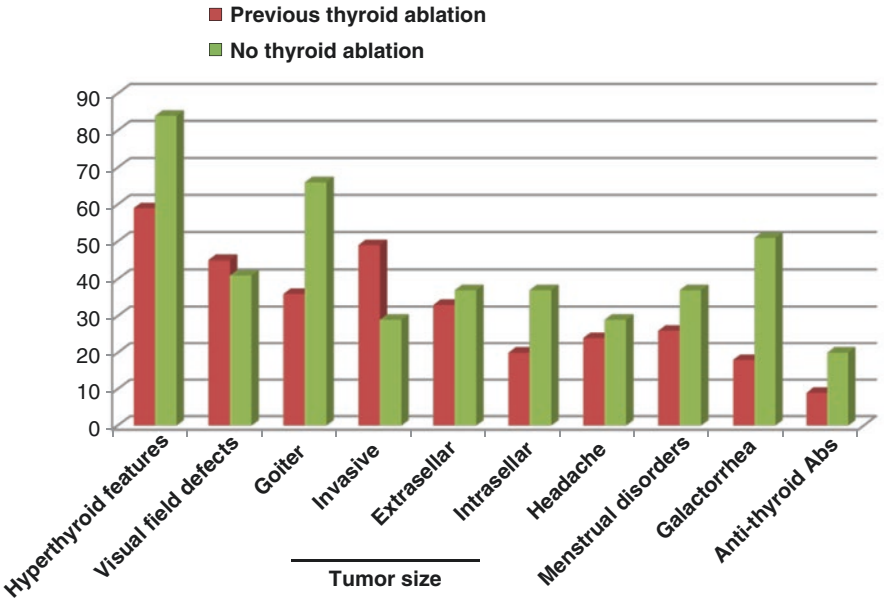

Fig. 8.1 Clinical manifestations in patients with TSH-omas. Based on previous thyroid ablation, patients have been divided into two groups. Patients usually present signs and symptoms of hyperthyroidism. As other pituitary adenomas, TSH-omas present clinical features caused by mass pressure effects. Invasive tumors are seen in about half of the patients with previous thyroidectomy and in 1/4 of untreated patients. TSH strong stimulation causes uni- or multi-nodular goiter. The prevalence of circulating antithyroid autoantibodies (Abs) is similar to that found in the general population (modified from Beck-Peccoz et al., Endotext, 2019)

noting that patients with mixed TSH/GH adenomas may have hyperthyroid features overshadowed by those of acromegaly [61–64]. This underlines the importance of TSH and FT4 systematic measurement in patients with pituitary tumors.

In about 72% of cases, the TSH strong stimulation causes uni- or multi-nodular goiter; however, the progression toward functional autonomy seems to be rare [3, 65].

Moreover, in sporadic cases cardiotoxicosis with atrial fibrillation and cardiac failure have been reported [66–70]. Two patients with typical episodes of periodic paralysis [71, 72] or a high prevalence of radiological vertebral fracture [73] have also been described.

Other symptoms can be tachycardia, tremors, heat intolerance, asthenia, and irritability.

As other pituitary adenomas, TSH-omas may present clinical features caused by mass pressure effects. The tendency to the invasiveness and the frequent over-sellar extension found in these adenomas increase the probability that the clinical symptomatology linked to the mass effect is manifest. Typical features that may occur are as follows:

1. Alterations in the visual field in about 50% of patients, due to the compression of the optic chiasm and the possible subsequent involvement of the optic nerve [74].
2. Ophthalmoplegia or diplopia due to compression of the cranial nerves at the level of cavernous sinus.
3. Hydrocephalus due to compression of the third ventricle and occlusion of foramen of Monroe.
4. Clear rhinorrhea due to erosion of the sellar floor with infiltration in the sphenoid sinus.

Furthermore, in 20–25% of patients frontal headache, continuous and resistant to analgesics can appear, caused by the distension of the sellar diaphragm that can be more rarely associated with other symptoms and signs of intracranial hypertension such as vomiting and edema of the papilla caused by the expanding intracranial mass.

Additionally, the compressive effect of adenomatous cells on surrounding adeno-pituitary cells can result in hypopituitarism in 25% of patients. The different sensitivity of adeno-pituitary cells to the compression effect establishes the progressive order according to which the secretory deficit occurs: first the GH-secreting cells, then the gonadotropin-secreting cells, then the TSH-secreting ones, and finally the corticotropic ones. Instead, the compression of the hypothalamic–pituitary peduncle is a cause of both the pseudo-hyperprolactinemia that is the consequence of dopamine-mediated inhibition on prolactin secretion and the diabetes insipidus due to the interruption of the axonic flow that carries the neurohypophysial hormones. Besides, dysfunction of the gonadal axis with menstrual disorders has been described mainly in mixed TSH/PRL adenomas and central hypogonadism, delayed puberty, and decreased libido in a number of male patients with TSH-oma and/or TSH/FSH adenomas [1, 49, 75, 67].

Finally, a recent publication has evidenced an estimated incidence of 4.8% of differentiated thyroid cancer (DTC) in patients who underwent surgery for TSH-oma, suggesting a possible role of TSH hypersecretion in the development of thyroid tumors [76].

8.5 Diagnosis

Patients harboring TSH-oma present with signs and symptoms of hyperthyroidism that usually are milder than expected owing to the levels of circulating FT4 and FT3, probably due to the long duration of the disease. From a biochemical point of view, the confirmed presence of elevated serum FT4/FT3 and measurable TSH levels (Fig. 8.2) is sufficient to exclude Graves' disease or other causes of primary hyperthyroidism. It is worth mentioning that a measurable TSH associated with high FT4/FT3 levels during levothyroxine replacement therapy may be caused by poor compliance or to the administration of levo-T4 (L-T4) before blood sampling.

A correct diagnosis of thyrotropinoma allows to avoid dramatic consequences, such as improper thyroid ablation (thyroidectomy or radioiodine) that may cause the pituitary tumor volume further expand. Since clinical features of hyperthyroidism can be overshadowed by those of acromegaly in case of mixed thyrotropin/GH-secreting tumors, systematic measurement of thyrotropin and FT4 is recommended in patients who have pituitary tumor.

Measurements of different parameters are proposed as quantifying the degree of tissue hyperthyroidism [2]. An unbalanced hypersecretion of circulating free α-GSU levels and elevated α-GSU/thyrotropin molar ratio (Table 8.1) is found in 80% of patients who have thyrotropinomas [3]. Accordingly to recently published data, such α-GSU hypersecretion is a phenomenon correlated with progressive tumor volume increase [16, 17, 20] since serum α-GSU levels are almost always normal in microadenomas.

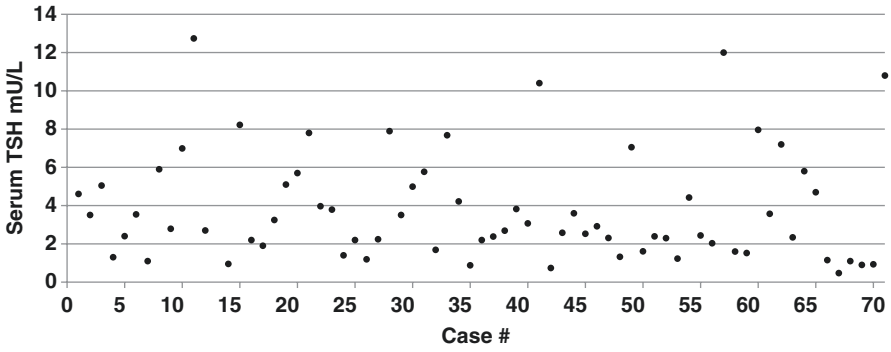

Fig. 8.2 Baseline serum TSH levels in TSH-omas from our case series. The biochemical hallmarks of TSH-omas are high serum free T3 and T4 concentrations while, as shown in the figure, TSH may be inappropriately normal or high

8.5.1 Dynamic Tests

TRH test and T3 suppression are recommended for the diagnosis of thyrotropinoma, although none of them is of clear-cut diagnostic value. Thus, the combination of both stimulatory and inhibitory tests increases the specificity and sensitivity of the diagnostic workup [3, 17, 20].

Classically, the T3 suppression test has been used to evaluate the presence of thyrotropinomas. The failure of a complete inhibition of TSH secretion after T3 suppression test (80–100 mg per day per 8–10 days) is typical of patients with thyrotropinoma (see Table 8.1). T3 suppression test is the most sensitive and specific test, particularly in patients who have had previous thyroid ablation [2, 77], whereas is contraindicated in elderly patients and in those with coronary heart disease.

As far as stimulatory tests are concerned, TRH injection (200 μg i.v.) does not increase either TSH or α-GSU levels in up to 85% of patients with thyrotropinoma [2]. Thus, this test has been used widely in the workup of thyrotropinomas. The finding of a discrepant response to TRH between thyrotropin and α-GSU (or GH in the case of acromegaly), however, is pathognomonic of pituitary tumors cosecreting thyrotropin and other hormones (Fig. 8.2) [16]. Interestingly, the administration of native somatostatin or its analogs (i.e., octreotide or lanreotide) induces a decrease in circulating TSH levels in normal and in the majority of the tumoral thyrotrophs [35, 36, 38, 53, 56, 65, 72], and this inhibitory response may predict the efficacy of long-term treatment with somatostatin analogs [78].

8.5.2 Differential Diagnosis

Primary hyperthyroidism in a hyperthyroid patient is ruled out by the presence of detectable TSH levels. However, in patients on L-T4 replacement for primary hypothyroidism, poor compliance is by far the most common cause of apparent inappropriate secretion of TSH (TSH still too high for the levels of the thyroid hormones). This underscores the importance of studying patients in steady state [79]. Inadequate measurement of TSH and peripheral thyroid hormones should always be taken into consideration. This may occur, for example, in medical therapy with amiodarone [79].

It is crucial to exclude the presence of methodological interferences due to the presence of circulating autoantibodies (e.g., against T3 and T4) or heterophilic antibodies (e.g., for TSH) that may give falsely elevated serum levels of thyrotropin or free thyroid hormones (i.e., rule out the presence of primary hyperthyroidism and the various forms of euthyroid hyperthyroxinemia) [2].

A thyrotropinoma must be suspected in the presence of neurologic signs and symptoms such as visual defects (25% of patients), headache (20%), and hypopituitarism (50%), all these being possible expressions of a tumor expansion [3, 17, 20]. Moreover, alteration of hypothalamic–pituitary–gonadal axis is frequent in case of pituitary tumors, with menstrual disorders present in all patients who have mixed thyrotropin/PRL tumors and in one third of those who have pure thyrotropinomas.

Delayed puberty, central hypogonadism, and decreased libido are also found in men with thyrotropinomas or mixed thyrotropin/FSH tumors.

Once the diagnosis of central hyperthyroidism is confirmed, additional diagnostic steps have to be performed to differentiate thyrotropinoma from RTH, in particular PRTH [2, 3, 7, 16, 17, 78, 80] (Table 8.2). Liver (sex hormone-binding globulin [SHBG]) and bone (carboxyterminal cross-linked telopeptide of type I collagen [ICTP]) parameters are successfully used to differentiate hyperthyroid patients who have thyrotropinoma from those who have PRTH. Higher serum SHBG and ICTP levels are common in patients with thyrotropinoma, whereas they are in the normal range in patients who have RTH. No statistically significant differences in terms of sex, age, TSH levels, or free thyroid hormone concentrations have been observed between patients with thyrotropinoma and those with RTH [2]. As far as dynamic tests are concerned, typically TSH-omas do not respond to TRH stimulation and/or to T3 suppression tests. Moreover, elevated α-GSU concentrations or high α-GSU/thyrotropin molar ratio and thyrotropin unresponsiveness to TRH stimulation or to T3 suppression tests, or both, favor the presence of a thyrotropinoma.

Another parameter that can be useful for the differential diagnosis is the evaluation of the sensitivity to long-acting somatostatin analogs [81]. More than 90% of

Table 8.2 Differential diagnosis between TSH-oma and PRTH

Parameter	TSH-omas	PRTH	*P*
F/M	1.3	1.4	NS
Familial cases (%)	0	85	<0.0001
Thyrotropin mU/L	2.7 ± 0.6	2.1 ± 0.3	NS
FT4 pmol/L	40.0 ± 4.2	30.5 ± 2.6	NS
FT3 pmol/L	14.5 ± 1.4	12.7 ± 1.2	NS
SHBG nmol/L[a]	113 ± 17	60 ± 5	<0.0001
Presence of lesion at CT scan or MRI (%)	98	5	<0.0001
High α-GSU levels (%)	65	3	<0.0001
High α-GSU/thyrotropin m.r. (%)	81	2	<0.0001
Abnormal thyrotropin response to T3 suppression test (%)[b]	100	100	NS
Blunted thyrotropin response to TRH test (%)	94	4	<0.0001

TSH-oma are sensitive, and two or more administrations of analog are usually sufficient to induce significant decreases or normalization of circulating free thyroid hormone. These modifications have never been observed in PRTH patients (Table 8.2). Finally, TRβ gene analysis may be useful in the differential diagnosis, as genomic TRβ mutations have been detected in patients with RTH only [7].

8.5.3 Imaging Studies and Localization of the Tumor

Nuclear MRI currently represents the imaging strategy of choice for the visualization of a thyrotropinoma. High-resolution CT is the alternative approach in case MRI is contraindicated (e.g., in the presence of a pacemaker). Although the diagnosis of TSH-oma is strongly supported by the presence of a pituitary lesion at neuroradiological imaging, a pituitary lesion has been identified at MRI in about 20% of RTH, indicating that a pituitary incidentaloma and RTH may coexist [82]. The differential diagnosis with PRTH may be problematic when the pituitary adenoma is small in size or in the case of confusing lesions, such as empty sella, pituitary incidentalomas, or ectopic tumors [81]. Pituitary scintigraphy with radiolabeled octreotide (Octreoscan) has been used to detect thyrotropinomas expressing somatostatin receptors [83]. Although the specificity of this procedure is low, it has been useful in the recognition of nasopharyngeal mass in few patients with clinical and biochemical features of central hyperthyroidism [84, 85].

8.6 Management

As recommended by the guidelines published by the European Thyroid Association [80], the first-line therapy for TSH-omas is surgical resection by transsphenoidal or subfrontal adenomectomy, the choice of the route depending on the tumor volume, and its suprasellar extension and invasiveness. This procedure aims to removing neoplastic tissue and normalizing normal pituitary/thyroid function. The operation may be difficult as the tumor may present a marked fibrosis, possibly related to high expression of basic fibroblast growth factor [28], and local invasion involving the cavernous sinus, internal carotid artery, or optic chiasm. Particular attention has to be paid to presurgical preparation of the patient, particularly in the preanesthetic period [86]: Antithyroid drugs along with propranolol should be used aiming at restoration of euthyroidism. Presurgical treatment with somatostatin analogs (octreotide LAR, lanreotide autogel) might be effective in reducing TSH-oma size and normalizing circulating thyroid hormones levels [87]. It should be noted that this approach may cause TSH secretion from normal thyrotropes to be re-activated, leading to the loss of a useful parameter to evaluate the complete removal of the adenoma, which is undetectable TSH levels few days after successful surgery. Neurosurgical intervention may cause a partial or complete hypopituitarism. However, a case of thyroid storm after pituitary surgery was reported [88]. In case of failure of pituitary surgery and in the presence of life-threatening hyperthyroidism, total thyroidectomy or

thyroid ablation with radioiodine is indicated [89]. According to the largest published series, pituitary surgery is effective in restoring euthyroidism in 75–83% of patients with TSH-omas [61, 90].

When the patients decline surgery or in case of surgical failure, radiotherapy and/or medical treatment with somatostatin analogs should be considered [80].

In case of radiotherapy, the recommended dose is no less than 45 Gy fractionated at 2 Gy per day or 10–25 Gy in a single dose if a stereotactic gamma unit is available [80, 91]. This procedure manages in normalizing thyroid function in 37% of patients within 2–4 years [61].

Some patients require medical therapy in order to control hyperthyroidism, although earlier diagnosis has improved the surgical cure rate of TSH-omas. The medical treatment of TSH-omas is based on long-acting somatostatin analogs, such as octreotide or lanreotide [27, 41, 42, 80, 92–94]. Treatment with these analogs leads to a reduction in TSH and alpha-GSU secretion in almost all cases, with restoration of the euthyroid state in about 95% of patients. Somatostatin analogs are safe even though side effects, such as cholelithiasis and carbohydrate intolerance, may appear. They are safe even during pregnancy [26]. Octreotide treatment in pregnant women was reported to be effective in restoring euthyroidism in the mother and had no side effects on development and thyroid function of the fetuses [7, 26, 48]. Many papers suggest the use of somatostatin analogs as first-line therapy for patients with TSH-omas, particularly for invasive macroadenomas [95–98]. During somatostatin analog therapy, tumor shrinkage occurs in about 50% of patients and vision improvement is seen in 75% [61, 90, 99]. Very rapid shrinkage of the tumor has been described [100]. Dose should be tailored for each patient, depending on therapeutic response. Tolerance is usually very good, as gastrointestinal side effects are transient with long-acting analogs [38, 41, 42, 99, 101].

Resistance to somatostatin analogs treatment has been documented in a minority of cases. The presence of dopamine receptors in TSH-omas was the rationale for the use of dopaminergic agonists, such as bromocriptine and cabergoline. A heterogeneity of TSH responses to these drugs has been described with the best effects achieved in mixed PRL/TSH tumors [69, 102, 103].

8.7 Follow-Up

Few data on TSH-oma recurrence in patients considered cured after surgery or radiotherapy have been reported so far. However, recurrence of the adenoma seems to be an infrequent event, at least in the first years after successful surgery [64, 104]. In general, postoperatively, the patient should be evaluated clinically and biochemically 2 or 3 times during the first year and then once a year. Pituitary imaging should be performed every 2 or 3 years, but should be promptly done whenever an increase in TSH and thyroid hormone levels, or clinical symptoms occur. In the case of a persistent macroadenoma, close visual field follow-up is required, since visual function could be threatened. Emergency surgical decompression is not always able to reverse even a recent visual deficit.

8.8 Prognosis

The criteria of cure of patients operated or irradiated for TSH-omas have not been clearly defined, due to the rarity of the disease and the great heterogeneity of the methods used. Some of these criteria are inapplicable if patients underwent previous thyroid ablation (Table 8.3).

A positive prognostic event is the absence of neurological signs and symptoms, but lacks both sensitivity and specificity, as even an incomplete debulking of the tumor may cause visual field defects and headache disappearance. It is logical that cured patients have clinical and biochemical reversal of thyroid hyperfunction after withdrawal from antithyroid medications. Nevertheless, the presence of normal free thyroid hormone concentrations or normalization of parameters peripheral thyroid hormone action (SHBG, ICTP, etc.) does not attest the complete removal or destruction of tumoral cells, since transient clinical remission accompanied by normalization of thyroid function is possible [32, 64, 104–106]. The criteria of normalization of circulating TSH are not applicable to previously thyroidectomized patients and to the 26% of patients with normal basal values of TSH. In our practice, undetectable TSH levels 1 week after surgery indicate complete adenomectomy, provided that the patient was hyperthyroid and presurgical treatments were stopped before surgery [64]. Normalization of alpha-GSU and/or the alpha-GSU/TSH molar ratio is in general a good index for the evaluation of therapy efficacy [16, 64]. However, both parameters are characterized by less than optimal sensitivity, as they are normal in about 25% of patients with TSH-oma. The most sensitive and specific test to document the complete removal of the adenoma, in the absence of contraindication, is

Table 8.3 Criteria for the evaluation of treatment outcome

Criteria	Comments
Remission from hyperthyroid manifestations (clinical and biochemical)	Clinical improvement may be transient *No predictive value*
Undetectable TSH 1 week after neurosurgery	Applicable to hyperthyroid patients that stopped treatments at least 10 days before surgery *Good prognostic value*
Normalization of circulating TSH levels	Not applicable to patients with normal TSH *Poor predictive value*
Normalization of free thyroid hormone levels	Biochemical remission may be transient *Poor predictive value*
Positive T3 suppression test with undetectable TSH and no response to TRH (or central hypothyroidism)	Not applicable to elderly patients or in those with cardiac diseases *Optimal sensitivity/specificity and predictive value*
Normalization of alpha-GSU levels and alpha-GSU/TSH molar ratio	Not applicable to patients with normal values before neurosurgery *Lack of sensitivity*
Disappearance of neurological manifestations (adenoma imaging, visual field defects, headache)	May be transient *Poor predictive value*

the T3 suppression test [64]: Only patients in whom T3 administration completely inhibits basal and TRH-stimulated TSH secretion can be defined as cured.

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Nonfunctioning Pituitary Adenoma

9

Maria Yavropoulou, Marina Tsoli, and Gregory Kaltsas

9.1 Introduction

Pituitary adenomas are predominantly monoclonal benign tumors that arise from expansion of single mutated precursor cells that possess a certain proliferative advantage. Due to their neuroendocrine component, the term pituitary neuroendocrine tumors (PitNETs) has recently been introduced [1]. Pituitary adenomas comprise different histological subtypes, according to their specific adenohypophyseal hormonal immunostaining and expression of pituitary-specific transcription factors. A number can present with a distinct clinical syndrome secondary to the secretion of a peptidic hormone to the circulation (functioning PitNETs). Nonfunctioning pituitary adenomas (NFPAs), in contrast to their functioning counterparts, are defined as pituitary tumors that are not associated with a secretory phenotype. The majority of these neoplasms are benign and are following a relatively indolent course although a subset may present a more aggressive behavior with resistance to employed treatment and/or early recurrences, whereas a small subset may become truly malignant developing distant metastases [2].

NFPAs are diagnosed either due to symptoms related to mass effect and compression to nearby vital structures and/or the presence of anterior hormonal deficiencies or incidentally during imaging investigations performed for unrelated purposes (pituitary incidentalomas). In the presence of hormonal deficiencies,

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adequate replacement is needed, whereas surgical resection is undertaken in cases of neurological abnormalities or visual impairment and/or in the presence of rapid tumor growth. Alternatively, active surveillance can also be considered in the absence of such manifestations. Radiotherapy (RT) may also be considered as adjuvant treatment in aggressive tumors and in case of significant growth of a tumor remnant or relapsing adenoma, while temozolomide, which is an alkylating agent, has been currently recommended as a therapeutic option for aggressive neoplasms or pituitary carcinomas [2].

The pathogenesis of NFPAs still remains elusive, but it has been proposed that disruption of cell cycle regulators, as the initiating event, can induce pituitary tumorigenesis. Classic oncogene mutations are rarely encountered in pituitary tumors. Nevertheless, a considerable number of pituitary-specific cellular disruptions have been uncovered and affect oncogenes, transcription factors, tumor suppressors, cyclins, inactivating factors, or proliferation inhibitors. Unraveling the pathogenesis of NFPAs is a critical step in the quest of new subcellular treatment targets that will decrease morbidity and mortality [3].

In this chapter, we outline current knowledge about the pathophysiology and management of NFPAs.

9.2 Epidemiology

Nonfunctioning pituitary adenomas (NFPAs) represent approximately 30–40% [4] of anterior pituitary tumors, ranging from 14% to 54% in national-wide population studies [4–8]. They present with a peak occurrence between forth and eight decades, but data on gender predominance are inconsistent [9, 10].

Most of the NFPAs are sporadic but may also occur as a component of hereditary syndromes, such as multiple endocrine neoplasia type 1 (MEN1), and multiple endocrine neoplasia type 4 (MEN4), the Carney complex, and the familial isolated pituitary adenomas (FIPAs). Data from patients with MEN1 from a French and Belgian multicenter study demonstrated that among pituitary adenomas only 14.7% are NFPAs and half of them are invasive [11]. In contrast, results from the Dutch MEN1 Study Group have revealed a increased proportion of NFPAs among pituitary tumors in patients with MEN1 (approximately 42.3%) in a systematic presymptomatic screening [12].

NFPAs represent less than 20% of patients with FIPA and, in this context, are diagnosed at an average of 8 years earlier compared with their sporadic counterparts [13, 14]. Patients with Carney complex most commonly develop functioning somatotropinomas or less frequently prolactinomas, but cases with pituitary tumors and asymptomatic elevations of growth hormone (GH), insulin growth factor 1 (IGF-1), and prolactin (PRL) have also been described [1, 15, 16].

9.3 Histopathological Classification

In the 2017 WHO classification for endocrine tumors, the adenohypophyseal cell lineage designation of the secreting and nonfunctioning pituitary adenomas based on the immunohistochemical (IHC) profile is considered the golden standard for the new classification system [17, 18] (Table 9.1). Moreover, the introduction of transcription factors as a complementary diagnostic tool in the classification of NFPAs in order to confirm the specific pituitary cell linage differentiation has paved the way toward a more precise classification of NFPAs and accurate characterization of their biological behavior. Three of the pituitary-specific transcription factors are currently recommended in routine diagnostics of PAs: pituitary transcription factor 1 (Pit-1), steroidogenic factor 1 (SF-1), and T-box family member TBX19 (T-Pit).

Pit-1 plays a role in the differentiation of somatotroph, lactotroph, and thyrotroph cells and in the development of the respective tumors [19, 20]. SF-1 acts as transcription factor for the differentiation of gonadotroph cells and is expressed in gonadotropinomas [17, 20], and T-Pit is required for the

Table 9.1 Classification of nonfunctioning pituitary adenomas based on hormone staining and nuclear expression of pituitary-specific transcription factors

Subtype	Hormone staining	Transcription factors
Silent Somatotroph adenomas	GH, α-subunit	PIT-1
Sparsely granulated	Diffuse and strong staining	
Densely granulated	Weak and; patchy staining	
Lactotroph adenomas	PRL	PT-1, ER a
Sparsely granulated	Perinuclear staining	
Densely granulated	Diffuse staining	
Acidophilic stem cell adenoma	Focal and variable staining of both PRL and GH	
Thyrotroph adenomas	TSHβ, α-subunit	PIT-1, GATA2
Silent Corticotroph adenomas	ACTH	T-PIT
Type 1 (densely granulated)	Diffuse and strong staining	
Type 2 (Sparsely granulated)	Weak and patchy staining	
Crooke -cell	Peripheral staining	
Gonadotroph Adenomas	FSH**β**, LH**β**, **α**-subunit	SF1, GATA2, ER**α**
Null cell adenomas	None	None
PIT-1-positive adenomas (plurihormonal)	GH, PRL, TSH**β**±, **α**-subunit	PIT-1

Modified by Mete et al 2017 (17).

transcription of the precursor polypeptide pro-opiomelanocortin (POMC) to adrenocorticotropic hormone (ACTH) and promotes the differentiation of corticotroph cells [21, 22].

In addition, estrogen receptor-α (ERα) and guanine–adenine–thymine–adenine binding protein 2 (GATA-2) transcription factors are also associated with the differentiation of gonadotroph, lactotroph, and thyrotroph cells, and may be pathogenetically involved in the development of the respective tumors [19, 23–25].

9.3.1 Subtypes of Nonfunctioning Pituitary Adenomas

NFPAs comprise different histological subtypes, classified according to their immunostaining to different adenohypophyseal hormones and transcription factors.

The current WHO classification system recognizes NFPAs as variants of their functioning counterparts, with the exception of null cell PAs, which is the only subtype without functioning counterpart [18, 26].

9.3.1.1 Nonfunctioning Silent Gonadotroph Adenomas

Silent gonadotroph adenomas (SGAs) comprise the vast majority (approximately 80%) of NFPAs and typically demonstrate, at least focally, immunostaining for beta-follicle-stimulating hormone (β-FSH), beta-luteinizing hormone (β-LH), and α-subunit [18]. Interestingly, it is the only type of NFPAs where the nonfunctioning variant predominates [27, 28].

Before the introduction of the SF-1, many silent gonadotroph adenomas that were LH/FSH immunonegative were mistakenly classified as null cell type pituitary adenomas [29, 30]. Although currently available anti-SF-1 antibodies exhibit several methodological difficulties, the SF-1 nuclear labeling is detected in a significant proportion of tumor cells, confirming the diagnosis in cases with sparse or no gonadotroph hormone expression [31]. The distinction between SGAs and true null cell adenomas is of significant clinical relevance since true null cell adenomas follow with a more aggressive biological behavior than SGAs [32]. In addition, low ERα nuclear expression in tumor cells of SGAs is considered a good predictive tool of aggressiveness in these tumors [33].

9.3.1.2 Nonfunctioning/Silent Corticotroph Adenomas

Silent corticotroph adenomas (SCAs) represent approximately 15–20% of NFPAs [34, 35], and it is second most common type after SGAs. The real proportion, however, is probably underestimated, since routine nuclear staining for the transcription factor T-Pit, which regulates corticotroph differentiation, is not widely available [36].

Despite the fact that clinical characteristics of Cushing's syndrome are not present by definition, SCAs can either present with normal cortisol secretion (totally silent) or slightly elevated ACTH levels (clinically silent) [37–40]. SCAs are usually macroadenomas associated with mass-related symptoms and cavernous sinus invasion and show a female preponderance [41].

Histologically, SCAs, similar to their functioning counterparts, can be further divided into two subtypes based on specific morphological and ultrastructural characteristics: type I densely granulated SCAs, which show strong ACTH immunoreactivity, and type 2 sparsely granulated SCAs, which are more common and demonstrate weak and focal ACTH staining. Both subtypes demonstrate a more aggressive biological behavior compared with their functioning counterparts [17]. In addition, POMC mRNA levels are higher in type I SCAs compared with type 2 SCAs, and similar to what is found in functioning corticotroph adenomas [42]. Crooke cell adenoma with a typical perinuclear ring-like accumulation of cytokeratin and relocation of ACTH positivity to the sub-membranous zone has been rarely documented in clinically silent corticotroph adenomas [43, 44] despite the absence of exposure to high circulating glucocorticoid levels.

In 2017 WHO Classification of Pituitary Tumors, SCAs are graded as "high-risk adenomas" due to their aggressive clinical behavior and high probability of recurrence. In a large cohort of patients with aggressive pituitary tumors and pituitary carcinomas, which investigated the efficacy of temozolomide as first-line chemotherapeutic treatment, corticotroph tumors prevailed accounting for approximately 45% of the cohort. Interestingly, a high proportion of the initially silent corticotroph tumors (26%) of this cohort were evolved in ACTH-secreting tumors [45]. Other studies, however, comparing recurrence rates of SCAs with other NFPAs subtypes have provided conflicting results. In a recent meta-analysis, based on studies with mean follow-up of more than 5 years, SCAs show recurrence of approximately 31%, similar to what has been reported to other subtypes of NFPAs [46].

Transformation of SCAs into functioning corticotroph adenomas (and vice versa), although rare, has also been reported [39, 43, 47–49]. In a recent study with 15 years of follow-up, only 4% of ACTH-immunohistopositive adenomas showed signs of transformation based on histology (2.8% adenomas transformed from SCA to ACTH-secreting adenomas and 1.1% transformed from ACTH-secreting adenomas to SCA).

The underlying molecular mechanisms that drive the tumorigenesis of these unique pituitary tumors remain largely unknown. The vast majority of SCA cells, however, exhibit multiple dark lysosomes, fusion of secretory granules with lysosomes, and extensive disposal of granules by lysosomes and autophagy [50] on electron microscope suggesting that either lysosome dysfunction or abnormal ACTH synthesis leads to destroy of ACTH before it is secreted [50].

Another hypothesis on the pathogenesis of these tumors is related to an impaired post-translational processing of the pro-hormone POMC in the biologically active mature hormone ACTH that is dependent on the expression of pro-hormone convertase 1/3 (PC1/3). SCAs show a significant decrease in PC1/3 expression compared with corticotroph adenomas associated with ACTH-secreting adenomas [51, 52].

In general, SCAs are pituitary adenomas that are biologically and clinically distinct from both functioning corticotroph adenomas and other NFPAs. Approximately one-third of patients with SCA may develop new-onset hypopituitarism and show tumor recurrence, underlying the need for close monitoring of these patients [40]. As in most NFPAs, silent CAs are diagnosed relatively late when they have already

formed invasively growing macroadenomas and, in rare cases, can transform to carcinomas [53, 54].

9.3.1.3 Nonfunctioning/Silent Somatotroph Adenomas

Silent somatotroph adenomas (SAs) represent approximately 2–4% of all pituitary adenomas in surgical series [55, 56].

Growth hormone immunostaining in silent SAs varies widely from very weak to strong positive but is usually less than somatotropinomas causing acromegaly [55, 57]. In cases with very low or absent GH staining nuclear expression of the transcription factor, Pit-1 is a valuable diagnostic tool, since all GH tumors express Pit-1 [56].

Patients with silent SAs usually present with normal GH and IGF-1 levels, but there have been few reports of "clinically silent" cases, with non-suppressible serum GH and elevated IGF-1 levels [58–61]. In addition, approximately 12% of silent SAs have been reported to progress in clinical acromegaly during long-term follow-up, particularly in women [62–64].

Similar to their functioning counterparts, silent SAs are classified as densely, sparsely, or intermediately granulated, based on the presence and pattern of the low molecular weight cytokeratin (CAM 5.2) staining. The sparsely granulated subtype is more frequently reported and is considered a more aggressive subtype, while more than 50% of the silent SAs (twofold higher than somatotropinomas causing acromegaly) are adenomas with mixed GH and prolactin (PRL) secretion [55, 62].

In addition, silent SAs exhibit distinct biological and epidemiological characteristics compared to their functioning tumors. They are usually larger, exhibit a more aggressive biological behavior, recur earlier and more frequently, and are more common in females, and at younger ages between 20 and 40 years [55, 62].

Both functioning and silent SAs express somatostatin receptors, 2 (SSTR2) and 5 (SSTR5), with SSTR2 expression being significantly lower in silent compared to functioning SAs [55]. In one series SSTR2 was expressed in all silent SAs reviewed, while in another one SSTR2 was expressed in approximately 50% of them [62]. Expression of SSTR5 was found similar between functioning and silent SAs. Nevertheless, silent SAs show a lower response to somatostatin analog therapy compared to their functioning counterparts [65].

The causes that lead to silence in these tumors remain unknown although several hypotheses have been suggested. Abnormalities downstream the Pit-1 signaling pathway [66], impaired synthesis or post-translational processing of GH leading to reduced circulating levels [30, 67], or synthesis of an immunoreactive form of GH without biologic activity has been proposed as the most plausible pathophysiological mechanisms in these tumors [48]. In addition, inhibition of GH release is highly unlike since cytoplasmic lysosome accumulation has not been described in silent SAs [57]. Mutations in the aryl hydrocarbon receptor-interacting protein (AIP) have also been reported in two cases of silent SAs [68]. Nevertheless, silent SAs usually present as giant tumors with higher recurrence rate and higher need for radiation therapy compared to other NFPAs, and thus, close follow-up is strongly indicated [56].

9.3.1.4 Nonfunctioning/Silent Thyrotroph Adenomas

Silent thyrotroph adenomas (STAs) are extremely rare but are reported more frequently compared to their functioning counterparts (thyroid-stimulating hormone, TSH-omas) associated with hyperthyroidism [17, 18]. STAs share many histopathological characteristics with TSH-omas and are almost indistinguishable on histology but display unique histopathological features compared to other types of pituitary adenomas. Both thyrotroph tumors present with TSH-β, and α-subunit immunohistochemical staining, express Pit-1 and GATA-2 transcription factors, and show significant membrane immunoreactivity for SSTR-2A and SSTR-5 [69]. Usually, they are larger and more invasive compared to TSH-omas causing hyperthyroidism [69], but they seem to behave similarly regarding treatment outcomes and recurrence rates [70]. A few cases of STAs evolving to functioning TSH-omas have also been described [33, 63].

9.3.1.5 Nonfunctioning/Silent Lactotroph Adenomas

Silent lactotroph adenomas (SLAs) are very rare in surgical series [29] although more frequently reported in autopsy series [71]. In addition to PRL-positive staining, SLAs also express Pit-1 and ER-a [17, 18]. Mono-hormonal SLAs are extremely rare and more often present as silent mixed somato-lactotroph adenomas in Pit-1-positive tumors [62].

As their functioning counterparts (PRL-omas), SLAs are classified into sparsely (with Golgi-like prolactin immunoreactivity) and densely (with diffuse cytoplasmic prolactin immunoreactivity) granulated subtypes [17, 18]. Despite that SLAs lack clinical symptomatology of hyperprolactinemia, high prolactin levels are reported due to stalk compression [71].

9.3.1.6 Null Cell Adenomas

Null cell adenomas (NCAs) are defined by the lack of immunohistochemical staining of any anterior pituitary hormone and pituitary-specific transcription factors [17, 18]. They present a very small proportion of all pituitary tumors [36], although their real frequency is probably overestimated due to methodological pitfalls in immunohistochemical protocols and lack of widely available and reliable antibodies to pituitary-specific transcription factors. The diagnosis of NCAs is usually made by exclusion and their differential diagnosis includes both adenohypophysial and non-adenohypophysial neuroendocrine tumors of the sellar region [72].

Data from a retrospective case series of 516 patients with NFPAs have shown that from the 23% of the tumors initially classified as NCAs by using only the classical pituitary hormone IHC, only 5% remained as true NCAs, when lineage-specific markers, such as Pit-1, SF-1, and T-Pit, were used [73].

9.3.1.7 Plurihormonal Pit-1-Positive

Pit-1-positive plurihormonal adenomas (previously named as silent subtype 3 adenomas) are a distinct entity, with reportedly aggressive behavior [17, 18]. In a single-center retrospective case series, their prevalence was less than 1% among resected pituitary tumors in a period of 13 years [74]. Histologically, these tumors

consist of large polygonal or spindle-shaped cells, with atypical nuclei, sometimes containing inclusions, that can be identified on routine HE stains or electron microscopy [17, 18, 75, 76].

They usually demonstrate immunoreactivity for GH, PRL, and TSH in different combinations, and despite their silent nature, approximately 30% of these tumors are associated with clinical signs of Pit-1 lineage hormones hypersecretion leading to hyperthyroidism, acromegaly, or hyperprolactinemia [77]. The correct diagnosis is very challenging in everyday clinical practice but of critical importance since these tumors are usually macroadenomas with aggressive biological behavior, high invasion rates to cavernous sinus and clivus, and high recurrence rates [75, 77].

9.3.2 Nonfunctioning Pituitary Carcinomas

Nonfunctioning pituitary carcinomas of gonadotroph or null cell type have rarely been reported, but their true prevalence is unknown since their metastases remain asymptomatic for many years [78, 79].

9.4 Pathophysiology

Despite recent advances in molecular biology and genetics, the pathophysiology of NFPAs is yet far from being fully elucidated. Many hypotheses, which mainly include genetic and epigenetic events, as well as hormonal stimulation, impaired intracellular signaling pathways, and microRNAs seem to finally converge in severely disrupted and uncontrolled tumor growth and proliferation of pituitary cells.

9.4.1 Genetics

Although the majority of NFPAs occur sporadically, recent advances in molecular genetics have identified an increased prevalence of inherited genetic susceptibility in PitNETs.

Genetic mutations in a single cell leading to overexpression of proto-oncogenes or inactivation of tumor suppressor genes, and functional or epigenetic alterations of transcription factors that regulate cell growth and differentiation are the most prominent events in the genetic background of pituitary tumorigenesis. Among the genes that have been implicated in the pathophysiology of pituitary adenomas, guanine nucleotide-binding protein, alpha-stimulating (GNAS), aryl hydrocarbon receptor-interacting protein (AIP), and pituitary tumor-transforming gene (PTTG) have been more frequently described in NFPAs [80, 81].

In a Brazilian cohort of patients with pituitary adenomas, GNAS mutations were found in much lower proportion in NFPAs compared to somatotropinomas (4.8% vs. 27%), whereas PTTG overexpression and AIP underexpression were reported in almost all cases without significant differences, however, between the NFPAs and

somatotropinomas [82]. Moreover, in this cohort neither GNAS mutations nor impaired expression of PTTG and AIP was associated with tumor clinical characteristics and treatment outcomes [82].

In the MEN1 syndrome caused by germline heterozygous mutations in the MEN1 gene, encoding for menin protein in chromosome 11q13, pituitary adenomas (that are present at approximately 15–50% of patients) coexist with parathyroid hyperplasia and neuroendocrine tumors of the gastro-enteropancreatic tract [83]. NFPAs are not the predominant type of Pit-NETs in patients with MEN1, but when they develop in this genetic background display a more aggressive behavior, similar to their functioning counterparts [84, 85].

Another genetic syndrome leading to the development of pituitary adenomas in association with other endocrine neoplasms is MEN4, caused by germline mutations in the CDKN1B gene, encoding for p27Kip1, a negative regulator of cell cycle progression. In patients with MEN4 that develop pituitary adenomas, NFPAs occur more frequently, [86] compared to MEN1. Whether CDKN1B mutations are also present in sporadic NFPAs is not yet known.

Carney complex is caused by inactivating mutations in the PRKAR1A gene that encodes for regulatory subunit type 1alpha of the cAMP-dependent protein kinase A (PKA) and acts as a tumor suppressor gene [1]. Almost half of the patients with Carney complex have germline-inactivating mutations in the PRKAR1A gene, and in those who develop pituitary tumors, the normal allele of PRKAR1A gene in the pituitary is lost. This loss of heterozygosity of the PRKAR1A gene leads to somatomammotroph hyperplasia, which in turn may lead to additional genetic changes at the somatic level, and the formation of adenomas [1, 15, 16]. Patients with Carney complex develop acromegaly at a 10–12%, but clinically silent GH and IGF-1 elevations have also been described [16].

The role of PRKAR1A somatic mutations in the development of sporadic pituitary tumors is currently unknown [87].

Although NFPAs account frequently in familial isolated pituitary adenomas (FIPA) (14.5% of the patients), the role of AIP germline mutations in the pathophysiology of NFPAs has demonstrated conflict results [88–91] and therefore does not seem to significantly contribute in the genetic background of NFPA tumorigenesis.

Coexistence of pituitary adenomas and pheochromocytoma/paragangliomas is very uncommon, but a germline mutation in two of the genes encoding succinate dehydrogenase SDHC and SDHD has been reported in a 30-year-old man with a NFPA [92].

In general, data reported so far underline the significant contribution of epigenetic alterations and somatic mutations rather than a role for germline mutations in NFPA tumorigenesis.

As such, the tumor suppressor protein p16, a cyclin-dependent kinase inhibitor encoded by the CDKN2A gene, is frequently reported to be downregulated in NFPAs [93]. Similarly, significant downregulation in NFPAs is reported for the DNA damage-inducible gene 45 g (GADD45g), due to promoter methylation [94], and the maternally expressed gene 3 (MEG3), which acts as a tumor suppressor

gene [95]. Both GADD45g and MEG3 are regulated by p53-protein, which negatively regulates pituitary cell growth, pointing the fact that impaired control of cell proliferation plays a key role in NFPA tumorigenesis. Activating somatic mutations of the PIK3CA gene that encodes for the catalytic subunit of PI3-kinase IA, within the AKT signaling pathway [96], have also been described in NFPAs.

9.4.2 Epigenetics

Despite the advanced progress in the identification of recurrent somatic alterations and rare inherited variants, their frequencies among pituitary adenomas are low, suggesting that factors other than gene mutations may be linked with the pathogenesis of sporadic pituitary adenomas.

In recent years, epigenetics present a new discipline that attempts to explain significant differences in phenotypes among patients with the same disease, such as diabetes mellitus or cancer [97]. There are two main types of epigenetic modifications—DNA methylation and histone modifications.

Methylation, in particular, changes the activity of a DNA segment without changing the sequence and it usually acts to repress gene transcription.

DNA methylation analysis in a cohort of NFPAs and functioning adenomas revealed tumor-specific genome-wide patterns of DNA methylation and gene expression between different subtypes of pituitary adenomas. In particular, genome-wide DNA methylation profile was very similar between silent and functioning corticotroph adenomas, whereas functioning SAs display higher levels of hypomethylated regions compared to functioning corticotroph adenomas and SGAs [98]. In addition, significant differences were noted in the methylated regions of SGAs (high proportion of hypermethylated DNA regions) and SCAs (higher proportion of hypomethylated DNA regions).

In contrast to the other epigenetic mechanisms that modulate gene transcription, microRNAs (miRs) are small noncoding single-stranded RNA molecules of approximately 22 nucleotides, acting at the post-transcriptional level and directly modulating gene expression of mRNA genes. MicroRNAs bind mRNAs through complementary base pairing, resulting in suppressed translation from the mRNAs or degradation of the mRNAs through the formation of an RNA-induced silencing complex (RISC). Although these molecules are reported to target both activating oncogenes and tumor suppressor genes, their exact role in pituitary tumorigenesis has not yet been clarified [99–104].

Several microRNAs have been found to be deregulated in NFPAs, compared to normal pituitary tissue (Table 9.2); however, a consistent molecular profile of specific microRNAs that could serve as novel biomarkers or as therapeutic molecular targets has not been identified. Nevertheless, a wide variety of target genes appear to be involved, with most being growth factors and regulators of cell cycle division. Interestingly, up to now research in microRNAs profile in pituitary adenomas has

Table 9.2 Differential expression of microRNAs in nonfunctioning pituitary adenomas

MicroRNA	Differential Expression	Target Gene
miR-107(186)	Increase	AIP
miR-598/181d/191-3p/ 181b-5p/3676-5p/383(103)	Increase	-
miR-23b(187)	Decrease	HMGA2
miR-130b(187)	Decrease	CCNA2
miR-424/503(188)	Decrease	CDC25A
miR-135a/429/140-5p/ 582-3p/938/582-5p(189)	Increase	Smad3
miR-197/33b(189)	Increase	DLK1
miR-15/16/26a/196a2, Let-7a(190)	Decrease	HMGA1/HMGA2
miR-133(191)	Decrease	FOXC1
miR-20a/17-5p(192)	Increase	PTEN/TIMP2
miR-106b(191, 192)	Increase	PTEN/TIMP2 PI3K/AKT
miR-124a(193)	Increase	-
miR-144/373/422b/202/520e/32/ 422a/181c/181b/520c/188/155/ 520 f/520b/182/10b/523/146a(193)	Increase	-
miR-31/506/218/503/513/514(193)	Increase	-
miR-128a/516a-3p/15(194)	Increase	Wee1
miR-195(194)	Increase	
miR-20a/93(194)	Increase	
miR-524-5p(195)	Decrease	PBF

failed to demonstrate epigenetic alterations on specific genes that have been traditionally linked to pituitary tumorigenesis. Thus, it appears that genetic mutations and epigenetic alterations involve distinct target genes but may act synergistically in regulating pituitary tumorigenesis.

9.4.3 Intracellular Molecular Signaling Pathways

Over the past 30 years, studies of developmental biology have identified the role of evolutionarily highly conserved intracellular signaling pathways such as Wnt, and Notch in pituitary development and disease. Through these pathways, specific secreted proteins control differentiation and function of recipient cells in a paracrine and/or autocrine manner. Various components of the Notch and Wnt signaling have been found deregulated in NFPAs, while data on the role of Hh signaling are currently missing.

9.4.3.1 The Role of Notch Intracellular Signaling Pathway in the Pathophysiology of NFPAs

Notch signaling pathway regulates cell fate through lateral inhibition and formation of boundaries, both of which represent patterning processes of critical importance in the regulation of spacing of different cell types within tissues [105, 106]. There are four Notch receptors in mammals (Notch1, Notch2, Notch3, and Notch4) and five classic DSL (Delta/Serrate/Lag-2) ligands: Jagged1, Jagged 2, Delta-like 1, Delta-like 3, and Delta-like 4.

Notch receptors are single-pass transmembrane proteins composed of a functional extracellular domain (NECD), a transmembrane domain, and an intracellular domain (NICD). Notch ligands are also transmembrane proteins in the signal-sending cells, and both ligands and receptors require a catalytic process to become active (Fig. 9.1).

Expression of Notch 3 and Jagged 1 protein has been found significantly elevated in NFPAs compared with normal pituitary tissue [107]. Gene microarrays and proteomic analyses have confirmed increased expression of Notch 3 gene and protein in human NFPAs [107, 108] but not in GH- and PRL-secreting adenomas. In addition delta-like 1 ligand is strongly downregulated in NFPAs and in PRL-secreting adenomas, while cyclin D1, one of the Notch target genes, is found overexpressed in NFPAs [109].

9.4.3.2 The Role of Wnt Intracellular Signaling Pathway in the Pathophysiology of NFPAs

Wnt forms a family of 19 highly conserved secreted signaling molecules, rich in cysteine, which play an important role in the development and function of multiple tissues [110]. The canonical Wnt signaling pathway involves the formation of a complex between extracellular Wnt proteins, and transmembrane co-receptors the

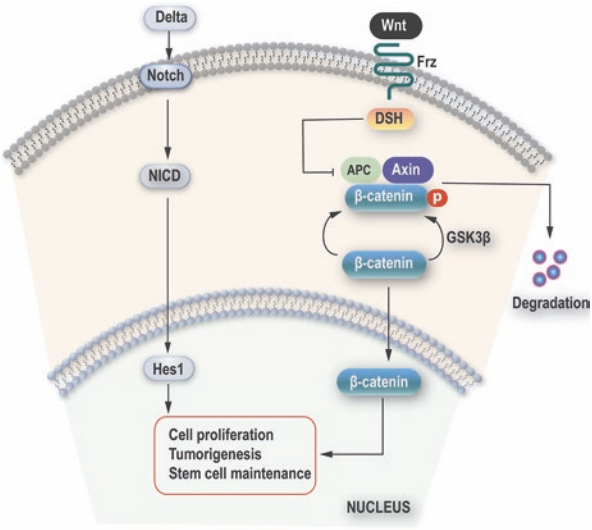

Fig. 9.1 An integrative diagram of Notch, and Wnt signaling crosstalk in the regulation of pituitary tumorigenesis. *NICD* Notch intracellular domain, *FRZ* frizzled receptor, *DSH* disheveled family protein, *APC* adenomatous polyposis coli, *GSK3b* glycogen synthase kinase 3b

Frizzled and the LRP5 or LRP6 receptors [111]. When activated, the ligand–receptor complex inhibits the cytoplasmic degradation of beta-catenin, promotes its translocation in the nucleus, and activates the transcription of target genes [112] (Fig. 9.1).

In a mouse model of MEN1 syndrome, Wnt proteins (Wnt4 and Wnt9a) and receptors (Fz6 and LRP2) were found significantly downregulated in all tumors related to this syndrome, from pituitary, pancreas, parathyroids, ovary, and testes [113].

Nuclear accumulation of β-catenin was found increased in 57% of pituitary tumors, with the higher expression reported in tumors originated from cells of the Pit-1 lineage [114], but this result was not confirmed in others [115]. Wnt inhibitors were also studied in human pituitary adenomas. Wnt inhibitory factor-1 (WIF-1) is a secreted protein that binds to Wnt proteins and inhibits their activities. WIF-1 was found significantly downregulated on a microarray analysis in a series of both functioning and nonfunctioning pituitary adenomas when compared with healthy pituitary tissue. Collectively, recent studies in vitro, in vivo, and in human tissue point to a potential role of Wnt signaling in pituitary adenomas both functioning and nonfunctioning, but further work is warranted in order to elucidate the exact molecular mechanisms involved [115].

9.5 Clinical Presentation

The clinical presentation of NFPAs varies from completely asymptomatic to panhypopituitarism and manifestations attributed to mass effect to nearby structures. The absence of clinical symptoms related to hormonal hypersecretion is associated with a significant delay in diagnosis estimated approximately to 1.96 ± 2.9 years [9].

Headache is a common neurologic symptom attributed to an expanding sellar mass and is observed in 19–75% of patients [116]. Possible mechanisms related to headache include increased intrasellar pressure and starching of dural membrane or invasion of cavernous sinus and trigeminal nerve irritation [14, 117]. A retrospective study found that 20% of the patients with incidentally observed NFPAs reported that suffered from headache [118].

Visual impairment is observed in 58% of patients with NFPAs [119]. Suprasellar extension of the pituitary adenoma can cause pressure on the optic chiasm, typically resulting to bitemporal hemianopia. Initially, the patients may be unaware of the visual deficit, but in case of severe and long-term compression visual acuity may be affected and optic atrophy or papilledema may occur [120]. Older age is considered to be associated with delayed diagnosis of NFPAs in patients with visual defects [121]. Visual field loss may be uni-, bilateral, or central as well as complete or partial depending on the site and degree of nerve compression [120]. In a recent study of 103 patients presenting to a neuro-surgical unit with a pituitary adenoma, it was observed that although bitemporal visual field loss was the most common defect (41%), a significant proportion of patients had unilateral or altitudinal defects [122]. Several mechanisms are involved in the pathophysiology of visual impairment that

may initially be reversible but becomes irreversible in case of long or intense chiasm compression. Reversible mechanisms include axoplasmic flow disorder, conduction blocking, and demyelinization. Axonal fiber degeneration is associated with irreversible visual defects [120].

Ocular motor impairment associated with large pituitary adenomas is attributed to cavernous sinus invasion and compression of ocular motor nerves III (common ocular motor nerve), IV (trochlear nerve), and VI (abducens nerve) [120]. The clinical manifestations vary according to the compromised nerve. Before true ocular motor impairment develops, some patients report frequent episodes of diplopia. The common ocular motor nerve is the most frequently affected resulting to eyeball shift outward, ptosis, and mydriasis. Trochlear nerve involvement causes upward and slightly inward deviation of the eyeball, while nerve VI palsy is related to abduction deficit [120, 123, 124].

In some rare cases, large aggressive NFPAs may invade other intracranial structures such as the posterior and anterior midbrain, the third ventricle or the brain causing the dorsal midbrain syndrome, intracranial hypertension, and hydrocephalus or temporal epilepsy. Invasion of the sellar floor may result to cerebrospinal fluid rhinorrhea. These tumors also display early recurrence after the initial surgical resection and rapid local growth and tumor extension [2, 120, 125].

Hypopituitarism is a very common manifestation in patients with NFPAs. Multiple cases are diagnosed when central hypothyroidism is observed during routine thyroid testing or in male patients who present with decreased libido and erectile dysfunction due to secondary hypogonadism. Pituitary hormone deficiency is attributed to compression of the normal anterior pituitary cells and/or pituitary stalk, affecting the stimulation from the hypothalamic factors and the secretion of pituitary hormones. GH deficiency is the most commonly observed followed by gonadotropin, corticotropin, and thyrotropin insufficiency [14, 126, 127]. In addition, disconnection hyperprolactinemia may be observed due to pituitary stalk compression as it prevents dopamine from reaching the anterior pituitary and could account for the observed cases of hypogonadism in the absence of gonadotropin deficiency. In patients with NFPAs, serum prolactin levels are rarely higher than 2000 mU/L (95 ng/ml) in the absence of medications affecting the prolactin levels [71]. Diabetes insipidus (DI) is an uncommon finding in case of NFPA and when encountered another than an adenoma pathology should be considered [128].

Pituitary apoplexy is a life-threatening situation that is attributed to acute hemorrhage or ischemic infarction of pituitary and is characterized by severe headache of sudden onset, nausea, vomiting, neuro-ophthalmologic symptoms, and impaired consciousness level. Furthermore, it may cause partial or complete hypopituitarism, particularly hypocortisolemia that is observed in 70% of cases [129, 130]. The real risk of apoplexy in NFPAs is largely unknown. In a retrospective study of 485 patients with NFPAs, pituitary apoplexy was the first presentation in 8% of cases [131]. A systematic review and meta-analysis regarding the natural history of pituitary incidentalomas and NFPAs reported an incidence of apoplexy in macroadenomas of 1.1% per year [132].

9.6 Diagnostic Evaluation

The identification of NFPA is based on the identification of a sellar mass on computed tomography (CT) or magnetic resonance imaging (MRI) of the sella turcica. The increased availability of CT and MRI and recent progress on neuroimaging have resulted in increased recognition of sellar or parasellar lesions, randomly, during imaging investigations performed for other reasons. These lesions are termed as pituitary incidentalomas [133].

MRI of the sella turcica with gadolinium is considered the gold standard modality for the initial evaluation or follow-up of pituitary lesions, while CT may display some advantage in the estimation of bone structures or calcifications [134]. Pituitary adenomas appear hypo- or isointense to normal pituitary on T1-weighted images, while on T2-weighted images they are isointense to the white matter [135] (Fig. 9.2**).** After the administration of gadolinium, the pituitary adenomas display low-contrast enhancement on T1-weighted images. There may be areas of necroses or hemorrhage that appear hyperintense on T1-weighted images without contrast. Microadenomas measuring less than 10 mm are typically small intrasellar lesions, while macroadenomas measuring above 10 mm may extend to the suprasellar cistern and compresses the optic chiasm or extend into the cavernous or sphenoid sinus [14, 135].

There are two systems of radiological classification of pituitary adenomas: Knosp's classification that divides pituitary adenomas into five categories according to parasellar extension and invasion of cavernous sinus (grades 0–IV) and Hardy's classification that comprises two subscales and estimates the sphenoid bone invasion (grades 0–IV) and the suprasellar extension of and adenoma (types A–E) [2, 136].

All patients with a pituitary incidentaloma or a clinically apparent NFPA should undergo a complete evaluation of anterior pituitary function as in many cases hormone hypersecretion or hypopituitarism may be subtle and slowly progressive

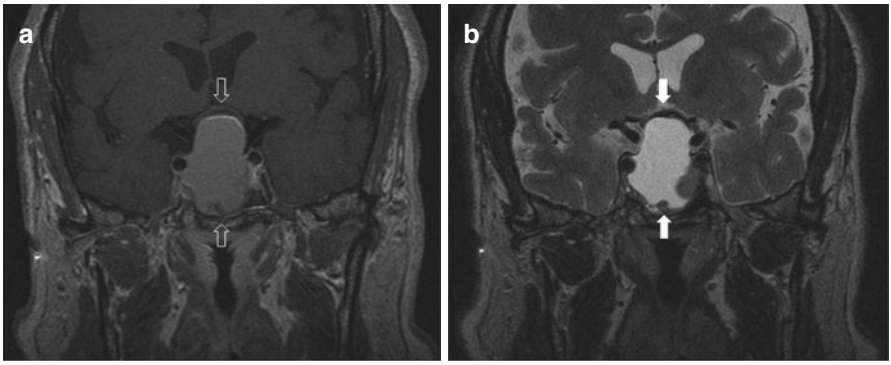

Fig. 9.2 T1 (**a**)-weighted and T2 (**b**)-weighted coronal MRI images showing a large pituitary lesion with suprasellar extension. The lesion causes elevation of the optic chiasm and contacts the right and left cavernous internal carotid artery

Table 9.3 Initial endocrine evaluation of clinically NFPAs

Evaluation for hormone hypersecretion
• Measure IGF-1
• Measure prolactin (in dilution in case of large macroadenomas)
• Screening for glucocorticoid excess in case of clinical suspicion (overnight dexamethasone suppression test, late-night salivary cortisol)
Evaluation for hypopituitarism
• Measure cortisol (09:00 a.m.), TSH, fT4, IGF-1, prolactin, FSH, LH, testosterone (males)
• A morning cortisol level < 3 μg/dl is suggestive of adrenal insufficiency, while a cortisol level > 15 μg/dl excludes the diagnosis. If cortisol level is between 3 μg/dl and 15 μg/dl, a corticotropin stimulation test should be performed
• In case, GH deficiency is suspected, and GH stimulation tests should be performed to exclude or confirm the diagnosis
• Gonadal function in premenopausal women is assessed through history and examination. Low gonadotropin levels in postmenopausal women suggest hypopituitarism, while in men exclude primary hypogonadism if testosterone levels are low

IGF-1 insulin-like growth factor 1, *TSH* thyroid-stimulating hormone, *fT4* free thyroxine, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *GH* growth hormone

(Table 9.3). Screening tests should involve serum PRL, IGF-1, TSH, fT4, FSH, LH, and testosterone for males. Gonadal function in premenopausal women can be assessed by history and clinical examination, while in postmenopausal women low gonadotropin levels suggest the presence of hypogonadism. In addition, screening for cortisol excess should be based either on midnight salivary cortisol or on overnight dexamethasone suppression test [14, 137, 138]. In case of suspected GH or adrenal insufficiency, GH stimulation and corticotropin/insulin stimulation tests are recommended, respectively [14]. In case of adrenal or thyroid deficiency, proper replacement should be initiated.

Ophthalmologic investigation should include assessment of visual acuity, pupil, and fundus examination, ocular motor evaluation, and visual field assessment [120]. According to Endocrine Society, a formal visual field examination is recommended in all patients with a pituitary incidentaloma that abuts or compress the optic chiasm [138]. The Congress of Neurological Surgeons has recently introduced guidelines regarding the pretreatment ophthalmologic evaluation in patients with NFPAs [139]. Their approach aims at identifying early and asymptomatic visual deficits as well as at providing prognostic factors of recovery and facilitating the postsurgical follow-up. Automated static perimetry is recommended for the initial assessment of visual fields, while visual evoked potentials may be used in order to evaluate the optic nerve function. Patients of advanced age or with a long duration (>4 months) of vision deficit present a low probability of visual recover postoperatively. Optical coherence tomography (OCT) of the optic nerve head is not yet standard of practice but is usually performed in case of visual impairment for prognostic purposes, to estimate the chance of postoperative vision improvement [120, 139].

9.7 Differential Diagnosis

The differential diagnosis of a pituitary incidentaloma involves multiple entities arising from the sellar or parasellar region (Table 9.4). Pituitary adenomas and Rathke's cleft cysts account for up to 90% of all lesions [133]. Adenomas and meningiomas represent the most common solid tumors in the sellar region. Imaging characteristics provide valuable help for the differential diagnosis, while clinical and endocrine evaluation may also point to different diagnoses. It is important that diabetes insipidus is not a common feature of pituitary adenomas and may be associated with lesions of non-pituitary origin [140]. Furthermore, α-subunit of the glycoprotein hormones may be elevated in 30% of patients with NFPAs, but normal values do not exclude the diagnosis of an NFPA and its measurement is not systematically recommended [141, 142]. The diagnosis of a truly malignant pituitary tumor (pituitary carcinoma) is based on the presence of craniospinal or systemic metastases [143].

In addition, it is very important to differentiate NFPA from a prolactinoma since sometimes the hormonal hypersecretion may not be clinically evident and the

Table 9.4 Differential diagnosis of sellar lesions

Pituitary tumors
• Pituitary adenoma
• Pituitary carcinoma
• Pituitary hyperplasia
• Pituicytoma
• Granular cell tumors
Other tumors
• Craniopharyngioma
• Meningioma
• Neurinoma
• Germ cell tumor
• Teratoma
• Glioma
• Chordoma
• Lymphoma
• Pituitary metastases
Cysts
• Cyst of Rathke's pouch
• Arachnoid cyst
• Epidermoid/dermoid cyst
Inflammatory/granulomatous lesions
• Lymphocytic hypophysitis
• Sarcoidosis
• Langerhans cell histiocytosis
• Tuberculosis
• Wegener's granulomatosis
• Pituitary abscess
Vascular lesions
• Aneurysms
• Cavernous angiomas

management of these two entities is significantly different. It has been observed that the incidence of hyperprolactinemia in histologically verified NFPAs is 25–65% [137]. PRL levels provide valuable help, while the hook effect may occasionally confound the diagnosis [144]. In a retrospective study, it has been observed that patients with NFPAs most often had PRL levels below 100 ng/ml, while levels >250 ng/ml were observed exclusively in patients with prolactinomas [145].

9.8 Treatment

9.8.1 Active Surveillance

Although few data are available regarding the treatment of asymptomatic NFPAs, the best strategy in these cases is considered the conservative follow-up. However, there is no established follow-up algorithm and guidelines are typically based on clinical experience [138, 146].

It is recommended that microadenomas are followed up in 6 months with MRI, and if there is no progression, MRI may be repeated at 2 years without visual or hormonal evaluation. Surveillance may be stopped if no progression is observed at 2 years. No surveillance is recommended for microadenomas with diameter lower than 5 mm [146]. In case of a macroadenoma that is not close to the optic chiasm, it is recommended follow-up with MRI 1 year after the initial diagnosis and if no progression is observed surveillance is recommended every 2 years. Visual field assessment is suggested if the tumor enlarges to abut or compress the optic chiasm during follow-up imaging. In case of a macroadenoma close to the optic chiasm, MRI should be performed at 6 months and annually thereafter. Visual assessment may be performed every 6 months [146].

In case of macroadenoma, it also recommended clinical and biochemical assessment of anterior pituitary function for hypopituitarism development 6 months after the diagnosis and annually afterward. Routine follow-up for hypopituitarism is not suggested for microadenomas whose clinical picture and MRI do not change over time [138, 146].

9.8.2 Surgery

Surgical treatment of NFPAs is recommended in patients with visual field deficits or other visual or neurological abnormalities associated with NFPAs abutting or compressing the optic nerves or chiasm and in case of pituitary apoplexy [138, 147]. In addition, Endocrine Society suggests that surgery should be considered in tumors that display significant growth or cause loss of endocrine function and in patients with persistent headache or planning to become pregnant and the NFPA is close to the optic chiasm [138]. Active surveillance may be a better approach for older patients as they display higher surgical intervention risk, while they also have a shorter lifetime probability of tumor enlargement.

Currently, the standard surgical technique is endoscopy or microscopy-assisted transsphenoidal surgery (TSS). Intraoperative MRI has been recently introduced as a mean to improve the surgical resection of the tumor, but its use remains controversial [148, 149].

TSS, when performed by an experienced surgeon, is a safe procedure associated with relatively low complication rates. A recent meta-analysis showed that total resection is achieved in 60–73% of NFPAs [150]. Immediate tumor volume decrease was observed in nearly all patients with a residual tumor rate of 10–36% [147]. Visual improvement is observed in 75–91% of patients, while 35–50% of cases display also hypopituitarism improvement [147]. It has been reported postoperative improvement in gonadal, thyroid, and adrenal axes in 64.9%, 71.9%. and 33.9% of cases, respectively [151]. Postoperative complications occur to less than 5% of cases and include cerebrospinal fluid leakage, meningitis, vision deterioration, persistent DI, or vascular injury. The mortality rate has also been demonstrated to be low (<1%) [152].

In the early postoperative period, the patients should be carefully monitored for water and sodium balance derangements. In approximately 4–20% of patients, a transient syndrome of inappropriate antidiuretic hormone secretion (SIADH) may be observed in the first 3–7 days postoperatively and should be properly treated that is related to intraoperative manipulations in the posterior pituitary or pituitary stalk [153–155]. DI may also develop in 18–31% of cases, is transient, and presents usually in the first 24–48 h [155].

Perioperative glucocorticoid treatment is frequently used to cover probable adrenal insufficiency development during the operation. Some centers interrupt glucocorticoid therapy 48 hours postoperatively, while others continue treatment until the evaluation of adrenal axis sufficiency [156, 157].

Assessment of pituitary function and visual field examination should be done 1–3 months postoperatively and treatment of probable hormone deficiencies introduced. A sellar MRI should be performed 3–6 months postoperatively to estimate tumor resection and to serve as baseline MRI during subsequent follow-up. If there is no residual adenoma, 10-year regrowth rate has been calculated to 0–6%. In case of residual adenoma, 10-year regrowth rate is 42–53% and 77–80% if the remnant is intrasellar or extrasellar, respectively [158–160].

9.8.3 Radiotherapy (RT)

In the past, RT was administered postoperatively in all patients to prevent recurrence or growth of residual tumor. However, today the role of RT as adjuvant treatment is under debate. Besides conventional radiotherapy, newer techniques are currently available such as fractionated stereotactic radiotherapy and Gamma Knife radiosurgery that aim to deliver high precision radiotherapy with lower complication rates [161].

Several studies have evaluated the efficacy of adjuvant RT on tumor growth or recurrence. A recent study has reported a 85–95% of tumor control at 5–10 years

postoperatively in patients with NFPAs [162]. Mean progression free survival (PFS) at 5 years postoperatively has been reported to be 95% after RT compared to 70% in patients not treated with RT. At 15 years postoperatively, mean PFS was calculated at 93% after RT compared to 33% if no RT was administered [163]. In addition, there are some reports of improved visual function in patients with visual impairment after surgery treated with RT [164]. However, there are no randomized controlled trials indicating the superiority of adjuvant postoperative RT compared to active surveillance, while the potential side effects of RT make the indication for RT debatable [165]. Furthermore, a recent meta-analysis reported that residual tumor growth occurs slowly as the tumor doubling time is 3.4 years, while no growth is observed during follow-up in 50–60% of patients [166].

Hence, there is no consensus regarding adjuvant treatment with RT after surgical resection of NFPAs. RT is considered at the time of disease progression during follow-up, while immediate postoperative treatment should be reserved for cases with significant tumor remnant and high risk of progression [147]. Furthermore, adjuvant RT should be considered for patients presenting with aggressive NFPAs, large tumors with suprasellar extension or cavernous sinus invasion or displaying aggressive histopathological characteristics such as labeling index Ki-67 > 3% or extensive immunostaining for p53 [165]. RT can also be used as primary treatment in cases when surgery is not feasible.

The most common side effect of RT is the high incidence of hypopituitarism. Five years after treatment with RT, the incidence of GH, gonadotropin, ACTH, and TSH deficiency has been calculated to 100%, 91%, 77%, and 42%, respectively [167]. Follow-up with assessment of anterior pituitary function is recommended every 6 months post-treatment with RT. Additional rare side effects include optic neuropathy, neurocognitive dysfunction, and a probably increased risk of secondary malignancies of the central nervous system [164, 168, 169]. Furthermore, various studies have shown an increased risk of cerebrovascular events in patients treated with RT [170, 171]. It has been observed that the relative risk of a first stroke in patients treated with RT compared to the general population was 4.1 [170]. The risk of these side effects can be reduced if limited radiation dose is administered.

9.8.4 Medical Therapy

The role of medical treatment of pituitary adenomas is not established. Based on observations that NFPAs express dopamine receptors and somatostatin receptors, multiple studies evaluated the efficacy of dopamine agonists and somatostatin analogs in patients with operated or nonoperated NFPAs [172–176]. Tumor control (shrinkage or stabilization) was observed in 87% of patients treated with dopamine agonists upon imaging detection of residual tumor, but tumor shrinkage was achieved in 29% of cases [176]. A recent case–control study evaluated the efficacy of long-acting somatostatin analog octreotide LAR in patients with residual tumors that displayed positive somatostatin receptor scintigraphy [177]. Tumor stabilization was observed in 81% of patients in the group that received treatment compared

with 47% in the control group. However, neither tumor shrinkage nor visual field and pituitary function improvement was observed in any patient after treatment with octreotide LAR.

Temozolomide (TMZ) is an alkylating agent currently approved for the treatment of brain gliomas and glioblastomas. Recently, the European Society of Endocrinology recommended the use of TMZ as first-line treatment of aggressive pituitary tumors and pituitary carcinomas [143]. The response rate has been observed to be approximately 42%, but tumor recurrence is common after cessation of treatment and 2-year PFS has been calculated to 47.7% [178–180]. In addition, there are some reports of disease stability achieved in patients with atypical adenomas or carcinoma treated with peptide receptor radionuclide therapy (PRRT) [181].

9.9 Quality of Life (QoL) and Mortality

Patients suffering from NFPA present morbidities associated with the tumor or with the treatment modalities offered. Multiple studies have observed that patients with NFPA display a mortality rate higher than the general population [182]. A series of 573 patients with NFPA reported a standardized mortality ratio (SMR) of 1.7 with the excess mortality attributed to respiratory and cardio/cerebrovascular deaths [183]. Another study of 2795 patients reported an SMR of 1.1, and mortality was associated with infectious and circulatory diseases [184]. Older age at diagnosis and high doses of glucocorticoid substitution therapy were identified as predictive factors of mortality [182].

Although it has been reported that following treatment for NFPA QoL displays significant improvement, the findings regarding QoL normalization remain inconclusive [185]. Multiple factors, including visual impairment, hypopituitarism, and the type of surgical technique, are associated with impaired QoL. Disease-specific questionnaires should be introduced in order to evaluate effectively and optimize QoL.

9.10 Conclusion

NFPAs are prevalent PitNETs that come to clinical attention either through the presence of compressive symptoms to surrounding tissue and/or anterior hormonal deficiencies or are discovered incidentally (Fig. 9.3). Although a number of genetic effects related to their pathogenesis have been identified their molecular pathogenesis has not been delineated as yet, the majority of these tumors follow a relatively indolent course; however, a subset may become more aggressive with early recurrences. Currently, existing biomarkers can help identify specific subtypes and predict tumoral behavior. Surgery remains the main treatment in the presence of symptoms related to compressive effects, whereas surveillance remains an option for incidentally discovered tumors. Radiotherapy is used for potentially aggressive and recurrent tumors and when needed systemic treatment with temozolomide may

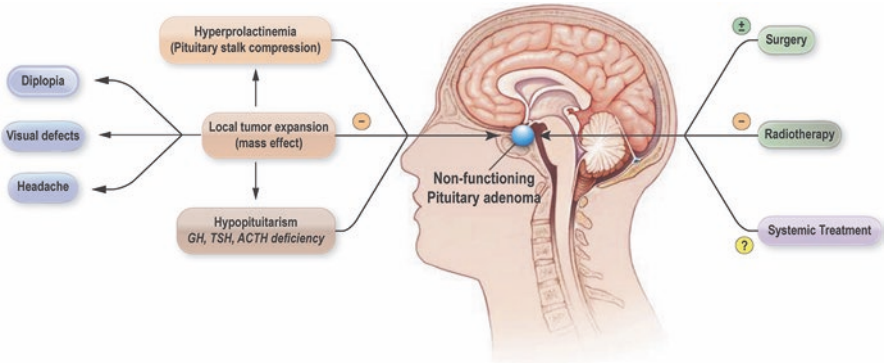

Fig. 9.3 Overview of clinical manifestations and management of nonfunctioning pituitary adenomas

be administered. Further studies are needed to help delineate their pathogenesis and provide specific tumor-directed treatment.

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Clinical Case 1

10

Ludovica F. S. Grasso, Renata S. Auriemma, Maria Cristina De Martino, Rosa Pirchio, Rosario Pivonello, and Annamaria Colao

10.1 Case Report

A 34-year-old woman with a history of oligo-amenorrhea, acne, and hirsutism by age 28 was admitted to the Neuroendocrinology Clinical Center in September 2014. At age 29, polycystic ovary syndrome (PCOS) was diagnosed and the patient started contraceptive pill for 2 years. At age 32, due to primary infertility, the patient conceived after ovarian stimulation, and she safely delivered a healthy baby. No complications were reported during pregnancy and delivery. The patient reported galactorrhea even though she stopped breastfeeding, and she had persistent amenorrhea after the birth of her child. The patient reported a 9.5 kg weight gain in the last year despite diet and exercise, and increased sweating for the last 6 months. She presented with gradually worsening headache in the last 6 months. The headaches generally occurred three times per week. The patient's past medical history included thyroid nodule and uterine leiomyomata diagnosed 4 years earlier.

Because of the persistent galactorrhea and amenorrhea, the patient underwent hormonal evaluation, reporting a mildly elevated prolactin (PRL) levels (68 ng/mL, normal range 5–25 ng/mL), low levels of luteinizing hormone (LH, 1.2 IU/L, normal range 2.4–13 IU/L), follicle-stimulating hormone (FSH, 2.3 IU/L, normal range 3.5–13 IU/L), and estradiol (16 pg/mL, normal range 20–240 pg/mL). The patient was subsequently referred to the Neuroendocrinology Clinical Center for further evaluation.

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At clinical evaluation, abnormal findings included obesity (body mass index 30.2 kg/m^2), acne, hirsutism (modified Ferriman Gallwey score 16/36), and neck and axillary acanthosis. She did not spontaneously complain about acral changes. However, after specific questions, she recognized that her shoe size had increased from size 37 to size 39 during the last 3 years.

She denied significant changes in facial appearance, new spaces between her teeth, snoring, fatigue, joint pain, and paresthesia.

10.2 Differential Diagnosis

Considering the main clinical presentation of the patient, including secondary amenorrhea, galactorrhea, and headache, the prolactinoma and clinically nonfunctioning pituitary adenoma should be considered in the differential diagnosis [1, 2]. Moreover, considering the PCOS-like phenotype associated with acral changes, insulin-mediated pseudoacromegaly should be excluded.

Prolactinomas are the most common of the hormone-secreting pituitary tumors [1] representing approximately 40% of all pituitary tumors, and occur most frequently among women aged 20–50 years [1, 3, 4]. In women of reproductive age, usually the most relevant clinical manifestations are oligomenorrhea or secondary amenorrhea, galactorrhea, and infertility, followed by decreased libido and weight gain [3, 4]. In clinical practice, macroprolactinomas are less common than microprolactinomas and occur more often in men than in women [3]. Mass effects cause headache, hypopituitarism, and visual field defects. Prolactinomas are the most frequent cause of PRL excess even if several other causes should be excluded before the diagnosis is made [4]. Besides prolactinomas, hyperprolactinemia can be associated with a variety of causes, such as pregnancy, hypothyroidism, PCOS, renal insufficiency, and PRL-stimulating drugs, which need to be considered in the differential diagnosis [5]. Since there are multiple causes of hyperprolactinemia, other than prolactinoma, a careful medical history, clinical examination, and measurement of serum thyrotropin and creatinine are required [3].

Clinically nonfunctioning adenomas account for 15–54% of pituitary adenomas [1]. Most patients present with symptoms of mass effect due to their large size (67% are macroadenomas), whereas some patients are completely asymptomatic, detecting the pituitary adenoma as incidental findings (incidentalomas) on magnetic resonance imaging (MRI) or computed tomography scans were performed for other reasons [6]. All patients with clinically nonfunctioning macroadenomas, symptomatic or incidental, should be evaluated for hypopituitarism [1]. Hyperprolactinemia may occur in these patients because of pituitary stalk dysfunction [3, 6].

Pseudoacromegaly is an extremely rare condition characteristic of some patients with physical features resembling acromegaly, usually affecting the face and extremities, without abnormalities in the GH/IGF-I axis [7]. Due to the rarity and variability of these conditions, its correct diagnosis can be challenging [7]. Insulin-mediated pseudoacromegaly is characterized by severe insulin resistance, acanthosis nigricans, and acromegaloid features in the absence of GH and IGF-I excess [7,

8]. Insulin-mediated pseudoacromegaly is associated with a selective post-receptor insulin signaling defect in which the insulin metabolic actions are impaired, but its mitogenic actions are preserved [7, 9]. Therefore, the metabolic actions of insulin are reduced resulting in hyperinsulinemia, while insulin mitogenic actions are preserved leading to acromegaloid features [7]. Insulin and IGF-I exhibit affinity for each other's receptor, and thus, high insulin levels resulting from insulin resistance may act on the type 1 IGF receptor [8]. Genetic abnormalities in the insulin receptor result in hyperinsulinemia, leading to diabetes mellitus and often acromegaloid features, a condition first described in 1976 by Kahn et al. [10]. In particular, the clinical presentation of insulin-mediated pseudoacromegaly may include face coarsening, frontal bossing, macroglossia, separated teeth, prognathism, large ears, acral enlargement, reduced subcutaneous fat on arms and legs, weight gain, acanthosis nigricans, skin tags, acne, hirsutism, hyperhidrosis, oligo-amenorrhea, and PCOS [7, 8, 11]. Adenomatous colonic polyps and multinodular goiter have been reported [8]. Clinical features of these patients overlap with those of acromegaly, but GH suppression on OGTT and IGF-I levels is generally normal. Moreover, these patients usually have increased LH levels and hyperandrogenism [7, 8].

10.3 Diagnostic Aspects

At the clinical evaluation, mild modification of nose, cheekbones, and lips was observed comparing previous photographs of the patient in order to detect the features of acromegaly. The evaluation of the pituitary function showed increased random GH (10.4 ng/mL) and IGF-I (874 ng/mL, normal range 80–290) levels. The oral glucose tolerance test (OGTT) was performed to confirm the diagnosis of acromegaly, resulting in GH nadir of 7.3 ng/mL. Other anterior pituitary hormone tests confirmed secondary hypogonadism (LH, 1.5 IU/L, FSH, 2.4 IU/L, estradiol, 16 pg/mL) and mildly elevated PRL levels (71 ng/mL). The MRI of the hypothalamus–pituitary region with gadolinium showed the presence of an intrasellar pituitary macroadenoma in the left side (14.5 × 13 mm), with a mild suprasellar extension (Fig. 10.1). Visual field testing was normal.

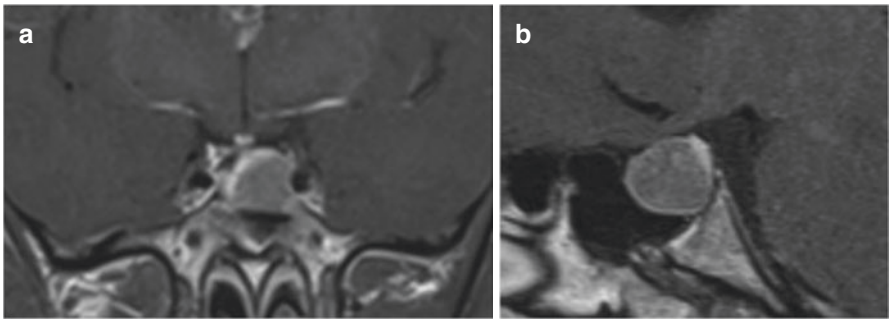

Fig. 10.1 Pituitary MRI images at baseline (coronal (**a**, left) and sagittal (**b**, right))

Table 10.1 Patient profile before and 3 months after neurosurgery

Parameters	Baseline	3 months after surgery
IGF-I (ng/mL)	874	592
GH nadir(ng/mL)	7.3	2.4
LH (IU/L)	1.5	4.6
FSH (IU/L)	2.5	6.1
Estradiol (pg/mL)	16	81
PRL (ng/mL)	71	13
Cortisol (ng/mL)	103	103
TSH (μU/mL)	2	2.4
Free T4 (ng/dL)	0.98	1.07

The assessment of acromegaly complications showed biochemical hyperandrogenism and confirmed the presence of an isoechoic thyroid nodule of 9 mm of maximal diameter on the thyroid ultrasound, uterine leiomyomata, and ovarian cysts on the pelvic ultrasound. In line with the standard diagnostic criteria, acromegaly due to a GH-secreting pituitary macroadenoma was diagnosed. Patient's profile at baseline is shown in Table 10.1.

10.4 Treatment

The patient underwent first-line transsphenoidal surgery of the pituitary macroadenoma in November 2014. Histology of the excised specimen revealed moderate amounts of sparsely granular eosinophilic cytoplasm. Immunohistochemistry was positive for GH and negative for other pituitary hormones. Ki 67 index was <2%.

Her 3 months postoperative evaluation revealed elevated IGF-I levels (592 ng/ml, normal range 80–290) and GH nadir during OGTT was 2.6 ng/mL, indicating persistent disease. Secondary hypogonadism resolved after surgery, and restoration of normal menstrual cycle was reported by the patient 2 months after surgery. Patient's profile 3 months after surgery is shown in Table 10.1. Postoperative MRI of the pituitary revealed millimetric parasellar area (4 mm in maximum diameter) in the left side of the pituitary (Fig. 10.2). Therefore, octreotide LAR treatment was initiated postoperatively, at the dose of 30 mg every 4 weeks. It was then increased stepwise, up to 40 mg every 4 weeks. The lowest IGF-I achieved during LAR treatment was 375 ng/mL (Fig. 10.3), indicating partial resistance to first-generation somatostatin analogs (SSA). LAR therapy was stopped in October 2015. Due to persistently uncontrolled acromegaly, despite high dose of LAR, pegvisomant (PEG) monotherapy was started, considering the small pituitary residual tumor. The dose of PEG was up-titrated up to 20 mg/day, achieving a disease control. Within a few months of PEG therapy, there was a significant clinical improvement.

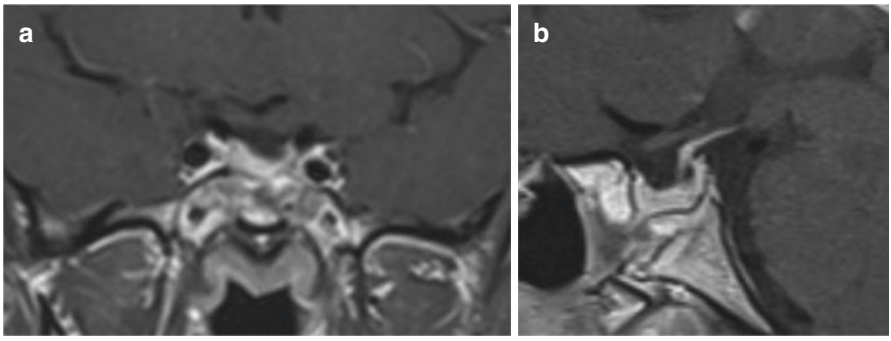

Fig. 10.2 Pituitary MRI images 3 months after surgery (coronal (**a**, left) and sagittal (**b**, right))

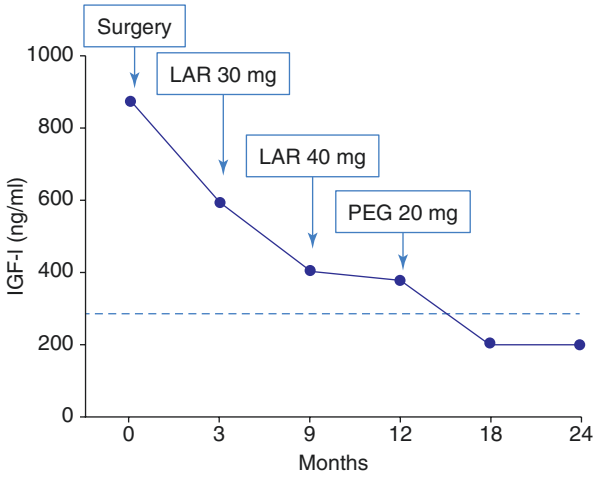

Fig. 10.3 IGF-I levels thought the follow-up. LAR: octreotide LAR; PEG: pegvisomant

10.5 Follow-Up

Within a month of starting PEG 20 mg/day, her IGF-I levels dropped to the normal range, remaining within the normal range for age over the follow-up, although tumor size remained unchanged. PEG was well tolerated. Patient's symptoms have significantly improved, and the patient reported normal menstrual cycle. At age 38, the patient spontaneously conceived. PEG therapy was discontinued after pregnancy confirmation, and the patient was regularly followed during pregnancy. She safely delivered a healthy baby; no complications were reported during pregnancy and delivery. The patient breastfed for 3 months, and then, PEG therapy was resumed.

10.6 Learning Points

- Although acromegaly manifests with distinct physical characteristics, diagnosis of the disease in its early stages can be difficult due to its insidious nature, meaning that neither the patient and their families nor their physicians may notice these changes [12].
- Acromegaly features develop insidiously over decades, often resulting in a delay of 7–10 years or more in diagnosis after the estimated onset of symptoms [13, 14]. Among clinical features, female reproductive disorders, including menstrual abnormalities, galactorrhea, and decreased libido, are commonly complained in acromegaly [13, 14]. Particularly, women with acromegaly often present with menstrual irregularity, mainly represented by oligo-amenorrhea, associated with anovulation and infertility [15, 16].
- A direct action of GH and IGF-I excess on the pituitary–gonadal axis and the tumor mass effect per se have been proposed as potential mechanisms responsible for the occurrence of hyperprolactinemia and for the impairment in gonadotrophin secretion, leading to ovarian dysfunction and infertility [16–19]. Moreover, IGF-I excess has been found associated with overt PCOS or to a PCOS-like phenotype in 50% of acromegalic women [20].
- Evidence from literature has shown menstrual disturbances to occur in 40–80% of acromegalic women [16]. This wide variation in prevalence has been attributed mainly to the insidious onset of the disease and to the delay in diagnosis [18]. However, an earlier diagnosis and the availability of a wide spectrum of effective treatments for acromegaly could positively impact on the female fertility outcome in acromegaly [21].
- Treatment of acromegaly aims to normalize GH and IGF-I levels, control tumor mass, and decrease the risk of developing systemic comorbidities, thereby reducing mortality [13, 22, 23].
- Transsphenoidal adenomectomy remains a cornerstone treatment for GH-secreting pituitary tumors and is the treatment of choice except in those patients with high surgical risk, who refuse surgery or who have invasive, unresectable tumors [22, 23]. In patients with persistent disease despite surgical resection of the adenoma, medical therapy is recommended and first-generation SSA are the first-line medical therapy in most patients with acromegaly [22, 23].
- According to Endocrine Society Clinical Practice Guidelines [22], the GH receptor antagonist PEG is indicated as a second-line or third-line therapy, mostly in patients in whom surgery has failed or in those who show a poor response to first-line SSA.
- Currently, no medication is officially approved and recommended for acromegaly during pregnancy [24, 25]; however, based on a case-by-case analysis of patients the use of medical treatment during pregnancy should be weighed upon the risk-to-benefit ratio, balancing the risk of tumor enlargement, acromegaly symptoms, and maternal/fetal complications [24, 25]. Safety of PEG during pregnancy is yet to be clarified, since only few case reports in the literature have documented uneventful pregnancies following treatment with PEG [26–28].

Questions and Answers

In pregnant women with acromegaly:

(a) Acromegaly medical therapy should be discontinued and administered only for patients with macroadenomas.
(b) **Acromegaly medical therapy should be discontinued and administered only for tumor and headache control.**
(c) Acromegaly medical therapy with somatostatin analogs and pegvisomant should be discontinued and dopamine agonist should be administered for tumor and headache control.
(d) The dosage of acromegaly medical therapy should be reduced in all patients.

The GH receptor antagonist (PEG) is indicated:

(a) As third-line therapy, after surgery in patients who show a poor response to first-line SSA.
(b) Only in patients with persistent disease after surgery.
(c) **As a second- or third-line therapy, mostly in patients in whom surgery has failed or in those who show a poor response to first-line SSA.**
(d) Only in patients resistant to first-line therapy with conventional SSA.

First-line surgery:

(a) Is indicated in all patients with visible pituitary adenoma.
(b) Is the treatment of choice in the majority of patients with acromegaly except in those patients with high surgical risk or who refuse surgery.
(c) Is the treatment of choice in the majority of patients with acromegaly except in those patients with invasive and unresectable tumors.
(d) **b + c.**

Among clinical features of acromegaly:

(a) **Female reproductive disorders are commonly complained in acromegaly women, including menstrual abnormalities, galactorrhea, and decreased libido.**
(b) Up to 80% of women with acromegaly present with menstrual irregularity and hyperprolactinemia.
(c) Menstrual disturbances occur in all young acromegalic women.
(d) PCOS-like phenotype has been reported in the majority of acromegalic women.

1) In women with acromegaly onset during the fertile period:
   (a) Menstrual cycle disorders do not improve despite disease control achieved with treatment.
   (b) The most frequent alteration of the menstrual cycle is polymenorrhea.

(c) **Menstrual irregularities are often associated with anovulation and infertility.**
(d) Hyperprolactinemia and galactorrhea rarely occur.

2) PCOS-like phenotype in women with acromegaly:
(a) Is rarely found at diagnosis in young women.
(b) **Is determined by the direct effect on the ovary of the IGF-1 excess and indirectly by the insulin resistance and hyperinsulinism.**
(c) Differs from classical PCOS due to the absence of clinical and biochemical hyperandrogenism.
(d) Seldom is associated with infertility in these patients.

3) Follow-up of acromegaly during pregnancy requires:
(a) Monthly IGF-1 evaluation.
(b) MRI during the second trimester.
(c) Monitoring of GH but not IGF-1.
(d) **Assessments of the possible growth of the adenoma using the visual field.**

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Clinical Case 2

11

Luiz Eduardo Wildemberg and Monica Gadelha

11.1 Case Report

A previously healthy 15-year-old female patient started to present irregular menses, weight gain, and edema in lower limbs and abdomen 4 months earlier. Later on, she noticed red striae in abdomen, and upper and lower limbs, increase in body hair in face and breasts, acne, mood instability, and fatigue. Previous history was

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unremarkable except for an isolated event of elevated blood pressure (150 × 90 mmHg). She denied the use of any medication.

At physical examination, she had a depressed mood, acne grade 3 in face and back, moon face and plethora, buffalo hump, and hirsutism (Ferriman–Gallwey score 16). Her height was 1.65 m and weight was 97.3 kg, with a BMI of 35.7 kg/m^2. Blood pressure (160/90 mmHg) and heart rate (122 bpm) were elevated. She had abdominal purple striae of approximately 2 cm.

At basal blood test, she had low potassium levels (3.1 mmol/L, NR 3.5–4.5), slightly elevated glycated hemoglobin (5.9%, NR < 5.7%), and elevated cortisol (45.8 mcg/L), and the rest of pituitary function was normal. A low-dose dexamethasone suppression test showed unsuppressed cortisol levels (22.9 mcg/L), and the free urinary cortisol (FUC) and salivary cortisol were very elevated, 720 mcg/24 h (NR 10–100) and 6.57 mcg/dL (NR < 0.35), respectively. So, the diagnosis of Cushing's syndrome was confirmed, and ACTH levels were elevated (89.9 pg/mL, NR < 46), in favor of ACTH-dependent Cushing's syndrome.

Due to the very rapid evolution of the case and to facilitate the diagnostic procedures, the patient was hospitalized. But during this period, she attempted suicide by throwing herself from the second floor. Luckily, she had no major injuries from this fall, but a chest computed tomography (CT) performed to investigate a thoracic pain after this episode revealed a 2.6 cm nodule in her left lung (Fig. 11.1).

A sella turcica magnetic resonance imaging (MRI) revealed a 0.4 cm lesion at right side of the adenohypophysis (Fig. 11.2). So, she was a patient with an ACTH-dependent Cushing's syndrome with lung and pituitary small lesions. Therefore, a simultaneous bilateral inferior petrosal sinus sampling (BIPSS) was realized, which confirmed central origin of the Cushing's syndrome (Table 11.1).

The patient was then submitted to microscopic transsphenoidal surgery. During surgical procedure, it was noted a liquefied material that was aspirated. Histopathological examination of the resected sample showed normal pituitary with Crooke's hyaline changes.

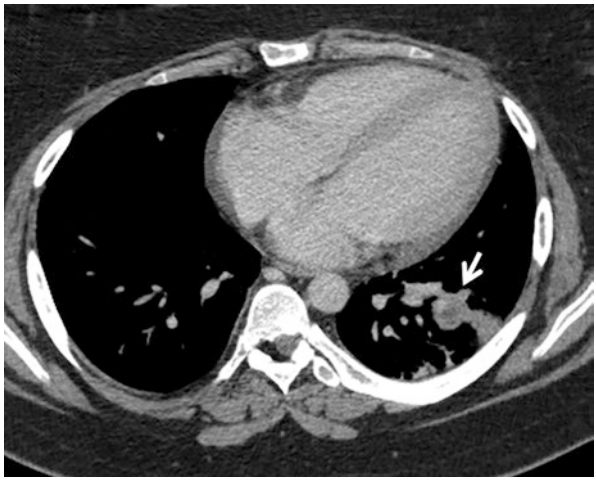

Fig. 11.1 Thorax computed tomography showing a 2.6 cm lesion in the left lung (white arrow)

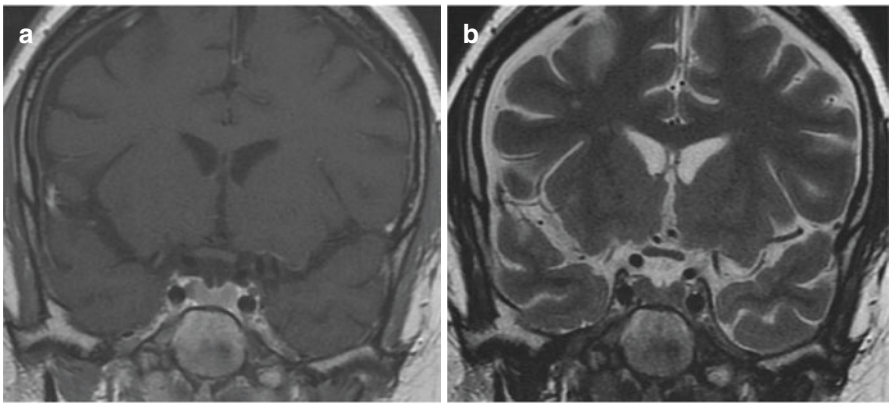

Fig. 11.2 Pituitary magnetic resonance imaging coronal view in T1-weighted after gadolinium (**a**) and T2-weighted (**b**) sequences. A small 4 mm lesion with no contrast enhancement can be seen in the right side of the adenohypophysis in contact with left carotid artery. The lesion cannot be visualized in the T2 sequence

Table 11.1 ACTH levels during simultaneous bilateral inferior petrosal sinus sampling

Time	Right	Periphery	Left
−5 min	672	56	5
0	6,4	52	45
3 min	889	46	53
5 min	696	48	50
10 min	1188	60	47

Blood samples were collected minus 5 min and immediately before the intravenous administration of 10 mcg of desmopressin, and 3, 5, and 10 min after it. Samples were collected from both right and left inferior petrosal sinuses and from a peripheric site. ACTH levels are expressed in pg/mL. Before desmopressin administration, central/periphery ratio was 12 and after it was 19.8, which confirms the central origin of the hypercortisolism

After surgery, the patient presented signs of adrenal insufficiency and was put on glucocorticoid treatment initially with hydrocortisone and later with prednisone. The patient lost weight and her mood was significantly improved, acne and hirsutism regressed, and regular menses returned. The patient used prednisone for 6 months, and after its withdrawal, a cortisol post-1 mg dexamethasone suppression test was 0.8 mcg/L and salivary cortisol was 0.31 mcg/dL and 0.29 mcg/dL. So, she had clinical and biochemical evidence of disease remission. Lung nodule was considered to be cicatricial and follow-up images revealed no increase.

Two years after surgery, she started to gain weight and presented irritability with fast progression. Laboratory examinations revealed disease relapse (post-1 mg test 20 mcg/L and FUC 1020 mcg/24 h). No lesion was observed in the pituitary MRI. She was reoperated transnasally, but no lesion was identified during pituitary exploration. After surgery, there was no improvement in the clinical signs of hypercortisolism, as well as in the laboratory examinations.

Due to the seriousness of the disease and the previous suicide attempt, bilateral adrenalectomy was performed. She is now stable under gluco- and mineralocorticoid substitutive therapy. Last ACTH levels were 165 pg/mL, and there was no tumor increase detected by MRI.

11.2 Differential Diagnosis

The main hypercortisolism etiology is external glucocorticoid use, so the first thing while dealing with a patient with clinical signs of hypercortisolism is to exclude exogenous corticoid use [1]. It is important to stress out that several dermatologic creams have glucocorticoids in their composition, so the use of these drugs must be extensively questioned. Moreover, inhaled glucocorticoids may also present systemic absorption leading to hypercortisolism in selected patients. Exogenous Cushing's syndrome may present a rapid course of development, similar to the present case. However, the patient denied chronic use of any medication, oral or topical, which excluded the hypothesis of exogenous Cushing's syndrome.

Endogenous Cushing's syndrome may be caused by excessive cortisol secretion due to adrenal tumor or hyperplasia or to excessive ACTH production. These Cushing's syndromes are of neoplastic origin and they must be distinguished from non-neoplastic hypercortisolism (NNH), also called pseudo-Cushing's syndrome (Table 11.2) [2]. NNH is usually associated with mild clinical and laboratory signs of hypercortisolism, which was not the case of our patient, making this diagnosis unlikely. Nevertheless, this was a teenage female patient with signs of androgen excess and menstrual irregularity, so the diagnosis of polycystic ovary syndrome (PCOS) must be considered [3]. The rapid course of the disease and the fact that she had previously regular menses make PCOS diagnosis very improbable. Psychiatric disorders are another important cause of NNH, and our patient had a depressive behavior. However, that started at the same time of other signs of hypercortisolism instead of being a previous condition, which may exclude depression as the cause of the clinical picture and makes it a part of the clinical picture of hypercortisolism. Other causes such as excessive alcohol intake, anorexia nervosa, and intense exercise were really unlikely [4].

After confirming endogenous hypercortisolism, it is necessary to differentiate between ACTH-dependent or ACTH-independent Cushing's syndrome [5]. The ACTH-independent cases are of adrenal origin, either caused by a cortisol-secreting

Table 11.2 Etiologies of non-neoplastic hypercortisolism

Depression and psychiatric disorders
Alcohol abuse
Hyperinsulinemia (obesity, type 2 diabetes mellitus, polycystic ovary syndrome)
Anorexia nervosa
Chronic kidney disease
Intense chronic exercise
Multiple sclerosis
Glucocorticoid resistance

adrenal adenoma or adrenal hyperplasia. ACTH-dependent cases are caused mostly by a pituitary adenoma (corticotropinoma), called Cushing's disease, or less frequently by an ACTH-secreting tumor located in organs other than the pituitary (ectopic Cushing's syndrome—ECS). This differential diagnosis will be further detailed in the section about diagnostic aspects.

11.3 Diagnostic Aspects

The diagnosis of Cushing's syndrome is a challenge, from the clinical presentation to laboratory and imaging examinations. The clinical picture may vary from a very mild and insidious presentation to a rich and/or fast evolution of symptoms [6]. Cushing's syndrome clinical picture includes several signs and symptoms with higher or lower specificity. The most specific signs are easy bruising, facial plethora, purple striae >1 cm, proximal muscular atrophy, and weight gain with growth retardation in children [1]. Other signs that are frequently found in patients with Cushing's syndrome but also may be encountered in other diseases are acne, hirsutism, peripheral edema, central obesity, "buffalo hump," and supraclavicular fullness. Some symptoms that are also—and still more—nonspecific include depression, fatigue, weight gain, menstrual abnormalities, decreased libido, cognitive deficits, and irritability [6]. Cushing's syndrome is also associated with a diversity of comorbidities: hypertension, glucose metabolism imbalance, osteoporosis, kidney stones, predisposition to thromboembolic events, and infectious diseases [7].

Our patient had a very severe and typical presentation, so laboratory examinations were performed to confirm a diagnosis that had been clinically made. However, as mentioned above, some patients may present mild symptoms, not as obvious as our patient, making the clinical suspicious not as simple. With the progressive increase in obesity and diabetes prevalence in world population, an important question is as follows: Who to screen for Cushing's syndrome? According to Nieman LK [7], some characteristics should raise the suspicion and make clinicians investigate it further:

- disease progression, with accumulation and worsening of signs and symptoms overtime;
- new or unexplained changes in cognition, mood, or memory;
- features incompatible with patient's age or population cohort (such as osteoporosis in young patient or recurrent infections in immunocompetent individuals);
- changes in fat distribution that suggest Cushing's syndrome such as supraclavicular and temporal fat (not dorsal pad or central obesity, which can be found in other causes of obesity);
- growth impairment with weight gain in children.

If there is clinical suspicion of Cushing's syndrome and there is no exogenous use of glucocorticoids, the next step is to confirm the hypercortisolism. For that, we have three main examinations that can be used: dexamethasone suppression test, late-night salivary cortisol, and 24-hour urinary free cortisol (UFC). It is necessary

to have two altered examinations out of the three [8]. Other tests that can be used as second-line examinations in selected patients are late-night serum cortisol, ovine CRH after longer low-dose dexamethasone suppression test, human CRH test, and desmopressin test [5]. Sensitivity and specificity of first-line tests can be found in Table 11.3.

Overnight, low-dose dexamethasone suppression test is performed by administration of 1 mg of dexamethasone between 11 p.m. and 12 p.m., with cortisol measurement the next day at 8 a.m. The test is considered positive for hypercortisolism if there is no cortisol suppression to 1.8 mcg/dL (i.e., 50 nmol/L) or lower. The physiopathology behind this test is that, in normal individuals, due to negative feedback in normal corticotrophs, the administration of dexamethasone leads to suppression of cortisol secretion, which does not occur in patients with either ACTH- or cortisol-producing tumors. Alternatively, a long low-dose dexamethasone suppression test can be performed. In this variant, dexamethasone 0.5 mg is administered every 6 h for 48 h and cortisol is measured after this period. The positive criterion is the same as post-1 mg test. Test can either start at 9 a.m., with last dexamethasone administration at 3 a.m. and cortisol collection at 9 a.m., or at 12 a.m., with last dexamethasone administration at 6 a.m. [5]. Several drugs may alter dexamethasone metabolism, either accelerating or reducing it, which may lead to false positive or false negative, respectively. Other causes of false positive are states that increase cortisol-binding globulin (CBG), such as estrogen use and pregnancy.

Late-night salivary cortisol evaluates the cortisol circadian rhythm. It is a test with high sensitivity and specificity, apart from being easier to perform than suppression tests and, especially, 24-h UFC. It measures free cortisol, so there is no influence of high CBG states. Causes of false positive include psychiatric disorders (depression, anxiety disorders, and obsessive–compulsive disorder), poorly controlled diabetes mellitus, gum disease, pregnancy, and alcoholism [9]. Patients should be oriented to not smoking in the day of the examination and to brush their teeth gently.

24-hour UFC is used both for diagnosis of hypercortisolism and for monitoring the disease. It measures the total cortisol production over 24 h. At least two or three samples should be collected consecutively or alternately to decrease false-negative results. Also, creatinine should always be measured to assure that sample was properly collected [5]. The performance of UFC in the detection of mild hypercortisolism is less than optimal, and salivary cortisol seems to be a better option in such cases [10].

Table 11.3 Sensitivity and specificity of first-line test for the detection of hypercortisolism

Test	Reference value	Sensitivity	Specificity
Post-1 mg dexamethasone suppression test	<1.8 mcg/dL	>95%	80%
Long low-dose dexamethasone suppression test	<1.8 mcg/dL	92–100%	92–100%
Late-night salivary cortisol	2× the ULN	88–100%	92–100%
24-h urinary free cortisol	3–4× the ULN	90–98%	45–95%

Adapted from reference 5
ULN: upper limit of normal range

After confirming hypercortisolism, ACTH levels should be measured in order to differentiate between ACTH-dependent or ACTH-independent causes. ACTH levels lower than 10 pg/mL indicate an adrenal cause for cortisol oversecretion, and an adrenal imaging (MRI or CT) should then be performed. ACTH higher than 20 pg/mL is compatible with ACTH-dependent Cushing's syndrome, and levels between 10 and 20 pg/mL are indeterminate and should be repeated [5]. In ACTH-dependent cases, the most frequent etiology is a corticotropinoma (90% of cases), so the next step is a pituitary MRI [8]. If an adenoma ≥6 mm is identified, the diagnosis of Cushing's disease is confirmed. If there is no visible adenoma or if it is smaller than 6 mm, the differential diagnosis between pituitary Cushing's syndrome and ectopic ACTH syndrome must be performed.

A BIPSS is the gold standard method to differentiate between these two causes of ACTH-dependent Cushing's syndrome. In this examination, blood samples are collected from both petrosal sinuses, by inserting a catheter through the femoral vein until the petrosal sinuses, and from a peripheral site. Samples are collected before and after stimulation with corticotropin-releasing hormone (CRH) or desmopressin [11]. A petrosal sinus/periphery gradient >2 before and >3 after stimuli indicates a central origin (CD).

In sites where a BIPSS cannot be performed, a high-dose dexamethasone suppression test can be used as an alternative. The rationale of using this test is that pituitary adenomas, although secrete ACTH autonomously, maintain some degree of suppression by cortisol, whereas non-pituitary tissues are unlikely to respond. It is performed by administration of dexamethasone 2.0 mg every 6 h for 48 h, and serum cortisol is evaluated immediately before and after dexamethasone use. A cortisol suppression >50%, and especially 80–90%, indicates that the etiology is a pituitary adenoma [8].

An algorithm for the diagnosis of Cushing's syndrome can be found in Fig. 11.3.

11.4 Treatment

Transsphenoidal surgery (TSS) is the treatment of choice for CD [12]. Success rates vary according to the size of the adenoma and vary from approximately 40% for macroadenomas to more than 70% for microadenomas [13]. A study evaluated 108 patients submitted to transsphenoidal surgery and found an overall remission rate of 69% [14]. In this series, endoscopic surgery results were slightly higher than microscopic surgery (71.4% vs 69.3%), but no statistical analysis was performed, probably because of the limited number of patients submitted to endoscopic surgery. Our patient was in remission after the first surgery. Evaluation of remission in the immediate postoperative period can be made by dosing cortisol levels in the first 7 days after surgery, especially in the first 2 days. Cortisol levels <5 mcg/L suggest remission. Cortisol levels were not evaluated in our patient since hydrocortisone was administered due to adrenal insufficiency symptoms, which is also a criterion of remission.

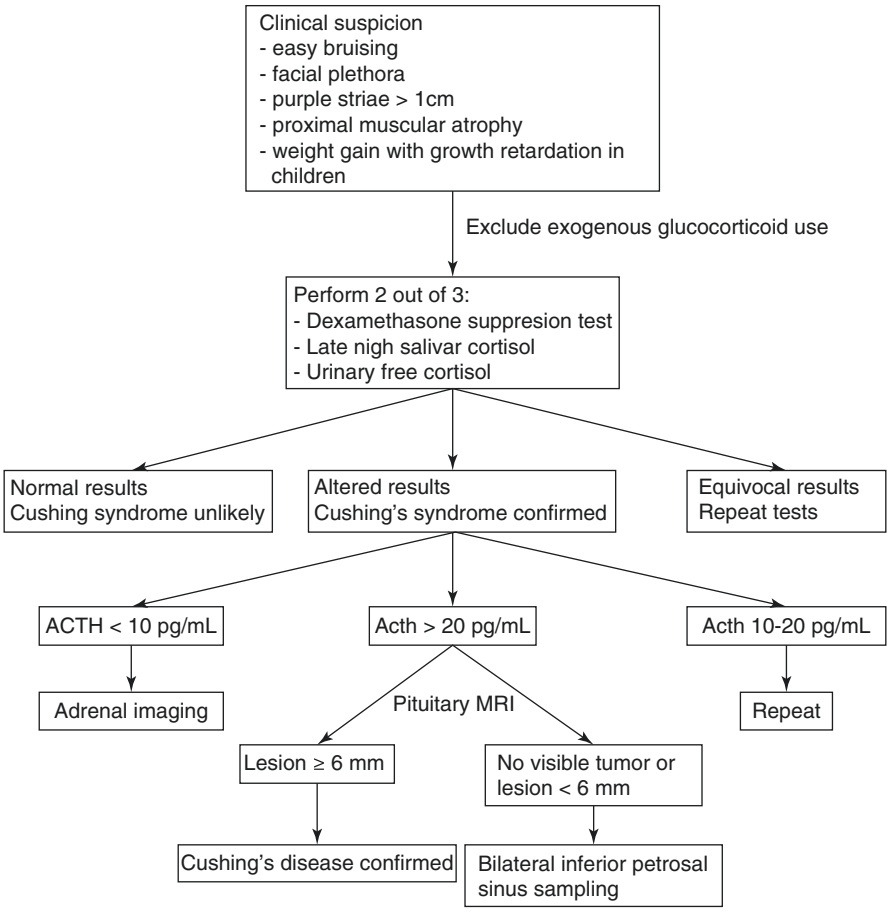

Fig. 11.3 Algorithm for the diagnosis of Cushing's syndrome

Although surgical success rate is high, especially for microadenomas, recurrence is an important problem in Cushing's disease. Recurrence rates vary from 15% to 66% within 5–10 years of a successful surgery, but can occur after up to 20 years or more [13, 15]. Our patient presented recurrence after 2 years of successful surgery.

In case of no remission or recurrence, options are a second surgery, medical treatment, bilateral adrenalectomy, or radiotherapy [13, 16]. In the previously cited study [14], efficacy of a second surgery was lower than first procedure, with remission rate of 50%. Burke and coll [17]. demonstrated remission in 60.8% of 51 patients submitted to a second surgery for treatment of recurrent disease. In the presented case, a second surgery was not successful, and the adenoma was not visualized during the procedure.

Due to the severity of the case, after this second surgical attempt, patient was submitted to bilateral adrenalectomy. The endocrine society guidelines for the treatment of Cushing's syndrome indicate bilateral adrenalectomy for occult or metastatic EAS or for patients with very severe ACTH-dependent disease who cannot be

controlled by medical therapy, as a life-preserving emergency treatment [13]. It is also a viable option for young female patients with a desire to be pregnant, in order to avoid the possibility of hypopituitarism associated with repeated surgery and radiotherapy. The main adverse effect of this treatment is primary adrenal insufficiency with the need of life-long administration of gluco- and mineralocorticoids [18]. Another important side effect is the development of corticotroph tumor progression, classically described as Nelson's syndrome, that is supposed to occur due to the absence of negative feedback from the hypercortisolism in the corticotropinoma [19]. ACTH levels should be monitored every 3 months for the first year, every 6 months for 3 years, and then annually. A rise up to 200 pg/mL is expected after bilateral adrenalectomy. Pituitary MRI should be done 3 months after surgery, then every 6 months for 2 years, and finally annually. Criteria for Nelson's syndrome is tumor increase detected in an imaging examination, ACTH levels >500 pg/mL with progressive increase >30% in three occasions and hyperpigmentation [20].

Another second-line treatment option is radiotherapy/radiosurgery. It was not a suitable treatment for our patient since it takes up to 2 years to reach goal and our patient had a severe disease, with risk of suicide, so it was not possible to wait for this long time. A study evaluated the efficacy of stereotactic radiosurgery in 18 patients [21]. The remission rates after 2 years of the procedure were 56% and after 5 years 77%, five patients needed additional therapy due to lack of biochemical control and tumor growth was detected in one patient. 24% of patients developed a new pituitary hormone deficit after radiosurgery.

Finally, medical treatment is also possible for the management of recurrent Cushing's disease, may be used drugs that act centrally inhibiting ACTH secretion (pasireotide, pasireotide LAR, cabergoline, temozolomide), that block adrenal cortisol secretion (ketoconazole, metyrapone, mitotane, etomidate), and that block glucocorticoid receptor (mifepristone) [22]. Dosage, efficacy, and safety of the drugs used for the treatment of Cushing's disease are summarized in Table 11.4. Osilodrostat and levoketoconazole, steroidogenesis blockers, are now in phase 3 trials with promising results [23]. Preclinical studies with tumor-directed therapeutic targets may help establish novel therapeutic options for the medical management of Cushing's disease [24].

11.5 Follow-Up

Patients with Cushing's disease have a standard mortality ratio (SMR) 2.5 times higher than general population [25]. This mortality was even higher for patients not in remission (6.9 times), but it is important to emphasize that even patients in remission had higher mortality (1.9 times). The main causes of death were myocardial and cerebrovascular infarction, but mortality due to infection, and respiratory and digestive diseases were also increased [25].

Due to this increased mortality, Endocrine Society Guidelines recommend treating specific comorbidities that are associated with Cushing's disease throughout patient's life. It is also recommended to monitor for disease relapse, since it can be detected up to 20 years after a successful treatment [13]. It is important to educate

Table 11.4 Dosage, efficacy, and adverse effects of drugs used for the medical management of Cushing's disease

Drug	Dosage	Efficacy	Safety
Pasireotide	0.3–0.9 mg SC bid	24-h UFC normalization in up to 26% of cases	Hyperglycemia common, gastrointestinal toxicity, gallstones
Pasireotide LAR	10–30 mg IM every 4 week	24-h UFC normalization in 41% of cases	Hyperglycemia common, gastrointestinal toxicity, gallstones
Cabergoline	0.5–7 mg PO week	24-h UFC normalization in up to 40% of cases	Nausea, orthostatic dizziness, nasal congestion, psychiatric manifestations
Temozolomide	150–200 mg/m^2/day PO for 5 days each month	Partial or complete tumor response in up to 80% of cases	Neutropenia, thrombocytopenia, gastrointestinal toxicity, hearing loss
Ketoconazole	200–600 mg PO bid–tid	24-h UFC normalization in 49% of cases	Gastrointestinal toxicity, liver enzymes increase, risk of hepatic failure
Metyrapone	250–1000 mg PO qid	24-h UFC normalization in 43%	Gastrointestinal symptoms, dizziness, hyperandrogenism, mineralocorticoid excess
Mitotane	0.5–3.0 g PO tid	24-h UFC normalization in up to 85%	Gastrointestinal, hepatic, metabolic, neurologic, adverse effects
Etomidate	0.03 mg/kg IV (bolus), followed by continuous infusion (0.1–0.3 mg/kg/h)	Highly effective in the short term	Sedation, nausea, vomiting, myoclonus, dystonia
Mifepristone	300–1200 mg PO daily	Improvement in hyperglycemia in 60%; decrease in blood pressure in 38%; global clinical improvement in 87%	Hypoadrenalism, hypertension, hypokalemia, endometrial thickening/vaginal bleeding

Adapted from reference 22
bid twice a day, *tid* thrice a day, *qd* once a day, *IM* intramuscular, *IV* intravenous, *SC* subcutaneous, *UFC* urinary free cortisol

patients and family members with respect to signs and symptoms of disease relapse, so it can be readily diagnosed. Monitoring of disease relapse can be made with either one of the three first-line examinations for the detection of hypercortisolism.

11.6 Learning Points

– Cushing's syndrome is a complex disease, with a myriad of clinical presentations that can vary from a rich clinical picture, which is readily diagnosed, to a mild presentation that needs a high suspicion in order to be sought.

– The diagnostic workup is also complex, involving a diversity of tests that may present false-positive or false-negative results, making it necessary to perform several examinations to reach a final conclusion.
– Treatment of Cushing's disease is multidisciplinary, involving endocrinologists, neurosurgeons, radiotherapists, and general surgeons. Recurrence after an initially successful treatment is not uncommon, so multimodal therapy is often necessary.
– Cushing's disease is associated with elevated mortality, even in patients in remission, so long-life monitoring of disease relapse and comorbidities is warranted.

Questions and Answers

(1) Which is the main cause of hypercortisolism?

(A) Cushing's diseases.
(B) Adrenal adenoma.
(C) Ectopic ACTH-producing tumor-directed.
(D) Exogenous glucocorticoid use.
(E) None of the above.

(2) Which of these signs are specific of Cushing's syndrome?

(A) Central obesity.
(B) Purple striae.
(C) Buffalo hump.
(D) Hirsutism.
(E) Acne.

(3) Which comorbidity can be associated with Cushing's syndrome?

(A) Hypertension.
(B) Diabetes mellitus.
(C) Venous thromboembolism.
(D) Osteoporosis.
(E) All of the above.

(4) Which one is a Cushing's syndrome differential diagnosis?

(A) Polycystic ovary syndromes.
(B) Depression.
(C) Anorexia nervosa.
(D) All of the above.
(E) None of the above.

(5) In the initial investigation of Cushing's syndrome what examinations can be used?

(A) Dexamethasone suppression test.
(B) Basal 8 a.m. cortisol levels.

(C) Late-night serum cortisol.
(D) ACTH levels.
(E) Magnetic resonance imaging.

(6) After confirming hypercortisolism, which examination should be ordered next?

(A) Magnetic resonance imaging of the sella turcica.
(B) Abdominal computed tomography.
(C) ACTH levels.
(D) Simultaneous bilateral inferior petrosal sinus sampling.
(E) Desmopressin test.

(7) Which gradient defines pituitary-dependent Cushing's syndrome in a simultaneous bilateral inferior petrosal sinus sampling?

(A) Petrosal sinus/periphery before stimuli >1.
(B) Periphery/petrosal sinus before stimuli >2.
(C) Petrosal sinus/periphery after stimuli >2.
(D) Periphery/petrosal sinus after stimuli >3.
(E) Petrosal sinus/periphery after stimuli >3.

(8) What is the first-line treatment for Cushing's disease?

(A) Medical treatment.
(B) Transsphenoidal surgery.
(C) Adrenalectomy.
(D) Radiotherapy.
(E) All of the above.

(9) Which drug is not used in the treatment of Cushing's disease?

(A) Octreotide.
(B) Pasireotide.
(C) Cabergoline.
(D) Ketoconazole.
(E) Metyrapone.

(10) Which is the main cause of mortality in patients with Cushing's disease?

(A) Infectious diseases.
(B) Respiratory diseases.
(C) Cardiovascular diseases.
(D) Cancer.
(E) Digestive diseases.

Answers:

1. D
2. B
3. E
4. D

5. A
6. C
7. E
8. B
9. A
10. C

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Clinical Case 3

12

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12.1 Case Report

A 57-year-old man with a past medical history of ulcerative colitis was referred for the evaluation of thyroid dysfunction concordant with secondary hyperthyroidism (TSH 6.44 uU/mL [reference levels 0.27–4.20] and FT4 5.07 [reference levels 0.93–1.70]). He recalled a 5-year history of similarly altered thyroid biochemical values that had never been previously considered. He did not smoke or drink alcohol, he denied intake of herbal or unknown products, his bowel habit was stable, and he had not undergone recent diagnostic tests with iodinated contrast media.

The patient acknowledged occasional tachycardia, anxiety, and sleeping difficulties, which he attributed to stress at work, and physical examination revealed normal blood pressure, a heart rate of 85 per minute, rapid pace of speech, mild diffuse goiter with no palpable nodules, and mild distal tremor and excess sweat in hands. Laboratory workup was consistent with secondary hyperthyroidism, but normality in the rest of the hormonal and biochemical parameters evaluated (pituitary hormones, testosterone, basal cortisol, IGF-I, ions, proteins, lipid, renal and hepatic profiles), and negative thyroid antibodies (anti-TPO, anti-Tg, and TRAb) (Table 12.1). Radioactive iodine uptake was diffusely elevated, suggesting global hyperfunction and thyroid ultrasound revealed a heterogeneous pattern. Pituitary magnetic resonance imaging (MRI) revealed an area of 14 × 5 mm on the left side of the pituitary, which was hypointense in T1-weighted images and isointense in

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Table 12.1 Patient's laboratory workup at the time of initial evaluation

Clinical parameter (units)	Result	Reference range
FT4 ng/dL	3.77	0.93–1.77
TSH uU/mL	6.06	0.27–4.30
Total T3 ng/mL	2.5	0.8–2.1
LH mU/mL	3.34	1.26–10.50
FSH mU/mL	5.20	1.37–13.58
Prolactin ng/mL	14.65	2.58–20.00
Total testosterone ng/mL	9.01	2.21–7.15
SHBG nmol/L	81.8	14.5–48.4
Calculated free testosterone pmol/L	377.2	228–720
Basal GH ng/mL	0.44	0.2–2
IGF-I ng/mL	171.2	55–186
Basal cortisol ug/dL	19.2	2.3–19.4
ACTH pg/mL	41.49	5–60

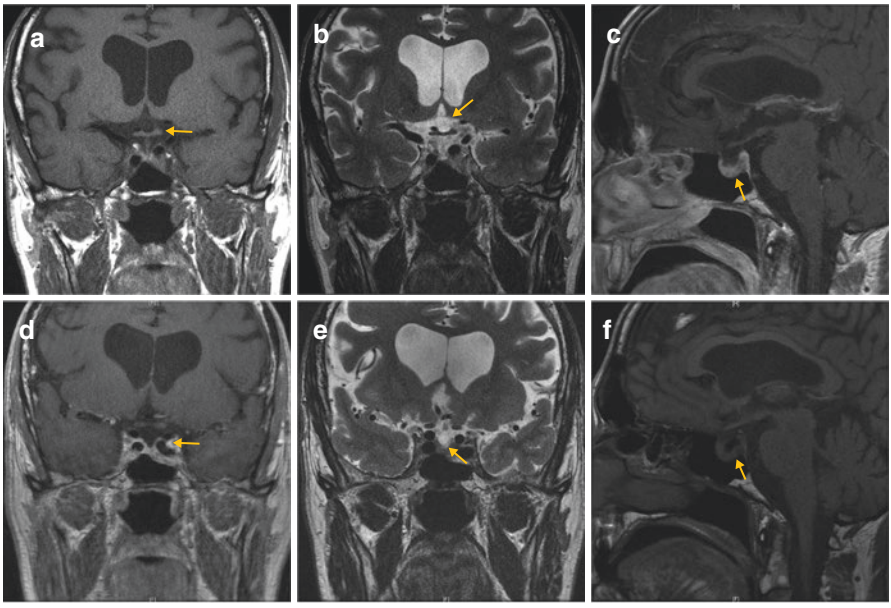

Fig. 12.1 Pituitary magnetic resonance imaging (MRI) sections. Findings revealed a hypointense in T1-weighted images and isointense in T2-weighted images area of 14 × 5 mm on the left side of the pituitary, which extended to the left cavernous sinus and contacted the left internal carotid artery (indicated with small arrows). The rest of the pituitary was mildly displaced to the right, with mild stalk deviation. (**a**) Frontal T1-weighted after gadolinium; (**b**) frontal T2-weighted; and (**c**) sagittal T1-weighted after gadolinium at diagnosis. (**d**) Frontal T1-weighted after gadolinium; (**e**) frontal T2-weighted; and (**f**) sagittal T1-weighted after gadolinium after 6 months of medical treatment with long-acting lanreotide

T2-weighted images, extended to the left cavernous sinus and contacted the left internal carotid artery. The rest of the pituitary was mildly displaced to the right, with mild stalk deviation (Fig. 12.1a–c). Neuro-ophthalmological evaluation, including optical coherence tomography, was unremarkable.

12.2 Differential Diagnosis

In the setting of this confirmed clinical setting of raised thyroid hormones with non-suppressed TSH, and once potential confounding factors have been reasonably ruled out (intercurrent non-thyroidal illness, drug interactions, altered protein profile, and assay interference), genetic and acquired disorders of the hypothalamic–pituitary–thyroid axis function should be ruled out.

Specifically, in this case, the differential diagnosis of what has been termed the "syndrome of inappropriate secretion of TSH" would include the existence of a TSH-secreting pituitary adenoma (TSHoma) versus resistance to thyroid hormone syndrome (RTH) plus pituitary nonfunctioning adenoma [1] or RTH plus pituitary enlargement, for example, after thyroid ablative treatment [2]. In these settings, although central hyperthyroidism is present, the clinical picture usually helps in differentiating them, since patients with TSHoma frequently exhibit hyperthyroid symptoms, while RTH patients are generally euthyroid (so-called generalized RTH). However, in a minority of RTH patients, thyrotoxic features are also present, mainly in those considered to have predominant central or pituitary resistance [3, 4], and the coexistence of a pituitary incidentaloma may entail a blurred differential diagnosis [5].

The stability of the clinical and analytical scenario could lean the diagnostic suspicion toward the diagnosis of RTH. However, on the other side, the mildly elevated levels of SHBG, the increased radioactive iodine uptake, and the existence of a pituitary macroadenoma favored the existence of a TSHoma. Sequencing of exons 1 through 10 of the TRβ gene was performed, which eventually resulted to be negative for the presence of specific known mutations. Sequencing of the TRα gene was not deemed necessary until other diagnostic tests had been performed [4, 6].

Regarding biochemical tests, serum TSH levels within the normal range are more frequently found in RTH, while elevated α-GSU concentrations and/or high α-GSU/TSH molar ratio are typical of TSHomas. However, no differences in terms of age, sex, TSH levels, or free thyroid hormone concentrations have been described as significant between patients with TSHoma and those with RTH [1]. In dynamic testing, the absence of TSH response to the T3 suppression test, combined with the absence of an increase in TSH or alpha-GSU after TRH administration, would be highly sensitive and specific for the diagnosis of TSHoma. However, dynamic testing may not always be recommended; for instance, the T3 suppression test is contraindicated in elderly patients or in those with coronary heart disease [1, 7, 8].

Dynamic testing or evaluation of alpha-GSU may not be readily available in all clinical settings. Thus, in the meantime, in our case, a pituitary indium-111 octreotide uptake (Octreoscan) was empirically performed [8], revealing a focal lesion of increased uptake of approximately 1.5 cm (Fig. 12.2), and a short-acting octreotide test was programmed [8]. Results of this dynamic test are shown in Table 12.2. We can observe how TSH and FT4 levels are normalized, PRL levels remained stable, and SHBG and total testosterone levels also decreased, with total testosterone remaining stable. Overall, this would imply a true effect of octreotide on TSH secretion, leading to a mild amelioration of the hyperthyroid status. The patient acknowledged a mild increase in his bowel habit with the first octreotide injections, which subsequently returned to normal, and, interestingly, referred a subjective impression

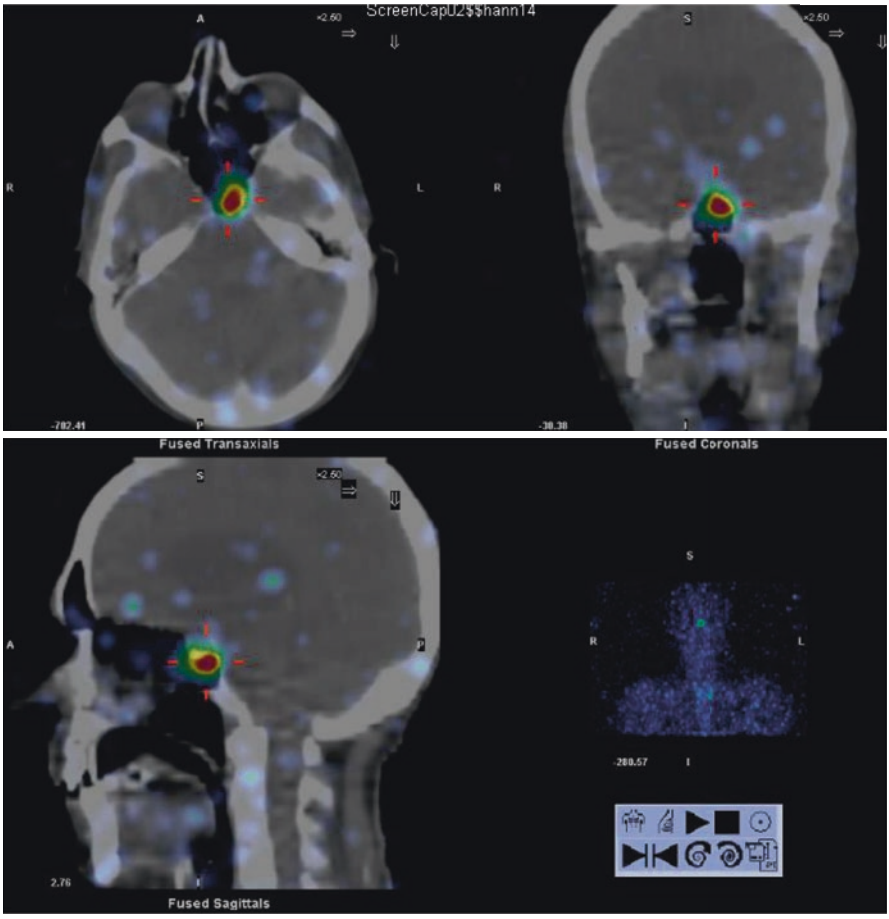

Fig. 12.2 Pituitary indium-111 octreotide uptake (Octreoscan) revealing a focal lesion of increased uptake of approximately 1.5 cm

Table 12.2 Results of the short-acting octreotide test

Clinical parameter (units)	Baseline result	Result after 2 weeks of 50 ug octreotide every 8 h sc	Reference range
FT4 ng/dL	3.89	0.98	0.93–1.77
TSH uU/mL	5.19	0.21	0.27–4.30
Prolactin ng/mL	13.91	10.11	2.58–20.00
Total testosterone ng/mL	11.13	5.46	2.21–7.15
SHBG nmol/L	119.8	47.0	14.5–48.4
Calculated free testosterone pmol/L	342.5	321.0	228–720
IGF-I ng/ml	177.9	85.03	55–186

of clinical improvement regarding his anxious symptoms. This combination of the use of somatostatin receptor imaging techniques (Octreoscan or Gallium-68-PET-DOTATOC scan) and the short-acting octreotide test reasonably ruled out the risk of pituitary incidentaloma (mainly clinically nonfunctioning pituitary adenoma), even though other classical thyroid hormone dynamic tests were not performed.

12.3 Diagnostic Aspects

The combined interpretation of clinical, analytical, and radiological findings favored the diagnosis of TSHoma. Specifically, the increased octreotide scintigraphy uptake where a pituitary adenoma was observed in the MRI [9, 10], and the significant decrease in hormonal values, normalization of SHBG levels, and subjective improvement of hyperthyroid symptoms (tachycardia, anxiety) following the octreotide test supported the existence of a TSH-secreting pituitary adenoma.

12.4 Treatment

The case was thoroughly revised by a multidisciplinary group of endocrinologist, radiologists, neurosurgeons, and radiotherapists. Because the lesion seemed to be in contact with the cavernous sinus and the patient was relatively reluctant to undergo surgery, medical treatment with long-acting somatostatin analogs was offered.

12.5 Follow-up

The patient was started on long-acting lanreotide 60 mg sc every 28 days [8, 11]. Initial follow-up laboratory workup 3 months after starting therapy revealed stabilization of thyroid function tests, as well as the rest of other hormonal and biochemical values: FT4 1.26 ng/dL, TSH 1.07 nuU/mL, total testosterone 6.29 ng/mL, SHBG 45.1 nmol/L, free testosterone 390.22 pmol/L, basal cortisol 17.7 ug/dL, and IGF-I 181.4 ng/mL (reference ranges as described in Tables 12.1 and 12.2). Follow-up pituitary MRI 6 months after revealed stability of the adenoma, with no change in its size or characteristics (Fig. 12.1d–f). Clinical, analytical, and radiological stabilization has endured over the 2-year follow-up that the patient has undergone up to now (Fig. 12.3).

12.6 Learning Points

– When a patient presents with high levels of circulating thyroid hormones and non-suppressed TSH, potential laboratory methodological interferences should be reasonably ruled out. Once the laboratory values have been confirmed, the differential diagnosis includes TSHoma and resistance to thyroid hormone syndrome (RTH).

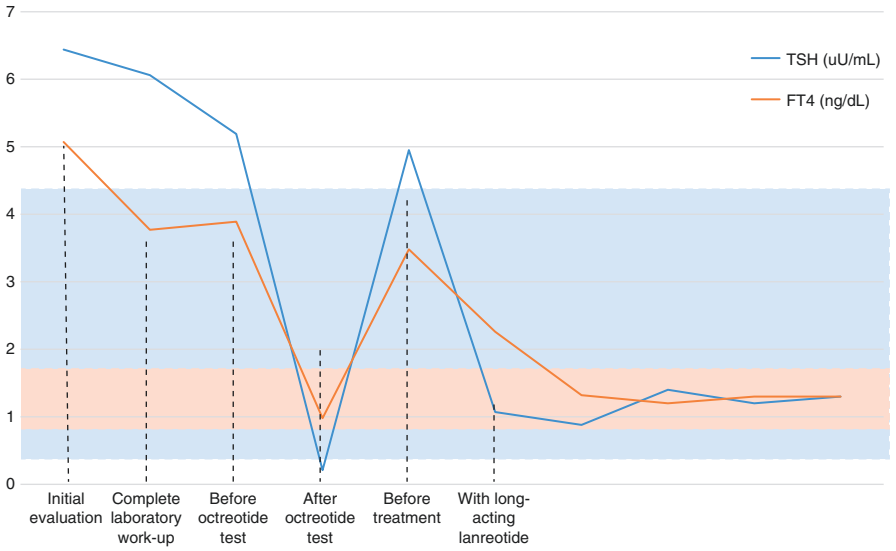

Fig. 12.3 Timeline summarizing TSH and FT4 values during the patient's follow-up. Values are shown as TSH uU/mL and FT4 ng/dL. Shaded blue box represents normal reference values

- The clinical appearance of hyperthyroidism may be mild, progressive, and relatively well tolerated.
- Dynamic tests, such as T3 suppression test and TRH stimulation test, may not always be available. In these cases, an octreotide test may help as a diagnostic and therapeutic approach to the diagnosis of TSHoma.
- Although the first treatment of choice for TSHomas is surgery, medical treatment with long-acting somatostatin analogs may be considered when the patient is not an appropriate surgical candidate.

Questions and Answers

1. When should we consider the possible existence of a TSHoma?
   - Hyperthyroidism.
   - Increased free thyroid hormone levels with detectable TSH.
   - Pituitary tumors.
2. Which diagnostic tests can help in the differential diagnosis between TSHomas and thyroid hormone resistance (RTH)?
   - History and physical examination.
   - Elevated free T4 and total T4, with non-suppressed TSH.
   - T3 suppression test.
   - TRH stimulation test.
   - Octreotide test.
   - Alpha-subunit evaluation.

- Pituitary magnetic resonance imaging.
- Pituitary indium-111 octreotide uptake (Octreoscan).

3. What is the main treatment of TSHoma?
   - Surgery.
4. What medical approach can we use when surgery is not feasible?
   - Long-acting somatostatin analogs.

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Clinical Case 4

13

Francesca D'Ercole, Irene Gagliardi,
Maria Rosaria Ambrosio, and Maria Chiara Zatelli

13.1 Case Report

We report the case of a male patient who was referred in 2003, at 59 years of age, for the incidental finding of a sellar mass, with partial sellar floor erosion, indentified after a computed tomography (CT) scan performed during investigation for sinusitis. Subsequently, the patient was submitted to sellar magnetic resonance imaging (MRI), showing a 29 × 28 × 20 mm lesion with intra- and extrasellar expansion, suprasellar cistern engagement, hourglass morphology, homogeneous contrast enhancement, optic chiasm compression, and invasion of the sphenoid sinus and of the right cavernous sinus most likely consistent with a pituitary neuroendocrine tumor (Pit-NET) (Fig. 13.1, sections 1a and 1b).

The patient complained of frontal headache, otherwise he was in good clinical condition, in particular he did not refer visual or neurological deficits. An endocrine workup was requested, with the evaluation of pituitary function, which was normal. The patient was submitted to visual field (VF) evaluation that showed few points of altered sensitivity, bilaterally.

The chapter has been endorsed by **Prof. Alberto Pereira**, A.M.Pereira@lumc.nl, Center for Endocrine Tumors, Division of Endocrinology, Department of Medicine, Leiden University Medical Center, Leiden, Netherlands

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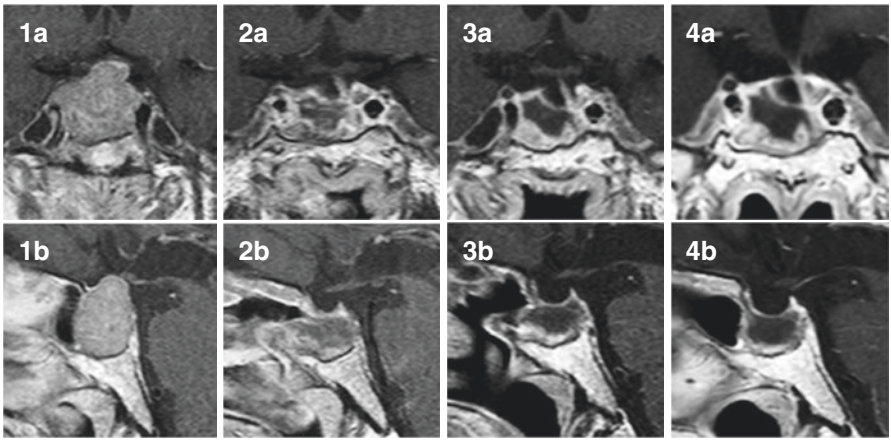

Fig. 13.1 (1**a**) Coronal and (1**b**) axial preoperative T1-weighted gadolinium contrast-enhanced MR image; (2**a**) coronal and (2**b**) axial postoperative T1-weighted gadolinium contrast-enhanced MR image; (3**a**) coronal and (3**b**) axial post-RT T1-weighted gadolinium contrast-enhanced MR image; (4**a**) coronal and (4**b**) axial T1-weighted gadolinium contrast-enhanced MR images at last follow-up

On the basis of the clinical and radiological findings, in February 2004 the patient was submitted to surgery by transsphenoidal adenomectomy (TSA) approach to remove the pituitary lesion. Histological examination disclosed a chromophobe cell adenoma with focal immunohistochemical reactivity for LH and FSH, consistent with a NFPA. Postoperative course was uneventful. Pituitary function assessed immediately after surgery and 2 months later was in the normal range. Three months after surgery, a control MRI showed a reduction in size of the sellar mass (maximum diameter ~ 20 mm); suprasellar cisterns and optic chiasm were no longer involved (Fig. 13.1, sections 2a and 2b). VF was normal and headache disappeared. Sellar MRI performed in 2005 (12 months after surgery) showed no significant changes and, due to the high risk of growth recurrence of the remnant tumor and the optimal distance from the chiasm after surgery, Gamma Knife stereotactic radiosurgery was performed in September 2005.

During the following year, the patient developed multiple pituitary hormone deficiencies, promptly replaced. The patient underwent annual follow-up with MRI, VF evaluation, and pituitary function assessment. Sellar MRI performed on March 2007 showed a ~ 20 mm mass with an irregular central area of liquid type signal, hypointense in T1, and hyperintense in T2 without contrast uptake, surrounded by parenchymatous tissue with contrast uptake. Pituitary stalk was clearly deviated to the left, and the optic chiasm had regular morphology (Fig. 13.1, sections 3a and 3b).

The last follow-up MRI, performed in September 2017, reported overlapping findings and pituitary function never recovered (Fig. 13.1, sections 4a and 4b). The patient reported physical well-being, but he considered his everyday quality of life (QoL) negatively affected by multiple pharmacological therapies including pituitary hormone replacement drugs.

13.2 Differential Diagnosis

Technological improvements in neuroradiology led to the identification of an increasing number of sellar/parasellar masses, often incidentally found during brain imaging performed for different diagnostic indications. Pit-NETs are the most common causes of sellar/parasellar masses, but other lesions could display similar clinical, endocrine, and radiological characteristics, indicating the need to consider them in the differential diagnosis. MRI is the gold standard radiological approach for sellar/parasellar region assessment, while CT scan could help to identify calcifications and bone remodeling [1, 2]. Pit-NET and Rathke's cleft cysts are the most common sellar/parasellar masses representing up to 90% of all lesions, followed by craniopharyngiomas (4.2–5.6%) and cystic malformations (2.9–5.2%) [2, 3] (Table 13.1).

Once the diagnosis of Pit-NET is established, it is well known that NFPAs occur more frequently as compared to functioning Pit-NET**.** The prevalence of NFPA varies between 60 and 100 cases per million inhabitants, with a bimodal peak incidence between the ages of 25–45 and 60–70 years. Less than 5% of Pit-NET occur as a component of hereditary syndromes, such as MEN1, MEN4, Carney complex, and familial isolated pituitary adenomas (FIPAs) [4].

13.3 Diagnostic Aspects

13.3.1 Clinical Presentation

NFPAs are benign Pit-NET not associated with clinical or biochemical evidence of hormonal hypersecretion. As a consequence, in many cases these lesions are found incidentally during radiological examinations (CT or MRI) performed for other reasons (i.e., pituitary incidentaloma). Moreover, the absence of endocrine symptoms often causes a diagnostic delay; as a consequence, NFPAs are frequently diagnosed when they are large enough to cause compressive effects on the surrounding structures. Besides, although usually benign, NFPAs represent a very heterogeneous group of tumors, with varying biological behaviors and clinical manifestations that range from completely asymptomatic lesions to rapidly growing neoplasms, causing mass effect, and neurological or endocrine symptoms. The most common neurological symptoms are visual defects and headache, usually leading to neuroradiological examination. The former occur due to optic chiasm compression; different types of alterations may arise depending on the degree and the site of compression: unilateral, bilateral, partial, or complete VF defects. The most typical VF alteration is bitemporal hemianopsia. Furthermore, lesions anterior to the optic chiasm can cause central scotoma, while posterior lesions can involve the optic tracts and lead to a homonymous hemianopsia. If compression is severe and prolonged, a decline in visual acuity may also develop. Optic atrophy and papilledema rarely occur [5].

Table 13.1 Sellar mass differential diagnosis

		Definition	Clinical manifestations	MR characteristics
Normal anatomical variant	**Small sella**	Pituitary pseudo-enlargement in a relatively small sella (due to sphenoid sinus hyperpneumatization, thicker dorsum sellae, or small/narrow sella)		• Normal T1 and T2 signals • Normal contrast enhancement
Pituitary hyperplasia	**Physiologic sex- and age-dependent hyperplasia**	Pituitary hyperplasia in young age or menopausal women Lactotrope hyperplasia in pregnant women		• Symmetrical pituitary enlargement and superior convexity • Normal adeno- and neurohypophysis signals • Homogenous contrast enhancement
	Pathologic hyperplasia	Thyrotrope hyperplasia	Severe primary hypothyroidism Hyperprolactinemia and related symptoms	
		Pituitary hyperplasia due to CRH or GHRH hypersecretion	Cushing or acromegaly disease McCune–Albright syndrome	
Anterior pituitary tumors	**Pituitary adenoma** **Pituitary carcinoma**	Deriving from adenohypophyseal cell proliferation	• Asymptomatic • Tumor mass effects • Hormonal hypersecretion symptoms	Intrasellar microadenomas (<1 cm): • Lateralization inside the gland • Possible sellar diaphragm deformation • Pituitary stalk displacement Macroadenomas (>1 cm): • Enlarged sella turcica • Not intense contrast enhancement • Possible areas of necrotic-hemorrhagic remodeling
Posterior pituitary tumors	**Pituicytoma**	Arising from neurohypophysis	• Headache • Hypopituitarism • Diabetes insipidus (rare)	• T1 isointensity • Possible displacement of the normal adenohypophysis
	Granular cell tumors	Arising from neurohypophysis or infundibulum		
Benign parasellar tumors	**Craniopharyngioma**	Epithelial tumors arising along the pathway of the craniopharyngeal duct. It can be predominantly solid, cystic, or mixed	• Headache • Nausea/vomiting • Visual impairment • Hydrocephalus • Endocrine dysfunction • Hypothalamic dysfunction • Papilledema • Cranial nerve palsies • Hydrocephalus	• T1 isointensity/ipointensity and T2 hyperintensity of the solid part • T1 hyperintensity of the cystic part • Calcifications
	Ependymoma	Embryonal brain tumor	• Compression symptoms • Endocrine dysfunction • Diabetes insipidus • Fever (lymphomas)	

Malignant tumors	**Primary CNS lymphomas**	Arising primarily in the craniospinal axis	• Visual impairment • Headache • Endocrine dysfunction • Dizziness, tinnitus, facial sensory deficits, ataxia, and hemiparesis (rare)	• Slightly T2 hypointensity • Homogenous contrast enhancement
	Glioma (hypothalamic or optic pathway)	Arising from hypothalamic or optic pathway		• T1 hypointensity • T2 hyperintensity
	Germinal cell tumors	Arising Around the third ventricle, followed by the suprasellar compartment and anterior hypothalamic regions		• T1 isointensity • T2 isointensity/hyperintensity • Homogeneous with a great contrast enhancement • Rarely cystic • Suprasellar infiltration
	Chordoma **Chondromas** **Chondrosarcomas**	Tumors that arise from the primary notochord Tumors that arise from cartilaginous remnants		• Bone erosion • Heterogeneous mass with internal septations and heterogeneous enhancement • Chordomas are typically midline lesions
	Metastases	Most common deriving from breast and lung cancers in women and men, respectively	• Rapid onset and progression of symptoms • Masse effect symptom • Endocrine dysfunction • Diabetes insipidus • Nerve palsies	• Loss of posterior pituitary bright spot and thickening of the pituitary stalk • Infiltration of the posterior pituitary and stalk • Bone erosions • Invasion of Sella diaphragm • Signal intensity varies depending on the primary tumor, but generally they present T1 hypointensity and T2 hyperintensity
	Plasmacytoma		Cranial neuropathies	• T1 and T2 hypointensity • Bony destruction of the sella

(continued)

Table 13.1 (continued)

		Definition	Clinical manifestations	MR characteristics
Malformative lesions	**Rathke's cleft cyst**	Originating from the remnant of the squamous epithelium of Rathke's pouch. Consist of a single layer of epithelial cells with mucoid, cellular, or serous components in the cyst fluid	• Usually asymptomatic • Compressive symptoms • Endocrine dysfunction • Diabetes insipidus	• Cystic lesions with variable signal intensity • Same density of CSF, with low intensity on T1-weighted and high intensity on T2-weighted images • Contrast enhancement is rare or confined to the cyst wall • T2 hypointense intracystic nodules due to cholesterol
	Dermoid and epidermoid cyst	Epithelial elements originating from incomplete separation of the neuroectoderm from the cutaneous ectoderm	• Hydrocephalus • Visual disturbance • Hypopituitarism • Diabetes insipidus • Cranial nerve abnormalities	• T1 and T2 hyperintensity (dermoid cysts) • Radiologic findings similar to cerebrospinal fluid with no contrast enhancement (epidermoid cysts)
	Arachnoid cyst	Herniation of the arachnoid diverticulum through the sellar diaphragm	• Increased intracranial pressure • Hypopituitarism • Visual impairment	• Well-defined lesions • T1 and T2 isointensity • No contrast enhancement
	Hamartoma	Congenital heterotopias usually located within the tuber cinereum	• Mass effect symptoms	• Pedunculated hypothalamic mass, isointense on MRI to gray matter. • No contrast enhancement
Vascular lesions	**Aneurysms** **Cavernous angiomas** **Cavernous sinus thrombosis**			

Inflammatory and granulomatous lesions	**Pituitary abscess**	Primary pituitary abscess Or Secondary pituitary abscesses in glands that harbor a preexisting lesion	• Headache • Visual impairment • Hypopituitarism • Signs of meningism, fever, or leukocytosis	• Cystic lesions with peripheral ring enhancement
	Tuberculosis		Signs of active TBC	
	Primary hypophysitis	Lymphocytic Granulomatous Xanthomatous	• Compressive symptoms • Hypopituitarism (most frequently ACTH deficiency)	• Pituitary enlargement with symmetrical suprasellar expansion • Optic chiasm compression and displacement • Thickened stalk • Intense and homogeneous enhancement of pituitary mass • Loss of neurohypophysis bright spot when diabetes insipidus is associated
	Secondary hypophysitis	Takayasu's disease Crohn's disease Langerhans cell histiocytosis Sarcoidosis Inflammatory pseudotumor Infective etiology Immune checkpoint inhibitor-induced hypophysitis		

In addition, ophthalmoplegia may occur due to compression of nervous structures contained in the cavernous sinus, with the development of different clinical pictures depending on the involved cranial nerves, most frequently the oculomotor nerve. Diplopia could be caused by paralysis of the III or VI cranial nerve. V cranial nerve involvement with consequent trigeminal neuralgia is rare. Similarly, the prevalence of oculomotor abnormalities due to the involvement of cranial nerves is very low (<5%). Indeed, the presence of these symptoms occurring in the context of a large pituitary mass should prompt the suspicion of alternative diagnoses such as apoplexy, infiltrative disorders (sarcoidosis or histiocytosis), and pituitary metastasis from other primary tumors [4].

In the case of NFPA, visual defects appear to be the most frequently reported disorders. However, patients frequently seek medical examination several months or even several years after symptom onset, since in most cases the gradual onset of visual impairments typically remains unnoticed for patients for a long time. The main risk factor for a delayed diagnosis seems to be the age of the patient, who tends to accept the decline of visual function as part of the natural aging process [6]. On the contrary, a timely diagnosis is fundamental because there is a linear correlation between visual symptoms duration and permanent damage probability, persisting after debulking surgery.

Therefore, an ophthalmologic assessment is essential for all NFPA patients, and it should include:

- sensory evaluation: At least one visual acuity (VA) measurement and VF examination, preferably central static + peripheral kinetic. Anterior segment and fundus examinations are also essential for interpreting VA and VF data; optic coherence tomography (OCT) is helpful but not essential for determining visual prognosis;
- oculomotor evaluation: Diplopia should be ruled out on interview, checking the patient's nine gaze directions, which may be completed by orthoptic examination and Lancaster's test.

Headache is another very common symptom regardless of adenoma size. A recent prospective study estimated a 48.5% headache prevalence in NFPA [7]. Clinical presentation of this symptom may widely vary, and it is often difficult to distinguish from primary headache (such as migraine or tension headache). Several mechanisms have been proposed to explain its pathogenesis: an increase in intrasellar pressure, stretching of the dural membrane, and activation of the trigeminal pain pathway for tumors with cavernous sinus invasion [5, 8]. The latter in NFPA is considered a risk factor for headache, which has been shown to be homolateral to the site of invasion of the cavernous sinus [7].

At time of diagnosis of macro-NFPA, 60–85% of patients present at least one pituitary deficiency. The risk of hypopituitarism correlates with tumor volume. Indeed, mechanical compression of the normal anterior pituitary gland or pituitary stalk hampering stimulatory hypothalamic factor delivery can result in partial or complete hypopituitarism. Partial hypopituitarism prevalence ranges from 37% to 85%; panhypopituitarism, instead, is found in 6–29% of patients with a

NFPA. Growth hormone (GH) deficiency and central hypogonadism have been reported to be the most common defects detected in NFPA patients, followed by corticotropin and thyrotropin deficiencies. Available data are sparse for microadenomas that can eventually lead to pituitary dysfunction [9].

Hyperprolactinemia is also a common finding in NFPA patients. Its prevalence in patients with histologically proven NFPA is 25–65% [10]**.** This "disconnection hyperprolactinemia" or nontumoral hyperprolactinemia is usually mild (<95 ng/mL) and is caused by pituitary stalk compression, which hampers dopamine delivery to the anterior pituitary (stalk effect) [11]**.**

The presence of diabetes insipidus (DI) at the time of clinical presentation is very rare in NFPA [5].

Patients with NFPA may rarely present with pituitary apoplexy, an acute clinical syndrome characterized by sudden development of headache, vomiting, altered consciousness, visual abnormalities, and hypopituitarism in the context of hemorrhagic infarction of a pituitary adenoma. Pituitary apoplexy occurs in 5–20% of all pituitary tumors and appears to be more common in NFPA than in hormonally active lesions [12]**.**

Rarer clinical manifestations are rhinorrhea, in the case of tumors that cause erosion of the sellar floor and extend inferiorly toward the sphenoid sinus, and occlusion of the internal carotid artery in the case of tumors extending to the parasellar region.

13.3.2 Hormonal Evaluation

Once a pituitary tumor is discovered, pituitary function should be tested looking either for a hyper- or hypopituitarism. All anterior pituitary axes should be evaluated, and when a hormonal hypersecretion is excluded, the tumor could be clinically defined as NFPA [3, 10].

However, hyperprolactinemia could be present at diagnosis due to pituitary stalk compression, and as outlined before, prolactin (PRL) levels are usually much lower as compared to "true" prolactinomas [13]. In addition, in the case of a large tumor with slightly elevated PRL levels and hyperprolactinemia symptoms, the diagnosis of macroprolactinoma should be taken into account due to the possibility of a "hook effect" in PRL level assessment. This laboratory artifact is due to high PRL levels that hinder the antibody–antigen–antibody sandwich complex formation. Serum dilution of the sample could avoid this bias. On the other hand, very high PRL levels in the absence of clinical hyperprolactinemia symptoms should prompt polyethylene glycol (PEG) precipitation to screen for macroprolactin interference [11, 14].

It is mandatory that all NFPA patients are investigated for pituitary hormone deficiencies, in order to promptly initiate hormone replacement therapy, especially in case of adrenal and thyroid insufficiency [10]. Very low 8–9 AM cortisol levels may be sufficient to diagnose central adrenal insufficiency; nevertheless, a dynamic test (Synacthen's test or insulin tolerance test, depending on general health status) is recommended. Stimulation tests are not required for the evaluation of other pituitary axes [11]. Low FT4 levels associated with low, inappropriately normal, or

mildly elevated TSH levels are consistent with central hypothyroidism. Hypogonadotropic hypogonadism is diagnosed by the presence of low serum testosterone levels associated with low or inappropriately normal LH/FSH levels, and hypogonadism symptoms in males. In premenopausal females presenting oligomenorrhea or amenorrhea, plasma FSH, LH, and E2 should be measured; low FSH and LH levels are consistent with central hypogonadism in post-menopausal women [15]. Finally, IGF1 concentration below gender- and age-specific lower limit of normal is consistent with GH deficiency, especially when three pituitary axis are compromised [11]. However, screening for GH deficiency is not recommended at diagnosis [9].

Regular monitoring of urine volume and serum sodium is necessary to identify hyponatremia and/or DI [13].

There is no evidence supporting the measurement of chromogranin A or routine genetic testing in patients with sporadic NFPA [10, 14].

13.3.3 Radiological Diagnosis

Gadolinium contrast MRI is the gold standard for sellar region evaluation since it provides high-resolution images of sellar contents and surrounding structures. On the other hand, CT is the preferred imaging technique for hospital emergencies or for surrounding bone structure evaluation. Thus, neuroradiological assessment of suspected NFPA is based on MRI, which should be performed according to specific criteria. In particular, MRI protocol should include:

- thin (≤3 mm) slices, high matrix, sagittal + coronal T1-weighted sequences with and without gadolinium injection;
- coronal 3D volume assessment with reconstruction;
- T2-weighted coronal slices;
- volume acquisition; otherwise, very thin slices are needed [9].

In T1-weighted images, adenomas can appear hypo- or isointense compared to nontumoral pituitary tissue with poor or absent gadolinium uptake. In T2-weighted images, adenomas appear isointense compared to the white matter. In the presence of tumor bleeding, such as pituitary apoplexy, hemorrhage appears as hyperintense in T1-weighted images without contrast. This characteristic hyperintensity may be absent in the early stage because hemorrhage is still in the form of deoxyhemoglobin. Hyperintensity of the optic chiasm on T2-weighted images can indicate a compromised visual function, which may persist even after prompt surgical removal of the pituitary adenoma [5].

The extension of the lesion should be determined:

- superiorly, toward the optic pathways;
- laterally, in the cavernous sinuses,
- inferiorly, in the sphenoidal sinus and posteriorly, toward the clivus.

The Knosp classification systems are currently mostly used for radiological classification, having a clinical and prognostic value. Knosp offered a grading system for predicting invasion of the cavernous sinus by pituitary macroadenomas based on MRI, describing tumor parasellar extension, which is a negative prognostic factor for surgical outcome. Briefly, the more laterally an adenoma grows and surrounds the internal carotid artery, the higher its grade level is, with grade 0 corresponding to an adenoma without any parasellar extension and grade 4 to the total encasement of the intracavernous carotid artery [13, 16]. There is insufficient evidence to indicate the use of MR spectroscopy, perfusion, positron emission tomography (PET), and single-photon emission computed tomography (PECT). Gradient echo and MR perfusion are promising tools to evaluate cavernous sinus invasion and tumor vascularity [17, 18], but need to be further confirmed.

13.4 Treatment

13.4.1 Surgery

According to recent guidelines issued by the Congress of Neurological Surgeons, surgery is the first-line treatment for symptomatic NFPA [18]. They state that the primary aim of surgery is relieving surrounding neural and vascular structures from pituitary tumor compression. Thus, visual abnormalities, optic nerves, or chiasm impairment, neurological deficit, and pituitary apoplexy with visual involvement are indicators for surgery. Even if a total tumor resection is ideal, an aggressive approach could compromise surrounding vital structures or damage healthy pituitary gland and its function [13, 19–21]. Indeed, pituitary surgery may also be indicated in the attempt to restore or preserve normal pituitary function in the presence of a large NFPA. However, surgery may not be effective in improving headache and/or hypopituitarism. Thus, treatment decision should be individualized, based on clinical context, multidisciplinary team (MDT) discussion, and patient preference [20].

Prior to surgery, correcting adrenal insufficiency with glucocorticoid (GC) replacement therapy and/or hypothyroidism with L-thyroxine replacement therapy is mandatory. Furthermore, pretreatment neuro-ophthalmologic evaluation is recommended to define the preoperative baseline and the urgency of surgery and to provide prognostic factors for recovery [19, 20, 22].

To date, evidence is insufficient to define a primary treatment strategy for asymptomatic lesions [18] and a nonemergency surgery should be evaluated case by case. Factors that should be taken into account are (1) patient age; (2) NFPA natural history; (3) risk of VF impairment, correlated with the rate of tumor growth and proximity to the optic pathways; (4) risk of pituitary deficiency development; (5) risk of apoplexy; and (6) risks associated with transsphenoidal surgery. Thus, surgery could be evaluated in asymptomatic pituitary tumors with anatomic signs of impending visual loss and in young patients with macroadenomas considering the higher lifetime probability of tumor growth [19, 20]. In other cases, a conservative approach

has been proposed [11]. In microadenomas, surgery is not indicated due to their slow progression [21]. Fernandez-Balsells et al. reported a greater tendency for tumor growth in macroadenomas in comparison with microadenomas (12.53 vs. 3.32 per 100 patient-years) [23].

TSA approach is the technique of choice for pituitary tumor removal and can be performed under endoscopic or microscopic visualization. No evidence confirms the superiority of one on the other, and the choice among them depends on surgeon's preference and skills. Microscopy offers a small vision and a limited field illumination with the risk of collision with other instruments. On the other hand, endoscopic approach decreases three-dimensional perspective by operating through a two-dimensional image but improves illumination and visualization of surgical field [19, 24]. The combined surgical strategy of TSA and transcranial approaches is recommended for invasive NFPA with significant suprasellar, frontal, or temporal extension. Surgery is defined as a safe and effective approach also in American Society of Anesthesiologists grade 1 to grade 3 elderly patients, as reported in recent guidelines [24]. Surgical indications are the same in older as compared to younger patients, but the higher comorbidity incidence in older patients should be taken into account. Thus, a careful anesthesia risk assessment is fundamental to guarantee the same mortality of the general population. Surgical approach should preferably be transsphenoidal, to lower the risk of complications. Some studies reported a longer hospitalization due to water and electrolyte disorders in older patients (10% vs. 6% of the general population), while other studies did not find any difference. Improvement of visual symptoms and pituitary deficiencies is comparable to that of younger patients. In young women with macroadenoma and no visual impairment, a pregnancy wish should be taken into account in the pituitary tumor management. Adenomectomy often recovers central hypogonadism due to disconnection hyperprolactinemia, but if hypogonadism is caused directly by tumor mass, it is effective only in about 30% of cases. Furthermore, lactotroph cell hyperplasia increases pituitary volume during pregnancy; therefore, any suprasellar extension toward optical structures must be taken into account. If the tumor mass is diagnosed during pregnancy, clinical, radiological (MRI without contrast enhancement), and ophthalmologic surveillance should be proposed [9, 20, 25]**.**

Surgical treatment is a safe procedure with adequate preoperative counseling. Surgery may be effective in symptom relief and is associated with low morbidity and mortality [19]. In a recent meta-analysis, TSA approach in NFPA patients was associated with 1% mortality [26]. Complication incidence is lower in hospitals with skilled surgeons, occurring in ≤5% of patients. The most common complications are cerebrospinal fluid leakage, fistula, meningitis, vascular injury, persistent DI, visual deterioration, temporary or permanent hypopituitarism, and sinonasal complications (crusting, septal perforation, epistaxis) [15, 19]. The more pituitary tumor is removed, the more symptoms relief is obtained. In a recent postsurgical series of 281 NFPA patients, 89.7% reported headache improvement and 70.1% achieved visual amelioration, while 5.1% had stable vision [19]. Visual recovery may be progressive and is probably correlated with duration and severity of visual

field disorders [27]. Multiple retrospective studies showed improved visual function in 75–91% of surgically treated patients and improved hypopituitarism in 35–50% of patients. However, surgery itself may worsen hypopituitarism in 10% of patients [20].

Pituitary surgery allows a gross total resection in 60–73% of patients with NFPA [28], but recurrences occur in about 10–20% of completely resected tumors after 5–10 years. Moreover, when residual tumor is left after surgery, tumor growth rate could reach 40% and 50% at 5 and 10 years, respectively [17].

13.4.2 Radiotherapy

Radiation therapy (RT) is an effective treatment for patients with NFPA unsuccessfully treated by surgery, resulting in local control of 90–95% of patients at 5–10 years [29]. Several RT techniques are currently available, including fractionated conformal RT, stereotactic radiosurgery (SRS), and fractionated stereotactic RT.

Fractionated conformal RT delivers high-energy photons, with a total dose between 45 and 50 Gy, fractionated in 25 sessions of 1.8–2 Gy [9]**.**

Stereotactic techniques, such as SRS or fractionated stereotactic RT, have been developed with the aim of delivering more localized irradiation and reducing long-term side effects, maximizing sparing of adjacent healthy structures. Stereotactic techniques represent the most commonly used radiation techniques in the treatment of NFPA [29]**.** Radiosurgery delivers radiation in a single session by using Gamma Knife and linear accelerator (LINAC). This type of RT is feasible only if the target volume is clearly defined, small (<2–3 cm on the long axis), and sufficiently remote from the optic pathways to ensure <8 Gy irradiation to the chiasm and optic nerves.

Fractionated stereotactic RT takes advantage of the ballistic precision and multiple beam entries of radiosurgery applied to healthy tissue radioprotection by fractioning. Hypofractionated stereotactic RT (in 3–5 fractions mainly, delivering a total dose of 20–30 Gy) may also be proposed [9].

The most frequent side effect of RT is the occurrence of anterior pituitary dysfunction, which develops in 5–40% of the cases and up to 80% of the patients over a 10-year period [30]**.**

Since the optic chiasm is the most radiation-sensitive structure, another potential, but rare, side effect of RT is optic neuropathy, which presents by an acute vision loss. This complication has been attributed to microvascular obliteration of the visual tract and is directly related to the radiation dose and the proximity of the tumor to the optic chiasm; an advanced age and the concomitant presence of diabetes mellitus appear to increase the risk of visual side effects [31]**.** Vascular complications such as stroke have been reported with conventional external RT but not with fractionated stereotactic RT [32]**.** In the long term, older RT techniques have been reported to associate with the development of secondary brain tumors, such as gliomas, glioblastomas, sarcomas, or meningiomas, even 20 years after irradiation. However, this increased risk is rare and was not confirmed in all studies [31].

In general, side effects of risk are lowered by reducing the dose to healthy tissues. Therefore, thanks to the new stereotactic techniques side effects that are becoming less frequent.

The choice of RT technique is made according to adenoma size, proximity to the visual pathways, potential risk to healthy surrounding tissues, and local availability. If available, SRS is preferred when the adenoma maximal diameter is <3 cm and is distant at least 2 mm from the optic chiasm (Minniti 2016 Rep Pract Oncol Radiother) to allow a <8 Gy irradiation to the optic chiasm and nerves [33]**.** On the contrary for large lesions involving the optic apparatus, conventionally fractionated RT is advised [29]**.**

The role of RT in the postoperative management of NFPA is still debated. Indeed, RT efficacy in reducing local tumor growth is indisputable, whatever the procedure used. Postoperative RT reduces NFPA recurrence rate from 40% in nonirradiated patients to <10% in those who have been irradiated [4]**.** On the other hand, the possible side effects must be taken into account. In addition, there are no randomized controlled trials showing the superiority of RT compared to a conservative approach after surgery for NFPA management. Therefore, at present, there is no consensus concerning the systematic use of RT in the postoperative period for patients with incompletely resected NFPA [34]. In most cases, regular surveillance can be proposed, postponing RT until recurrence is confirmed [9, 35]**.**

Adjuvant RT may also be considered for patients who, at diagnosis, already present aggressive tumors, such as those invading parasellar structures or with high Ki-67 labeling index, a proliferative marker significantly associated with NFPA recurrence [36]**.** There is no consensus concerning the optimal timing for adjuvant radiotherapy. The risk of side effect development increases with longer time since RT, suggesting to postpone this intervention until tumor regrowth, since late RT seems as effective as early treatment in terms of local control [37]**.**

In summary, systematic postoperative RT is not indicated following complete resection. In case of tumor remnant after initial surgery, the beneficial effects of RT need to be weighed against the other possible alternatives, such as wait and scan and reoperation**, which** is advised to be discussed within an experienced multidisciplinary pituitary team. However, RT and in particular stereotactic irradiation remain an important pillar in the management of patients with residual or recurrent NFPA, due to its efficacy in inhibiting tumor growth and reducing tumor volume [37]**.**

Clearly, a short- and long-term assessment of the risk–benefit profile is always required, considering the patient's age, clinical history, and comorbidities. When considering patients already presenting with hypopituitarism, the occurrence of pituitary dysfunction after RT carries less concern, which may shift the benefit/risk ratio. The occurrence of optic neuropathy needs to be counterbalanced by the potential visual improvement that can be observed due to reduction in tumor volume.

Another type of RT approach is represented by proton beam radiotherapy, which takes advantage of the physical properties of protons, offering better dose distribution and normal tissue preservation as compared to photons. However, at present, the superiority of protons over photons in terms of long-term efficacy and toxicity

remains unsubstantiated [29]**.** Furthermore, this therapeutic approach is not widely available.

There are several case reports describing the use of peptide receptor radionuclide therapy (PRRT) in Pit-NET. Some patients may respond to this treatment modality, but its overall efficacy cannot be established based on the small available clinical experience [38].

13.4.3 Medical Therapy

The recent Guidelines by the Congress of Neurological Surgeons state that there is no clinical evidence supporting medical therapy as primary treatment for NFPA. Indeed, dopamine agonists (DAs) and somatostatin analogs (SAs) demonstrated inconsistent tumor response rates (12–40% and 0–61%, respectively) [5]. However, several studies focused on a potential DA therapeutic role in the medical management of NFPA. Dopamine 2 receptors (DR2) are expressed on all anterior pituitary cell types, including NFPA cells [39–41]. Some authors demonstrated a positive effect of cabergoline on tumor shrinkage, which was evident after a 6-month treatment [42, 43]. Greenman et al. reported the largest series of postoperative NFPA patients with tumor remnants treated with DA. They compared the outcome between patients receiving DA therapy immediately after surgery and those who started therapy after tumor growth confirmation. Tumor control was achieved in 87% and 58% of cases, respectively. Thus, they suggested that DA therapy might avoid additional invasive treatment such as surgery and RT [44]. Young age, male gender, and a larger preoperative tumor size were associated with a lower response rate to DA treatment [38]. Cabergoline therapy is usually started with 0.5 mg weekly, increasing 0.5 mg each week until a maximum dose of 3 mg weekly [11]. In patients resistant to standard dose, higher doses did not prevent tumor progression. With regard to cardiac safety, reliable data on valvulopathy development in NFPA are not available. Periodic echocardiogram probably should be reserved only to patients taking >3 mg/week of cabergoline for prolonged periods [38, 44]. In conclusion, even if evidence is contrasting, recent data seem to suggest cabergoline as a safe and cheap alternative in cases where surgery is contraindicated, in patients refusing surgery and as adjuvant therapy in patients with postsurgical residual tumors. Further randomized controlled trials are needed to identify the optimal clinical scenario in which DA could be used [38, 39, 42]. NFPAs express also different somatostatin receptor (SSTR) subtypes, but their significance is unclear [39, 45]. Somatostatin receptor scintigraphy is nonpredictive of SA therapy response in NFPA. The significant antiproliferative and antisecretory effects of somatostatin on NFPA cells demonstrated in vitro were not confirmed in vivo. Further long-term well-designed studies are needed to better define SA treatment for NFPA tumor control [38]. To date, pasireotide demonstrated positive effects in vitro, but in vivo trials are still not available. Combination therapy with DA and SA did not show better efficacy [17].

Finally, temozolomide (TMZ) is an alkylating chemotherapeutic drug proposed by the European Society of Endocrinology Guidelines as first-line chemotherapy in aggressive pituitary tumors showing progression [46]. Low expression of O-methylguanine DNA methyltransferase (MGMT) correlates with a better response rate [11]. In progressive and regrowing NFPA, low MGMT expression was found, suggesting TMZ as an alternative treatment after conventional therapy failure [47].

13.5 Follow-up

Recent Guidelines and Expert's Consensus offer some recommendations about post-treatment NFPA follow-up, even if there is no high-grade level of evidence defining duration and frequency [9, 14, 48, 49]. NFPA recurrence rate was reported to be 15–66% for surgery alone and 2–28% for surgery followed by RT; therefore, a long-term radiologic follow-up of at least 10 years is reasonable [35, 50]. A postsurgical radiological tissue or tumor remnant and younger age are risk factors associated with recurrence. In the early postoperative period, signs and symptoms of water balance disorders (diabetes insipidus DI, syndrome of inappropriate ADH secretion [SIADH], and cerebral salt-wasting syndrome) and hypocortisolism should be promptly detected by measuring plasma electrolytes, urine specific gravity, water balance, osmolality, and early morning cortisol levels [13, 35, 48]. Central DI is a condition characterized by an inappropriate hypotonic polyuria (urine output >3 l/24 h and urine osmolality <300 mOsm/kg) associated with a high or normal serum sodium [48]. It may occur 12–48 hours after surgery and is generally due to a transient dysfunction of ADH-secreting neurons [13, 48], but it could persist in 2–7% of patients. Risks factors for permanent DI are young age, male gender, large intrasellar masses and postoperative CSF leaking, preoperative DI, and repeated pituitary surgery [48]. Sometimes (3–4% of cases), postsurgical water–electrolyte imbalance could present with a triphasic trend: DI occurs in the first 5–7 days, followed by an uncontrolled release of ADH (SIADH phase) lasting up to 2 weeks, and a final condition of permanent DI [13]. Immediate postsurgical DI should be treated with vasopressin with frequent retesting of response to avoid ADH administration during SIADH phase or after DI resolution [15, 48]. SIADH, instead, leads to a condition of euvolemic hyponatremia that should be treated in the first place with fluid intake restriction in the milder cases (Na 134–125 mmol/l), or with hypertonic saline or ADH receptor antagonists in more severe cases (Na < 125 mmol/l). Rapid decreases in serum Na and/or symptomatology (headaches, nausea, vomiting) also affect the action plan.

More rarely, a postsurgical hypovolemic hyponatremia may be due to a cerebral salt-wasting syndrome mediated by brain natriuretic peptide action [48]. As concerns the risk of secondary adrenal insufficiency, corticosteroid supplementation is recommended in the perioperative period in patients showing preoperative or immediate postoperative (day 2) hypocortisolemia [49]. Nevertheless, in clinical practice, the management of NFPA patients with normal preoperative HPA axis is still controversial. Prophylactic GC coverage is also controversial: Available data are not

clear concerning the efficacy of this approach in all patients, specifically in patients found to be adrenal insufficient after surgery [48, 51–53]. Since hypoadrenalism is a life-threatening condition, Prete et al. proposed hydrocortisone e.v. therapy to all patients in the first 24 h after pituitary surgery (50 mg every 6 h), rapidly tapering to standard oral replacement doses (15–20 mg/daily) during the following 48 hours, in the absence of complications. The same authors consider morning plasma cortisol levels (assessed before oral GC therapy) tested 1 week after surgery, as predictive of HPA axis integrity: Cortisol levels <5 μg/dl are consistent with secondary adrenal insufficiency, whereas cortisol levels >15 μg/dl should rule out adrenal insufficiency. Intermediate values need to be confirmed with dynamic provocative tests after at least 6 weeks from surgery [48]. In case of persisting deficit, pituitary function should be assessed at least 3 months postoperatively, to screen for late recovery [9]. The insulin tolerance test (ITT) is considered the gold standard to evaluate HPA axis integrity. However, this test may cause serious side effects and is contraindicated in specific patients (older patients, patients with a history of epilepsy or ischemic heart disease). As an alternative, high-dose (250 μg) or low-dose (1 μg) Synacthen test can be proposed, but there is no evidence to date supporting one or the other dose. During the first year, after surgery regular evaluation of morning cortisol should be recommended [13]. Retesting of all pituitary axis (thyrotroph, gonadotroph, and somatotropic) should be completed 4–6 weeks after surgery, unless acute complications occur [15, 48]. If a preexisting deficiency is confirmed or it is newly diagnosed, pituitary function should be reassessed in order to adapt replacement therapy [9]. GH deficiency treatment should be evaluated case by case, also taking age into account, considering that, to date, there is no evidence supporting tumor remnant growth promotion or recurrence induction by rhGH replacement therapy in patients with NFPA treated exclusively by surgery. If pituitary function is conserved, re-evaluation should be performed only in case of recurrence/residual tumor progression or after adjuvant RT. In the latter case, pituitary function should be explored at least once yearly for at least 10 years [14].

Generally, first postoperative MRI is performed 3–6 months after surgery and the second one after 12 months. The acquired images are considered as reference for subsequent follow-up [9, 13, 49], which needs to be individualized. In the absence of residual tumor, MRI is suggested to be repeated annually for 5 years and then at 7, 10, and 15 years. If no clinical signs, residual disease, or suspect images are present, radiological follow-up may be terminated. In case of adenomatous remnant or suspect image, MRI is suggested to be repeated annually for 5 years and then every 2 or 3 years in the absence of progression [9]. In case of recurrence or tumor remnant growth, a multidisciplinary re-evaluation (endocrinologist, neuroradiologist, neurosurgeon, radiotherapist) should discuss tumor morphology and pathology characteristics, patient age, presence of hypopituitarism, and center experience, which are important issues in decision making. Considering patient preference, surveillance or adjuvant therapy may be proposed. Second surgery may be considered an option: (1) when a complete resection is possible; (2) in case of optic pathway compression; and (3) to ensure 3–5 mm safety margins between superior tumoral dome and optic pathway for complementary RT [9].

Ophthalmologic re-evaluation is advised 3 months after surgery and repeated every 6 months when a preoperative visual impairment is found. When the maximum visual function improvement is reached, assessment intervals may be lengthened. Ophthalmological follow-up can stop when first postoperative visit is negative for visual impairment, in the absence of suprasellar remnant threatening the optic pathways. In case of RT, prolonged annual follow-up is mandatory [9, 13].

13.6 Learning Points

1. Multimodal therapeutic approaches are needed in NFPA management.
2. Differential diagnosis should always be explored.
3. NFPA clinical presentation is highly variable, according to mass symptoms.
4. Hyperprolactinemia should be accurately evaluated due to possible laboratory bias.
5. Hypopituitarism should always be investigated, since it significantly influences further therapeutic management.
6. MRI represents the imaging gold standard, together with an expert radiologist.
7. Surgery is the first-line treatment for macroNFPA. Accurate patient preparation is mandatory.
8. The role of radiation therapy is still debated.
9. Post-radiotherapy hypopituitarism may develop after many years.
10. Medical approaches are still controversial.
11. Long-term follow-up by MRI imaging, endocrine workup, and ophthalmological evaluation is warranted.
12. Multidisciplinary team approach is necessary in order to achieve a correct diagnosis and plan an appropriate treatment.
13. Patient QoL must be always taken care of when considering NFPA management and follow-up.

Questions and Answers

Q: *When should we suspect the presence of a nonfunctioning pituitary adenoma?*

A: The absence of endocrine symptoms often delays a diagnosis, until mass effects reveal neurological symptoms, headache, visual disturbances, or secondary endocrine symptoms. The most common symptoms are visual defects and headache, possibly associated with hyperprolactinemia (and related hypogonadism), or pituitary deficiencies. Diabetes insipidus is rare and rhinorrhea even rarer.

Q: *Which are the recommended tests to establish the diagnosis of a nonfunctioning pituitary adenoma?*

A: Sellar MRI is the gold standard for diagnosis, interpreted by an expert radiologist. Hyperprolactinemia should be accurately evaluated to exclude the presence of a high-dose hook effect or macroprolactinemia.

Q: *Which is the optimum treatment for nonfunctioning pituitary adenomas?*

A: Transsphenoidal tumor resection is the first-line treatment for nonfunctioning pituitary macroadenomas (diameter > 1 cm), in order to relieve mass symptoms. Surgery may be associated with the development of hormonal deficiencies in the short term.

Q: *After surgery, how is hypopituitarism diagnosed?*

A: HPA axis should be assessed immediately after surgery. Pituitary function should be assessed within 4–6 weeks after surgery and at least 3 months postoperatively. The insulin tolerance test (ITT) is considered the gold standard to evaluate HPA axis integrity. However, this test is contraindicated in specific patients (older patients, patients with a history of epilepsy or ischemic heart disease). Synacthen test can be proposed as alternative. During the first year after surgery, regular evaluation of morning cortisol should be recommended. If a preexisting deficiency is confirmed or it is newly diagnosed, pituitary function should be reassessed in order to adapt replacement therapy. GH-deficit treatment should be evaluated case by case. If pituitary function is preserved, re-evaluation should be performed only in cases of recurrence/residual tumor progression or after adjuvant RT.

Q: *How long is follow-up and monitoring of a nonfunctioning pituitary adenoma?*

A: There is no firm evidence defining duration and frequency of follow-up for patients with nonfunctioning pituitary adenomas. Recurrence rate is significant (either after surgery alone or after surgery + RT); therefore, a long-term radiological and clinical follow-up of at least 10 years is advisable. In addition, hormonal deficiencies occurring after initial treatment should be supplemented and retested in order to detect pituitary function recovery, which, although rare, may occur.

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Questions and Answers

14

Gianluca Tamagno and Manuel D. Gahete

14.1 Questions

1. **Why the secretion of pituitary hormones is controlled by a widely and complex landscape of central and peripheral modulators?**

2. **Pituitary tumors are mainly:**
   A. Sporadic
   B. Familiar

3. **What gene mutation should be considered in a 3-year-old female patient with gigantism/acromegaly?**

4. **Which of the following sentences is true about somatotroph adenoma pathogenesis?**
   A. With the current genetic advances, a predisposing germline mutation is found in the majority of patients harboring somatotroph adenomas.
   B. Despite advances in genetics, the majority of somatotroph adenomas have only somatic (i.e., nongermline) mutations.
   C. With current histopathological staining techniques, the majority of somatotroph adenomas coexpress other pituitary markers, such as prolactin, glycoprotein alpha and beta subunits, and ACTH.
   D. The pure or mixed GH-PRL somatotroph adenomas are polyclonal in origin, indicating pituitary hyperplasia as a pretumoral lesion.

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5. **What is the cutoff point to be defined as a macroprolactinoma?**
   A. 2 cm
   B. 5 mm
   C. 4 cm
   D. 10 mm
   E. None of the above

6. **Who should be tested for Cushing's syndrome?**

7. **Can all the GH-modulating factors be able to act synergistically?**

8. **Pituitary tumors are:**
   A. Monoclonal
   B. Polyclonal

9. **What gene mutation should be considered in a 10-month-old child with ACTH-dependent Cushing's syndrome?**

10. **What is the current evidence about cardiac ischemic disease in patients with acromegaly?**
    A. Acromegaly is associated with a decreased Framingham risk and a lower risk of myocardial infarction and cardiac ischemic disease than that of the general population.
    B. Acromegaly is associated with an increased Framingham risk but no increase in myocardial infarction or cardiac ischemic disease compared to the general population.
    C. Acromegaly is associated with increased Framingham risk and consequently with a higher risk of myocardial infarction and cardiac ischemic disease than that of the general population.
    D. Despite a lower Framingham risk, patients with acromegaly have an increased risk of myocardial infarction and cardiac ischemic disease than the general population.

11. **What pituitary cells do prolactinomas arise from?**
    A. Somatotrophs
    B. Lactotrophs
    C. Gonadotrophs
    D. Thyrotrophs
    E. Corticotrophs

12. **Once hypercortisolism is confirmed, how Cushing's disease is identified?**

13. **Does PRL exert a feedback regulatory loop to modulate its own expression controlling its regulators?**

14. **Pituitary tumorigenesis is triggered by:**
    A. Aberrant hypothalamic factors causing hyperplasia
    B. Disrupted negative feedback from the periphery
    C. Overexpressed growth factors and cytokines
    D. Genetic hit (mutation) transforming a single cell

15. **What are genetic causes of 3Pa?**

16. **Which of the following sentences concerning acromegaly and neoplasms are true?**
    A. The prevalence of cancer in cohorts of patients with acromegaly is approximately 20%.
    B. Reinforced cancer screening must be adopted in patients with acromegaly, such as colonoscopy, cervical ultrasound, mammography, and frequent PSA samples.
    C. Following the latest evidence, concern for neoplasms in acromegaly is currently restrained to colorectal and thyroid cancer.
    D. The prevalence of certain cancer subtypes in individuals with acromegaly may be overestimated by systematic screening procedures and physician awareness.

17. **What factors might affect basal prolactin value?**
    A. Sertraline
    B. Exercise
    C. Pituitary stalk compression
    D. Venipuncture
    E. All the above

18. **Which are the recommended initial tests to establish the diagnosis of hypercortisolism or Cushing's syndrome?**

19. **Is there any relationship between metabolic status and PRL secretion?**

20. **Mouse models of genes involved in cell cycle regulation present with pituitary hyperplasia and tumors; which of these genes are frequently mutated in human pituitary tumors?**
    A. *RB*
    B. *CCNE1* (cyclin E1)
    C. *CDKN1B* (p27)
    D. *CDKN2C* (p18)
    E. All of the above
    F. None of the above

21. **What are the most common somatic mutations among sporadic pituitary adenomas?**

22. **Please identify the correct statement about radiating techniques in acromegaly**
    A. Radiating techniques are considered third-line treatments because they have been roughly the same over the last decades.
    B. Radiating techniques are considered third-line treatment because of the improvement in surgical techniques over the last decades.
    C. Radiating techniques are considered third-line treatment because of the improvement in medical treatments over the last decades.
    D. Radiating techniques have consistently improved over the last decades and are now considered as first-line treatment along with other treatment approaches.

23. **Which imaging modality is of choice in characterizing a prolactinoma?**
    A. Ultrasound
    B. CT scan with contrast
    C. Nuclear medicine scans
    D. MRI scan
    E. None of the above

24. **Which is the optimum treatment for CD?**

25. **Does the ghrelin/GHSR1a system modulate the same signaling pathways in all pituitary cells to control hormone secretion?**

26. **Which gene is mutated in ~40% of sporadic somatotroph tumors?**
    A. *PRKAR1A*
    B. *GNAS*
    C. *GNAI2*
    D. *GPR101*
    E. *AIP*

27. **Identify the correct statements about acromegaly management**
    A. Stepwise treatment in acromegaly, according to the latest guidelines, indicates medical treatment as a first-line strategy, followed by selective pituitary surgery and radiotherapy.
    B. Mixed GH-prolactin adenomas may benefit from a first-line medical treatment by dopamine agonist, which may lead to IGF-I normalization and tumor shrinkage.
    C. According to the latest guidelines, the treatment approach in acromegaly is multimodal by delivering selective pituitary surgery, medical treatment, and radiotherapy simultaneously.
    D. First-line medical treatment is preferred in inoperable patients or in those with highly morbid concomitant diseases.

28. **Which of the dopamine agonists has higher chances of therapeutic success for the treatment of prolactinomas?**
    A. Bromocriptine
    B. Pergolide
    C. Cabergoline
    D. Octreotide
    E. Dopastatin

29. **For how long is long-term follow-up and monitoring after remission required in Cushing's disease?**

30. **Has the administration of opioids had an effect on the OT system?**

31. **Mutations in the *USP8* mutational hotspot can be found in:**
    A. Most pituitary tumor types
    B. Only in ACTH-secreting tumors of both pituitary and non-pituitary origin
    C. Only in corticotroph tumors

32. **What are the principal side effects of dopamine agonists?**
    A. Nausea
    B. Postural hypotension
    C. Mental fogginess
    D. Compulsive eating
    E. All the above

33. **Melanotrophs and corticotrophs can both synthesize POMC. However, the main end products derived from POMC are different in these cells. How can this phenomenon be explained?**

34. **How many genes are known to be mutated in >5% of sporadic pituitary tumors?**
    A. <5 genes
    B. Between 5 and 10 genes
    C. >10 genes

35. **In which situations would surgical management be considered as an option in the management of prolactinomas?**
    A. Dopamine agonist resistance
    B. Pituitary apoplexy
    C. Both answer A and answer B
    D. Old age
    E. Shrinking microadenoma

36. **What is the prevalence of TSH-omas with respect to all pituitary tumors?**
    A. 10%
    B. Below 3%
    C. 50%
    D. The same prevalence of GH-omas
    E. TSH-omas are the most frequent pituitary tumors

37. **What pituitary cells do TSH-omas arise from?**
    A. Somatotrophs
    B. Lactotrophs
    C. Thyrotropes
    D. Gonadotrophs
    E. Corticotropes

38. **When diagnosed, most TSH-oma have:**
    A. A diameter size <1 cm
    B. A diameter size <1 cm with invasive features
    C. A diameter size >1 cm
    D. A diameter size >1 cm with invasive features
    E. GH cosecretion

39. **Which are the classical signs and symptoms of TSH-omas?**
    A. Elevated thyroid hormones and elevated or inappropriately normal TSH levels
    B. If diagnosed as a silent adenoma, no clinical or biochemical evidence of central hyperthyroidism
    C. Mass pressure effects
    D. Answers a, b, and c
    E. None of the above

40. **Which parameters are useful for the differential diagnosis between TSH-oma and PRTH?**
    A. SHBG and ICTP levels
    B. FT4 levels
    C. Abnormal thyreotropin response to T3 suppression test
    D. Female gender
    E. All the above

41. **Which is the first-line treatment for TSH-omas?**
    A. Radiotherapy
    B. Medical therapy with somatostatin analogs
    C. Medical therapy with dopamine analogs
    D. Levo-T4
    E. Pituitary surgery via transsphenoidal route

42. **Which is the most sensitive and specific test to document the complete removal of a TSH-oma?**
    A. Positive T3 suppression test
    B. Normalization of alpha-GSU levels and alpha-GSU/TSH ratio
    C. Normalization of circulating TSH levels
    D. Normalization of free thyroid hormone levels
    E. Both answers A and D

43. **What percentage of anterior pituitary adenomas are nonfunctioning pituitary adenomas?**
    A. Lower that 10%
    B. Approximately 50–60%
    C. Approximately 30–40%
    D. Approximately 85%
    E. None of the above

44. **Which of the following hereditary syndromes may include nonfunctioning pituitary adenomas?**
    A. Multiple endocrine neoplasia type 1 (MEN1) and type 2
    B. Von Hippel–Lindau syndrome
    C. Mc–Cune Albright syndrome
    D. Multiple endocrine neoplasia type 1 (MEN1) and type 4
    E. *Paraganglioma syndrome type* 1

45. **Which of the following transcription factors are currently recommended in routine diagnostics of pituitary adenomas?**
    A. Pituitary transcription factor 1 (Pit-1) and steroidogenic factor 1 (SF-1)
    B. T-cell factor/lymphoid enhancer factor (TCF/LEF) transcription factors
    C. Runt-related transcription factor 2 (RUNX2)
    D. Guanine–adenine–thymine–adenine binding protein 2 (GATA) transcription factors
    E. Thyroid transcription factor **1 (TTF1)**

46. **What is true for null cell adenomas?**
    A. Are defined by the lack of immunohistochemical staining of any anterior pituitary hormone
    B. Represent the vast majority of nonfunctioning pituitary adenomas
    C. Are defined by the lack of immunohistochemical staining of any pituitary-specific transcription factors
    D. A and C
    E. They usually present with aggressive behavior

47. **Which of the genes that have been implicated in the pathophysiology of pituitary adenomas, have been more frequently described in nonfunctioning pituitary adenomas?**
    A. Aryl hydrocarbon receptor-interacting protein (AIP) gene
    B. Menin 1 gene (MEN1)
    C. Neurofibromatosis type 1 (NF1) gene
    D. Rearranged during transfection (RET) gene
    E. None of the above

48. **Which of the following symptoms are most frequently described in nonfunctioning pituitary adenomas**
    A. Cerebrospinal fluid rhinorrhea
    B. Obstructive hydrocephalus
    C. Pituitary apoplexy
    D. Headache and visual impairment
    E. All of the above

49. **Which of the following are considered criteria for the surgical treatment of nonfunctioning pituitary adenomas?**
    A. Age above 50
    B. Patient that are planning to become pregnant
    C. Visual field deficits at presentation
    D. Tumors larger than 10 mm
    E. Extension within the sphenoid sinus

14.2 Answers

1. The pituitary is a central gland in the organism that controls a plethora of biological processes important for growth, reproduction, metabolism, stress, etc.; therefore, pituitary cells act as metabolic sensors of the general status of the organisms in order to modulate pituitary secretions and maintain whole-body well-being. To do that, pituitary cells are able to sense and integrate multiple central and peripheral signals and respond by finely modulating pituitary secretions.
2. A.
3. In early-onset (<5-year-old) gigantism, X-linked acrogigantism should be strongly considered. McCune–Albright syndrome can also cause very young onset GH excess, and single case of *AIP* mutation-positive patient with age of onset of symptoms age 3 has also been described. Childhood-onset GH-secreting adenoma in MEN1 is very rare, but GHRH-secreting pancreas tumors have been described in children.
4. B.
5. D.

6. After excluding iatrogenic CS, it is recommended to test for CS patients if unusual features for age are present (osteoporosis, hypertension) or features suggestive of CS (easy bruising, proximal muscle weakness, reddish-purple striae), children with decreasing height percentile despite obesity, and patients with adrenal adenoma.
7. Somatotroph cells are able to sense different GH-modulating factors and integrate all these signals to adapt GH secretion to specific situations of needs. In this sense, it has been shown that GH-modulating factors do not always produce synergistic effects. This can be explained by the fact that many of these factors share several intracellular signaling pathways, which allows the somatotroph cells to integrate all the signals and coordinate their response to the extracellular milieu.
8. A.
9. In early-onset Cushing's disease (<2-year-old), *DICER1* mutation testing should be performed. Even if the penetrance of pituitary tumors is very low (1%), a pituitary blastoma could be a single and pathognomonic manifestation of DICER1 syndrome.
10. B.
11. B.
12. It is essential to measure ACTH (at least twice). Undetectable ACTH levels or <1.1 pmol/L suggest an adrenal etiology, and ACTH levels >4.4 pmol/L are suggestive of an ACTH-dependent CS. A pituitary MRI is mandatory to assess for a pituitary adenoma. If there is no visible tumor on the MRI or the lesion is <6mm, inferior petrosal sinus sampling is considered the gold standard test to identify a pituitary cause for CS. However, IPSS is not always available and noninvasive tests (high-dose DST, CRH, or desmopressin test) might be also useful.
13. It is well known that PRL may upregulate the expression of dopamine, its main negative regulator, triggering a negative feedback loop of its own production. Although this negative feedback loop has not been demonstrated for rest of the PRL regulators, different evidence suggests that this could be also the case for PACAP signaling and other potential factors.
14. D.
15. The association of Pit-NET and pheochromocytoma/paraganglioma is caused by germline mutation of *SDHx* and *MAX*. As MEN1 syndrome can be associated with pheochromocytoma, this must also be considered in 3Pa. Single cases of *RET* and *VHL* associations have been described, but it is unclear if these are coincidences or truly genetically related.
16. C.
17. E.
18. After excluding exogenous GC administration, it is recommended to use a combination of test with high diagnostic accuracy such as 24-h urinary free cortisol, late-night salivary cortisol, or 1 mg overnight dexamethasone suppression test. A second test should be repeated either UFC, LNSC, or DST to confirm hypercortisolism.

19. PRL secretion is strongly influenced by the metabolic status. The most obvious example is body weight. In fact, there is a clear association between obesity and PRL secretion since it has been reported that PRL levels are increased in plasma from obese patients as compared to those from individuals with normoweight. Indeed, these differences have been also observed between rapid gain weight women compared to stable weight women.
20. F.
21. Somatic *GNAS* mutation is one of the most common genetic causes for isolated somatotroph adenoma. Among sporadic corticotrophinomas, the most frequent cause is *USP8*.
22. C.
23. D.
24. Transsphenoidal selective tumor resection is the first-line treatment for CD. Successful tumor resection leads to low concentrations of ACTH and cortisol and therefore to remission. In case, transsphenoidal surgery cannot be done or is unsuccessful, medical therapy (steroidogenesis inhibitors, tumor-directed therapy, GC receptor antagonists), radiation, or bilateral adrenalectomy might be indicated.
25. Ghrelin/GHSR1a interaction has been shown to activate multiple signaling cascades. In the case of somatotroph and corticotroph cells, ghrelin can modulate phospholipase C (PLC), protein kinase C (PKC), PKA, intracellular and extracellular calcium, or MAPK pathways. However, the specific mechanisms involved in the regulation of gonadotrophs remain still unclear, although some reports have suggested that the ghrelin effects on gonadotropin secretion are mediated through nitric oxide, and calcium-dependent and cGMP-independent mechanism in rats.
26. B.
27. B, D.
28. C.
29. Cushing's disease patients should be follow-up life-long even if remission is obtained at the first therapy. Recurrences of the disease have been described even after 20 years of initial therapy. Although comorbidities improve after remission of hypercortisolism, most of them are not completely reversible; therefore, monitoring and treatment of glucocorticoid-related comorbidities are of great importance.
30. Chronic administration of morphine induced a significant decrease in OT immunoreactivity in the hippocampus, decreased OT mRNA levels within the SON, median eminence, and arcuate nucleus of the hypothalamus, and reduced

brain OT synthesis and plasma OT levels. This general downregulation of the oxytocinergic system following chronic opioid administration, in comparison with the acute stimulatory effects of opioid administration in different brain regions, may be a result of several neuroadaptive changes in the oxytocinergic system caused by chronic exposure to opioids.

31. C.
32. E.
33. **Although melanotroph and corticotroph cells produce POMC, the main end product in corticotrophs is** ACTH, while in melanotrophs are α-MSH, *β*-MSH, and γ-MSH. This phenomenon is the result of a differential posttranslational processing, inasmuch as these cell types express different patterns of proteolytic enzymes.
34. A.
35. C.
36. B.
37. C.
38. D.
39. D.
40. A.
41. E.
42. A.
43. C
44. D
45. A
46. D
47. A
48. D
49. C