# Chapter One

## General Introduction

## Organization of the Thesis

The **first chapter** consists of a general introduction and outline of the aims of the research undertaken and the structure of the dissertation.

### *Part One: Scientific Background*

This is followed in the **second chapter** by an introduction to the anterior pituitary gland (*adenohypophysis*) and describes the various cell types that are found.

In the **third chapter**, an outline of the types of pituitary adenomas that occur is presented, followed by an overview of the genetic patho-physiology of pituitary adenomas.

The **fourth chapter** contains a detailed description of the hereditary or familial forms of pituitary adenomas that are recognized to occur, along with their characteristic clinical and pathological features.

The **fifth chapter** is devoted to an overview of previous results in the literature regarding the epidemiology of pituitary adenomas as detailed in cancer databases, autopsy studies, radiological screening studies and a handful of population-based studies.
The results of the research undertaken are described in depth in the next five chapters.

### *Part Two: Personal Contribution*

In the **sixth chapter** the methodology and results of a cross-sectional epidemiological study of the prevalence of pituitary adenomas in the Province of Liège are described and discussed.

The **seventh chapter** presents the clinical description of a new familial pituitary tumor syndrome, termed familial isolated pituitary adenoma (FIPA), including details of patient characteristics, tumor types, and disease severity markers in 64 families.

Thereafter, the **eighth chapter** concerns a study of the role of mutations in the *aryl hydrocarbon receptor interacting protein* gene in the etiology and pathogenesis of FIPA among an international cohort of 73 families.

In the **ninth chapter**, a detailed study is presented regarding the genetic, pathological and clinical features seen in a large family with an *aryl hydrocarbon receptor interacting protein* gene mutation. This description presents the first evidence that endocrine conditions other than pituitary tumors may be found in FIPA families with *aryl hydrocarbon receptor interacting protein* gene mutations.

The minor role of germline and somatic *aryl hydrocarbon receptor interacting protein* gene mutations in the pathogenesis of non-FIPA pituitary adenomas is described in **chapter ten**.

A discussion of the impact of the results presented in the previous chapters is contained in **chapter eleven**, which summarizes the current knowledge in the field of the epidemiology and genetics of

pituitary tumors and suggests future directions for research.

Studies by the candidate, related to the epidemiology and genetics of pituitary adenomas, and supportive of the core studies contained in the thesis are included in the **Appendix**.

## Aims of the Research

To undertake the first comprehensive, cross-sectional study of the epidemiology of pituitary adenomas in a tightly defined region of a developed European country in order to determine the true prevalence of clinically apparent pituitary adenomas.

To collect and characterize for the first time kindreds exhibiting pituitary adenomas of all phenotypes in multiple family members in the absence of diseases known to be associated with an increased risk of pituitary tumors.

To compare the demographic, radiological and pathological characteristics of patients with the new clinical classification, familial isolated pituitary adenomas (FIPA), with those with non-inherited pituitary tumors.

To determine the role played by genetic risk factors in the etiology and pathogenesis of FIPA kindreds and in patients with non-familial sporadic adenomas.

# Chapter Two.

## The Anterior Pituitary Gland

### Embryology

The pituitary gland consists of two parts, the anterior (adeno-hypophysis) and posterior (neuro-hypophysis) divisions, which have distinct embryonic origins and discrete functional roles. The adenohypophysis is comprised of three sub-regions, the pars distalis, the pars intermedia and the pars tuberalis. It develops during embryonic life from Rathke's pouch, an endodermal region of the embryonic oral cavity. The posterior pituitary develops from the diencephalon as the median eminence, the neural stalk and the posterior lobe. The definitive structure of the pituitary forming the anterior lobe (pars distalis), pituitary stalk (neural stalk and pars tuberalis) and the posterior lobe begins to form during the second month of embryonic life. The development of the anterior pituitary is orchestrated by the activation of a series of transcription factors in a tightly regulated manner. This process begins with Rathke's pouch homeobox protein activation, which directs development of Rathke's pouch from the oral cavity ectoderm, aided by a number of other factors such as the LIM homeobox proteins and Prophet of Pit-1. Specific sub-populations of differentiated glandular anterior lobe cells develop initially from precursor stem cells. Somatotrope (growth hormone (GH) secreting), somatomammotrope (GH and prolactin secreting), lactotrope (prolactin secreting) and thyrotrope cells develop under the control of the transcription factor Pit-1 and subsequently differentiate into final distinct cell types under the control of stimulatory or repressor proteins. Gonadotropes (follicle stimulating hormone (FSH) and luteinising hormone (LH) secreting) and corticotropes (that secrete adreno-corticotropic hormone (ACTH) and melanocyte stimulating hormone (MSH) from pro-opiomelanocortin) differentiate from Rathke's pouch precursor cells under separate transcription factor cascades. Pituitary cells are functional by the end of the second month of gestation.

### Anatomy

The adult pituitary measures approximately 13 mm across by 6 mm high and is 9 mm in antero-posterior depth. In adults the pituitary weighs from 0.5-1.0 g, the gland being larger in younger individuals and in females. Anatomically the pituitary sits in a region of the skull base called the hypophyseal fossa of the sella turcica ("Turkish saddle"). Inferolaterally the pituitary is bounded by sphenoid bone and the sphenoid sinus; the pituitary is covered superiorly by dura mater (the diaphragma sellae), through which the pituitary stalk passes. Superiorly lies the optic chiasm, while the lateral walls of the sella turcica are formed by the cavernous sinuses, which contain the internal carotid artery, and cranial nerves III, IV, $V_1$, $V_2$, and VI.
Anatomically, the anterior pituitary consists of zones of glandular epithelium that are served by a complex sinusoidal vascular network. The blood supply of the

anterior pituitary provides the means by which regulatory factors from the hypothalamus can modulate the secretion of anterior pituitary hormones, which in turn are secreted into the systemic circulation. The hypophyseal arteries feed the median eminence and pituitary stalk and then develop into long portal vessels that terminate as a plexus abutting the anterior pituitary glandular epithelium. Short portal vessels from the posterior lobe also feed the anterior lobe. The long portal vessels supply three quarters of the blood to the anterior lobe and the short portal system the other quarter. Anterior pituitary hormones are secreted into a venous system that drains into the internal jugular vein.

## Pituitary glandular cells

The constitutive cells of the anterior pituitary can be classified functionally by hormone production or histologically according to staining properties. Traditional histological classification dates from late nineteenth century and is derived from the appearance of pituitary cells following staining on light microscopy. On that basis, cells were called "acidophilic", "basophilic" or "chromophobic", and tumors of the anterior pituitary followed this classification until the identification of individual hormones in the mid-twentieth century. Cells are now named functionally based on their respective hormonal production characteristics.

### *Somatotropes*

Somatotropes produce growth hormone (GH), which regulates growth and metabolism directly and via the stimulation of insulin-like growth factor I (IGF-I) by the liver and other target organs. Somatotropes are acidophilic calls and constitute about half of anterior pituitary cells. They occupy a lateral position within the gland, are of medium size and display a large amount of rough endoplasmic reticulum and Golgi apparatus as befits their secretory potential. Large GH positive secretory granules (commonly dense) are prominent.

### *Lactotropes*

Lactotropes (sometimes termed mammotropes) secrete prolactin, the physiological role of which is to stimulate breast development and lactation in females. Lactotropes constitute a widely varying proportion of the anterior pituitary cell population, which is sexually dimorphic (10% in males, 30% in pregnant females). They are located throughout the anterior lobe but are concentrated in the pars nervosa (postero-laterally). Structurally, lactotropes can be sparsely or densely granulated, and have varied size and morphology which is particularly marked in pregnancy. Lactotropes are typically chromophobic.

### *Somatomammotropes*

A subset of cells generate and secrete both GH and prolactin and are hence termed somatomammotropes. They share ultrastructural and secretory features of lactotropes and may have larger sized granules than somatotropes.

### *Corticotropes*

Corticotropes comprise around 15% of cells of the anterior pituitary and are centrally located in the gland. These cells produce proopio-

melanocortin (POMC), which is a precursor that is cleaved to form ACTH, MSH, β-lipotropin and β-endorphin. The physiological function of ACTH is to stimulate cortisol production and secretion by the adrenal cortex, while MSH stimulates melanin production by melanocytes; β-lipotropin and β-endorphin play roles in lipid energy homeostasis and pain modulation, respectively. Corticotrope secretory granules are highly variable in morphology. Corticotropes are basophilic cells.

### *Gonadotropes*

Gonadotropes comprise about 10% of the anterior pituitary cells, and are scattered throughout the anterior lobe. These round cells contain small secretory granules; gonadotropes can produce LH, FSH or both hormones together.

LH functions to stimulate testosterone secretion by Leydig cells in males and a surge in LH stimulates ovulation in the female. FSH plays a crucial role in the maturation of gametes in males and females. Gonadotrope cell size can vary with circulating sex hormone levels and enlarge after the menopause.

### *Thyrotropes*

Thyrotropes are the least common secretory cell of the anterior pituitary (5% of anterior lobe cells) and stimulate the production of thyroid hormones by the thyroid gland. Thyrotropes are located in relatively well-defined area of the anteromedial portion of the anterior lobe. Secretory granules are small and thyrotropes can change in morphology depending on thyroid hormone status; in hypothyroidism thyrotropes demonstrate increased prominence of the Golgi apparatus and rough endoplasmic reticulum.

# Chapter Three

## Adenomas of the Anterior Pituitary

### Introduction

Multiple pathological conditions can affect the anterior pituitary leading to a disruption of function, which can take the form of increased or decreased hormone production or symptoms due to impingement of an abnormally sized pituitary on local neurological, vascular or bony structures. Tumoral pathology of the anterior pituitary consists overwhelmingly of benign adenomas of the glandular epithelium; malignant transformation of pituitary epithelium as a pituitary carcinoma is extremely rare. Anterior pituitary adenomas, by virtue of their origin in secretory glandular epithelium, often demonstrate positivity for one or more pituitary hormones on pathological analysis (e.g. immunohistochemistry). Anterior pituitary adenomas may also retain the ability to secrete hormones, which can lead to typical clinical syndromes such as excessive secretion of GH in acromegaly or ACTH in Cushing's disease. However, a proportion of anterior pituitary adenomas do not secrete intact or functional pituitary hormones. These tumors, termed non-secreting (NS) or non-functioning pituitary adenomas tend to manifest due to growth and impingement on healthy pituitary, leading to hormonal hyposecretion (hypopituitarism) or pressure on local structures like the optic chiasm. Pituitary adenomas have been classified in a variety of different ways, including by tumor size/extent, the hormone(s) secreted, the histological staining characteristics, the ultrastructural elements, the immunohistochemical profile and the molecular genetic features. The practical classification of anterior pituitary adenomas in the clinical research setting requires aspects of all of these systems, which are described concisely below.

### Classification

#### *Radiological classification*

Neuroradiological studies using magnetic resonance imaging (MRI), often with gadolinium enhancement, are used to detect or confirm the existence of a pituitary adenoma. The MRI images are also used to assess the size of the pituitary adenoma, its extension to sites outside of the sella turcica, the relationship to nearby structures (sinuses, cranial nerves and blood vessels) and the presence of invasion of other tissues by the adenoma.

The classification system for pituitary adenomas using neuroradiological images and surgical findings is relatively straightforward. Pituitary tumours are initially classified into those <10 mm in maximum diameter (microadenomas) and those ≥10mm in diameter (macroadenomas). A third classification, namely "giant pituitary adenomas" is sometimes used for rare cases in which the tumour has a maximum diameter of >40 mm. A more detailed

classification was developed by Hardy and colleagues using a combination of radiological and surgical data. In their system, sella turcica involvement was used as a reference point to grade pituitary tumors.

MRI classification is clinically relevant as characteristics such as tumor size (microadenoma versus macroadenoma), extension and invasion of local structures are predictive of the success of therapeutic outcomes (e.g. transsphenoidal neurosurgery).

**Table 1. Radiological-operative classification of pituitary adenomas**

| |
|---|
| Grade 0: enclosed adenoma with normal intact sella |
| Grade 1: enclosed (intrasellar) microadenoma with minor bulging of the sella |
| Grade 2: enclosed macroadenoma, generalised sellar enlargement |
| Grade 3: macroadenoma, focal sellar invasion |
| Grade 4: macroadenoma, generalised sellar invasion |
| |
| Larger or more extensive tumours beyond grade 4 are sub-classified as follows: |
| |
| *Suprasellar or symmetrical extension* |
| A. 10mm: fills the chiasmatic cistern. |
| B. 20 mm: lifts the recesses of the third ventricle |
| C. >30 mm: fills the anterior third ventricle |
| |
| *Parasellar or asymmetrical extension* |
| D. Extends intracranially |
| E. Lateral extension outward from cavernous sinus |

## Clinicopathological classification [1, 2, 3]

Pituitary adenomas are commonly classified in terms of their hormonal secretion patterns, which can vary across a spectrum from discrete hypersecretion of a single hormone to multiple hormonal hypersecretion. A sizeable proportion of pituitary adenomas are non-secreting or non-functioning pituitary adenomas (NFPA) which can be entirely hormonally inactive, or secrete inactive hormone subunits or may synthesize but not secrete intact hormone. As they grow, NFPA can impinge on normal pituitary cells and gradually impair hormonal function leading to varying degrees of pituitary hormone insufficiency, termed hypopituitarism.

### Secreting pituitary adenomas

#### *Prolactinoma*

Prolactinomas derive from the lactotrope cell population and lead to increased levels of circulating prolactin. The biological consequences of hyperprolactinemia from a prolactinoma are manifested as disturbances in fertility, the breast and sex hormones in both sexes. Prolactinomas cause oligomenorrhea or amenorrhea in premenopausal females due to inhibition of estrogen. This may give rise to an earlier diagnosis in younger women than in males, who more typically suffer more non-specific symptoms like loss of libido or impotence due to decreased testosterone. In males this later diagnosis may explain why tumors tend to be larger than in females. Both sexes may suffer from the highly suggestive sign of galactorrhea, which is more frequent in females.

Prolactin secretion is mainly under the negative control of dopamine, with physiological dopamine stimulation leading to inhibition of prolactin. Symptomatic hyperprolactinemia (both tumoral and non-tumoral) is managed pharmacologically using oral dopamine agonists as first-line therapy. Dopamine agonist therapy can also produce shrinkage of the prolactinoma, an effect which is caused by a reduction in size of individual cells. In cases of large or invasive prolactinomas that do not exhibit sufficient shrinkage on dopamine agonist treatment, transsphenoidal neurosurgery to debulk or remove the tumor is an option. Radiotherapy is used only in rare cases of aggressive prolactinomas that are not amenable to surgical resection. Prolactinomas can be exquisitely sensitive to dopamine agonist therapy, and in large tumors there can be a danger of infarction if too high a dose of dopamine agonist is used at the outset; hence dopamine agonist therapy is begun at a low dose and titrated up gradually.

Prolactinomas generally appear in the posterolateral region of the anterior pituitary, where lactotropes are concentrated. On light microscopy, prolactinomas may have a papillary or fibrotic appearance, with large tumor cells being delineated by an indistinct cell membrane and containing irregularly shaped nuclei. Electron microscopic study shows prolactinomas to consist of sparsely granulated and densely granulated types. The former is more common and is consistent with the classical chromophobic staining pattern. Densely granulated prolactinomas

are uncommon and in this type cells are reported to be polyhedral with oval nuclei. Prolactinoma cell may extrude secretory granules laterally, a distinctive feature known as misplaced exocytosis. Another particular structural feature of note in sparsely granulated prolactinomas is the presence of elaborate whorls of rough endoplasmic reticulum called *Nebenkerns*. The granules in prolactinomas are generally about 200 nm in diameter.

### *Somatotropinoma* [4]

Somatotropinomas are derived from somatotrope cells and are associated with excess secretion of GH, which in turn stimulates elevated peripheral IGF-I secretion. Such tumors lead to increased somatic growth, which manifests as tall stature or gigantism in younger individuals that have not undergone epiphyseal fusion of their skeleton. In adults, somatotropinomas lead to acromegaly, which is typified by overgrowth of the mandible, the frontal bones of the skull, the extremities and is associated with widespread organomegaly and metabolic disturbances. Acromegaly has a gradual onset and patients typically suffer from signs and symptoms of the disease for a period of seven years before a diagnosis is made. The long-term effects of elevated GH on the cardiovascular system, particularly the heart, leads to cardiomyopathy, which may play a role in the increased mortality seen in acromegaly. Treatment of acromegaly is often multi-modal in nature. Transsphenoidal neurosurgical resection is the mainstay of patient management, and in cases of microadenomas and uncomplicated macroadenomas, cure can be achieved by surgical resection of the tumor. However, many tumors are macroadenomas, and while they may benefit from surgical intervention, medical therapy using somatostatin analogs is frequently necessary. Somatostatin analogs like octreotide or lanreotide target the somatostatin receptor subtype 2 that is predominantly expressed on the somatotrope cell membrane surface, thereby reducing GH secretion. Dopamine agonists may have some effect in acromegaly, particularly in patients that have tumors that co-secrete GH and prolactin. In patients with inadequate responses to other forms of treatment, pegvisomant a pegylated adapted form of GH that antagonizes the GH receptor, can be useful. Radiotherapy may be useful in very aggressive cases that do not respond adequately to the above surgical and medical options. Somatotropinomas can display a variety of morphological features. While all types are immunohistochemically positive for GH staining, five different pathological subtypes have been described. Somatotropinomas may be densely or sparsely granulated. Densely granulated adenomas have acidophilic cytoplasm on classical staining and the cells are polyhedral and medium sized with round nuclei; these tumors are quite vascular in nature. The granules are numerous and round/fusiform in shape and range from 350-500 nm in size. Sparsely granulated somatotropinomas are, in contrast, chromophobic on classical staining and the cells are variable in size with irregular single or multiple nuclei. These

tumors have fibrotic inclusion bodies in the cytoplasm. The secretory granules in sparsely granulated somatotropinomas are small at 200 nm. Mixed GH and prolactin cell adenomas consist of two cell types and thus have cellular patterns and features of both somatotrope and lactotrope cell lines. Somatomammotrope tumors are derived from the somatomammotrope cell population, in which the cells produce both GH and prolactin. There are two granule types, one of 400 nm in diameter and round in shape and larger, variably-sized granules that are generally 1200-1500 nm in diameter. It is noted that extruded secretory granules can be seen intracellularly, a feature that assists in pathological classification. Acidophil stem cell adenomas are generally strongly prolactin positive with weaker GH staining. These cells contain large vacuoles and mitochondria which may aid in the diagnosis.

### *Corticotropinoma*

Corticotropinomas are associated with excess production of ACTH and related hormones and peptides from POMC, although significant hormonal abnormalities and clinical effects are due almost entirely to ACTH-related effects. Cushing's disease is caused by a corticotropinoma and is associated with hypersecretion of glucocorticoids (cortisol) from the adrenal glands. Patients suffer from the end-organ effects of hypercortisolism, which include hypertension, altered body habitus and composition, skin changed (thinning and hyperpigmented striae), osteoporosis, psychiatric problems and impaired carbohydrate metabolism. Cushing's disease, if not adequately treated, is associated with increased mortality. A condition known as Nelson's syndrome can occur after bilateral adrenalectomy for the treatment of endogenous hypercortisolism in which a corticotropinoma has not been identified. As corticotropinomas are usually microadenomas, this was a more frequent occurrence in the pre-MRI era. In Nelson's syndrome the removal of the adrenals leads to expansion of the corticotropinoma, which can be massive in nature. This is thought to be due to a loss of feedback inhibition of the corticotropes. Hyperpigmentation occurs in Nelson's syndrome due to hypersecretion of MSH, leading to activation of melanocyte activity in the skin. Corticotropinomas are treated neurosurgically as first-line therapy. Current medical therapies are poorly effective and radiotherapy may be necessary in some resistant cases.

Corticotropinomas occur in the central anterior pituitary and as mentioned above, are most often microadenomas. Indeed, corticotropinomas can frequently be very difficult to distinguish from the surrounding normal tissue. The constituent cells are medium to large in size, of uniform shape and are often centered around blood vessels. Corticotropinoma cells have a basophilic cytoplasm and can be either densely (200-500 nm diameter) or sparsely (200 nm diameter) granulated. An interesting feature of corticotropinomas is the presence of changes in corticotropes not involved with the tumor, in which a glassy cytoplasmic change called Crooke's hyaline is seen; this can aid the pathological diagnosis. Rarely,

ACTH-positive but clinically silent pituitary tumors can occur; these silent corticotropinomas may stain weakly positive for ACTH but not secrete intact hormone at levels sufficient to produce clinical features of hypercortisolism.

### *Gonadotropinoma*

Gonadotropinomas are derived from gonadotrope cells that occur throughout the anterior lobe. In contrast to the other anterior pituitary adenoma types described above, the clinical features of gonadotropinomas are much less tied to hormonal hypersecretion. A significant proportion of non-functioning pituitary adenomas stain positively for LH or FSH. When functional, hypersecretion of LH/FSH may lead to disturbance of reproductive organ function. Gonadotropinomas tend to be large in size and frequently have extension and impingement upon or invasion of local structures. Treatment is neurosurgical and recurrence is common. In some cases treatment with dopamine agonists or somatostatin analogs can have some efficacy, while radiotherapy may be required for aggressive and resistant cases.
Pathologically, small to medium sized gonadotropinoma cells are organised commonly into rosette formations around a central capillary and the cytoplasm is usually chromophobic. Granules are smaller in males than in females (50-150 nm versus up to 300 nm, respectively), are infrequent and are more strongly positive for FSH than LH. Gonadotropinoma cells in females are closer in appearance to normal non-adenomatous cell than in males, which are more poorly differentiated.

### *Thyrotropinoma*

Thyrotropinomas are extremely rare tumors, with the two largest published series accounting for 52 and 43 cases each [5, 6]. Thyrotropinomas are usually macroadenomas and local invasion is frequently seen. As such, these tumors present with a combination of hyperthyroidism in the presence of an elevated TSH/glycoprotein α-subunit, which may also be accompanied by symptoms due to local invasion or expansion of the tumor. Thyrotropinoma cells are usually chromophobic on classical staining. TSH positive secretory granules are found close to the cell membrane and are infrequent and small in size.

### *Pituitary adenomas secreting multiple hormones*

Pituitary adenomas that exhibit multiple pituitary hormones are termed plurihormonal adenomas. Plurihormonal adenomas that secrete multiple intact functional hormones are rare; more frequently, such tumors will exhibit immunohistochemical evidence of a multiplicity of pituitary hormones. Their clinical presentation is dependent on whether hormonal hypersecretion sufficient to produce clinical features occurs. In the case of tumors that are positive on staining for multiple hormones but are non-secreting, presentation is more likely due to impingement of local structures when the tumor becomes a macroadenoma. Most commonly these tumors will co-secrete or synthesize TSH, GH and FSH, although other rarer patterns

such as prolactin and LH have been reported [7]. Somatomammotropinomas and mixed GH and prolactin secreting adenomas, while technically plurihormonal adenomas, are considerably more common than other types and are noted above.

### *Non-secreting adenomas*

Pituitary adenomas that do not synthesize or synthesize but do not secrete functional hormones are generally classified as non-secreting (NS) or non-functioning pituitary adenomas (NFPA). They can arise as adenomatous growth from a variety of anterior pituitary cell types. Due to their lack of signs and symptoms linked to hormonal hypersecretion, NS-adenomas tend to present in older patients and cause hypopituitarism and symptoms due to tumor expansion (visual disturbances, headache).

Null cell adenomas are the simplest form of NS adenoma, in that they entirely lack evidence of hormonal synthesis or staining. Null cell adenomas typify the presentation of NS adenomas in that they present predominantly in elderly patients with hypopituitarism and local symptoms. Pathologically, null cell adenomas display frequent cystic or hemorrhagic regions. They are chromophobic or oncocytic in classification. Pituitary oncocytomas are characterized by the presence of large aggregations of mitochondria that can comprise up to half of the cytoplasmic volume.

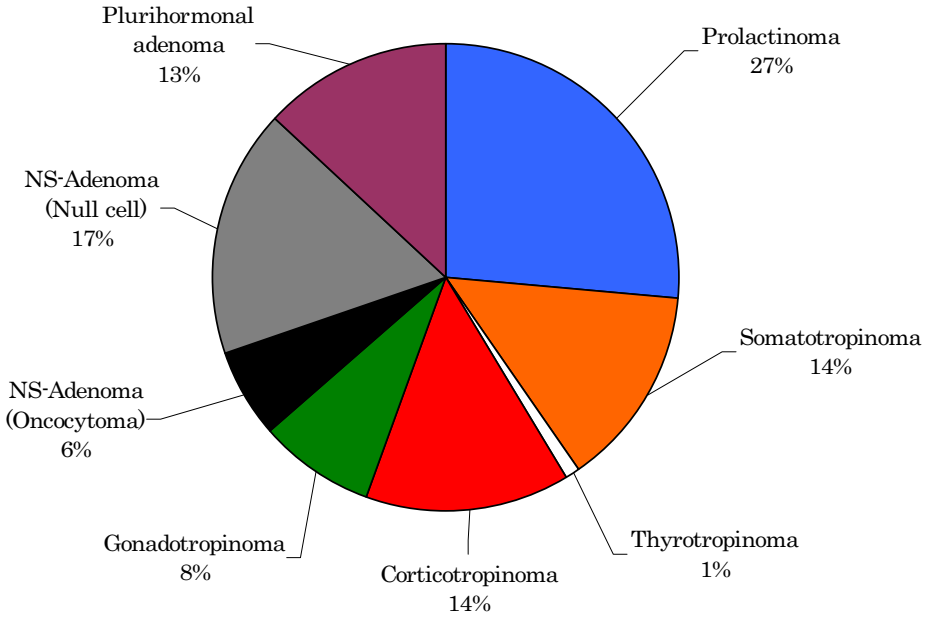


**Figure 1. Pituitary adenoma sub-type frequencies according to histological subtypes** [1, 2, 3]

## Genetic Pathophysiology of Pituitary Adenomas

It is now widely accepted that most pituitary adenomas arise as a clonal expansion from a single mutated anterior pituitary cell. Previously an alternate theory suggested that pituitary adenomas arose due to stimulation by factors from the hypothalamus. This latter theory has less evidential support due to molecular genetic advances that have identified a wide variety of genetic mutations and subsequent molecular alterations in adenomatous pituitary tissue. The clonal nature of pituitary adenomas is supported by a series of methodologies, among these being X-chromosome linked genetic inactivation patterns [8,9,10]. However this is not to say that the process is entirely straightforward at the tissue level, as a single pituitary can contain multiple tumors or hyperplastic areas, each with its own clonal origin, each with their own specific pattern of growth, apoptosis and pathological features.

The final development of a pituitary adenoma is also dependent on the activation or inactivation of a variety of tumor suppressor genes and oncogenes (Table 2). Among the oncogenes the most important for sporadic pituitary tumorigenesis is *gsp*, which encodes the Gsα subunit, a stimulatory guanine binding protein that regulates hypothalamic GH-releasing hormone (GHRH) effects in somatotropes. Biallelic expression of mutated gsp can lead to endogenous activation of adenylate cyclase and elevated levels of cyclic adenosine monophosphate (cAMP). Mutations in *gsp* have been most closely associated with somatotropinomas, and they are found to occur in up to 40% of these tumors. The oncogene *ras* has also been implicated in pituitary tumorigenesis, although in a very small number of cases. Mutations in *ras* appear to be associated with high levels of tumor aggression and have been noted to occur among rare pituitary carcinomas [11, 12]. Pituitary tumor transforming gene (PTTG) is a gene that is usually poorly expressed in normal pituitary, but has been reported as being upregulated in most pituitary tumor types [13, 14].

In addition to oncogenes, mutations in tumor suppressor genes have been identified in the setting of pituitary adenomas and tumorigenesis. The best known among these is the gene *MEN1* that is responsible for multiple endocrine neoplasia type 1 (MEN1) and which is discussed in detail in a later chapter. Retinoblastoma gene (*Rb*) is a classical tumor suppressor gene that plays an important role in tumorigenesis in a variety of tissues. Pituitary adenomas develop in mice that are heterozygous carriers of certain *Rb* mutations [15]. In humans the role of *Rb* is less certain; Rb is lost in tumor tissue in few pituitary adenomas, although *Rb* promoter hypermethylation has been reported [16, 17].

Cell cycle regulators have also been implicated in pituitary tumorigenesis and development. For instance, cyclin D1, which regulates the transition from $G_1$ to S-phase is overexpressed in nearly 70% NS-pituitary adenomas and about 40% of somatotropinomas. *CCND1* genotypes are related to tumor grades seen in pituitary

adenomas [18, 19]. Much oncology research has been devoted to cyclin-dependent kinase inhibitors, and their role in the regulation of the cell cycle in tumor tissue. In the case of pituitary adenomas, the cyclin-dependent kinase inhibitor p16 is heavily downregulated due to gene promoter hypermethylation [20, 21]. Another cyclin dependent kinase inhibitor, $p27^{kip1}$ appears to play an important role in pituitary tumorigenesis, as evidenced by data from a knockout mouse that show the development of specific patterns of pituitary adenomas and other abnormalities [22]. More recently mutations in the *CDKN1B* gene that encodes $p27^{kip1}$ have been shown to be associated with the rare occurrence of familial and sporadic endocrine cancers, including pituitary adenomas; this is discussed in more detail in a later chapter.

The protein ZAC (standing for zinc finger protein inducing apoptosis and cell cycle arrest) is normally expressed at high levels in healthy pituitary tissue. In pituitary adenomas (predominantly non-secreting tumors), ZAC expression is strongly reduced. The somatostatin analog, octreotide, may function in somatotropinomas in part via ZAC as it increases the expression of the gene *Zac1* [23, 24]. Fibroblast growth factor receptors (FGFR) play a role in the growth and development of many tissues. A specific truncated pituitary tumor-derived form of FGFR4 has been identified in humans and was reported be associated with invasive pituitary tumorigenesis in a transgenic mouse model [25].

MEG3, appears to play a role as a potential growth suppressor in pituitary tissue; a pituitary-derived variant is absent from NS-adenomas and functioning aden-omas, possibly due to pro-moter hypermethylation [26, 27]. Compared with normal pituitary, expression of the *growth arrest and DNA damage-inducible gene* (GADD45G) is decreased in somatotropinomas, prolactinomas and NS-adenomas [28]. Other factors such as bone morphogenetic protein-4, which may indirectly stimulate c-myc expression, is overexpressed in prolactinomas as compared with other tissues [29].

| Gene | Defect |
|---|---|
| Cyclin D1 | Overexpression in non-secreting adenomas and somatotropinomas |
| Gsp | Somatic activating mutations in up to 40% of somatotropinomas<br>Mosaicism in McCune–Albright syndrome (somatotropinoma, somatomammotropinoma, and Cushing's syndrome in association with precocious puberty, hyperthyroidism, and dermal and bony lesions), |
| PRKAR1A | Truncation mutations in Carney's complex leading to somatolactotrope hyperplasia and adenomas |
| Pdt-FGFR4 | Alternative transcription initiation in pituitary adenomas |
| PTTG | Increased expression in more aggressive pituitary tumors |
| BMP-4 | Diminished expression in prolactinoma |
| GADD45G | Promoter methylation in non-secreting adenomas, prolactinomas, and somatotropinomas |
| MEG3a | Promoter methylation in non-secreting adenomas and gonadotropinomas |
| MEN1 | Inactivating mutations in all pituitary adenoma types |
| PKC | Point mutations in invasive pituitary adenomas |
| p16 | Promoter methylation in pituitary adenomas |
| CDKN1B (p27Kip1) | Germline heterozygous nonsense mutation in MEN4, a novel, rare MEN1-like syndrome |
| Retinoblastoma | Promoter methylation in pituitary adenomas |
| ZAC | Promoter methylation in non-functioning adenomas |
| AIP | Germline mutations and loss of heterozygosity in 15% of FIPA cases. Seen in familial/sporadic somatotropinomas, somatolactotrope adenomas, prolactinomas, non-secreting adenomas, and Cushing's disease (sporadic only) |

**Table 2. Germline and somatic genetic abnormalities associated with pituitary adenomas**

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# Chapter Four.

## Familial Pituitary Tumor Syndromes

### Introduction

Familial or inherited pituitary adenomas are uncommon and occur in the setting of a number of tumor syndromes. Among these, multiple endocrine neoplasia type 1 (MEN1) and Carney complex (CNC) are quite well defined pathologically. Another inherited tumor syndrome, multiple endocrine neoplasia type 4 (MEN4, also known as MENX), has been described recently in one family and one unrelated subject and is at an early stage of characterization. Finally, the rare occurrence of acromegaly-gigantism in families has been reported in the lay press for hundreds of years, and more recently has been codified as isolated familial acromegaly.

Pituitary tumors that occur in a familial setting due to MEN1, CNC or familial acromegaly account for a minority of pituitary tumors. Scheithauer et al estimated that 2.7% of pituitary adenomas were due to MEN1 [1], while experience from the Departments of Endocrinology and Neurosurgery at the CHU de Liège, suggests that a further 2% of pituitary tumor cases have family links. In studies described in following chapters, it has now been recognized that pituitary tumors of all types can occur in a familial setting, termed familial isolated pituitary adenomas (FIPA), and are not limited to the phenotype of acromegaly. It is useful to recapitulate the history and current state of understanding of the clinical features and molecular pathology of other familial pituitary tumor syndromes, MEN1, CNC, MEN4 and familial acromegaly.

### Historical Background

The occurrence of tumors in a syndromic setting has always piqued the interest of researchers due to the increased potential for discovering a common pathophysiological explanation. The endocrine arena is no exception, and in some ways can be considered to be a pioneering specialty in this regard. As most endocrine tumors are relatively slow-growing adenomas and have quite typical associated clinical features, has meant that before the advent of modern molecular genetic methods, endocrine tumor syndromes were described.

A common thread among the various endocrine tumor syndromes described to date is that a comprehensive clinical description is a vital first step. In the past, scientific communications were more limited than today and the accrual of cases and families with common clinico-pathological features was slow. In particular the *familial* occurrence of endocrine tumor syndromes permits greater focus for research into genetic pathophysiology. In 1903, Erdheim described a case of adenomas in the parathyroid and pituitary [2] that is typical of what would later be described as Wermer's syndrome [3], eventually becoming MEN1. Indeed, in his original 1954 description of multiple endocrine adenomas, Wermer noted that "*Proof of the genetic* (inherited) *character of a disease lies in its familial occurrence.*" In his study Wermer described a family with four sisters affected with pituitary

adenomas (one had acromegaly), hypercalcemia, and adenomatosis of the pancreas and gut. Further investigation revealed that the father had evidence of multiple pancreatic and gut islet cell tumors on autopsy (he had died of peritonitis and had a past history of a gastric resection for a perforated ulcer). After carefully reviewing the familial case and the more than a dozen isolated cases described over previous decades in the literature, Wermer correctly posited a autosomal dominant mode of genetic inheritance for this condition, outruling the potential for serendipitous or environmental causes. As described below, such a description has been shown to hold up well even under intense molecular genetic scrutiny more than 50 years later.

In the case of other endocrine tumor syndromes that are related to the pituitary, a similar path has been followed consisting of painstaking clinico-pathological study, case accrual, leading to clinical characterization and the eventual identification of a molecular genetic cause. In relation to pituitary tumors, this pathway is exemplified by the work of J. Aidan Carney at the Mayo Clinic, which led to his description in 1985 of a complex of myxomas, spotty pigmentation, and endocrine overactivity that included four (of a total of 40 cases) with pituitary adenomas causing acromegaly or gigantism [4]. Similar progress has led to the identification of other non-pituitary endocrine-related tumors syndromes, such as Carney-Stratakis syndrome [5] and the Carney triad [6].

Focussing on the pituitary alone, familial links in certain cases have been suggested for over a century. While it is not possible to outrule the involvement of conditions described above (MEN1/CNC) in the genetic pathophysiology of familial pituitary tumors, some reported cases lack any evidence of multiple endocrine organ involvement. In essence, a familial form of isolated pituitary adenomas appears to have been in evidence for some time. The type of pituitary tumors involved in these early familial cases appears to be limited to somatotropinomas, particularly acrogigantism. One controversial description is Friedreich's report of the brothers William and Carl Hagner, which was published in 1867 [7], nearly 20 years before the seminal report on acromegaly by Marie. This study is focussed on a description of skeletal changes that involved widening of the hands and feet, but also with involvement of the knees and other more proximal joints. The face of one of the brothers that is illustrated in detail by Friedreich is not suggestive of acromegaly and it may be that these brothers suffered a skeletal disorder rather than acromegaly

The interest in reporting cases of acrogigantism in the medical literature in the late 19th and early 20th centuries was quite intense. In 1908, Dr. VC Thomas, writing about the disease in the California State Journal of Medicine [8] remarked that "*No disease of such comparative rarity has attracted such universal attention of the most able and eminent men in the profession as acromegaly."* In the years preceding the definition of acromegaly as a disease entity, reports of individuals with gigantism were common in the lay press. Interest in the extremes of stature has always existed, with both individuals with dwarfism and gigantism meeting curiosity and attention, often not entirely benign [9]. Reviewing the reports and descriptions of the experiences of

people with gigantism, scattered cases that suggest familial links can be found. For instance the Knipe brothers were identical twins from Ireland born in 1761 and they appeared in London in 1785 when they were both reported to have a height equivalent to 218 cm. The Hugo brothers represent one of the best recorded early cases of familial pituitary tumors, and were the subjects of many photographs and exhibitions in early years of the 20th century. In one of the brothers, an autopsy demonstrated a pituitary adenoma measuring 5.0 x 2.5 x 2.3 cm which extended in suprasellar and parasellar directions. In 1925, Bailey and Davidoff reported a landmark study of clinical and surgical pathological findings in 35 patients with acromegaly treated by Harvey Cushing at the Peter Bent Brigham Hospital in Boston, U.S.A.[10] In the description of one of the patients (Case III), a 25-year-old man who was referred in 1914, it was noted that the patient had striking acromegalic features that had begun to develop at 16 years of age. Regarding the patient's family history, Bailey and Davidoff noted "*He came of a family of tall people. … His paternal great uncle was 7 feet 1 1/2 inches tall* (217 cm) *and was exhibited in a traveling circus under the title of the "Kentucky giant.*" As was the case with "Irish giants" in Great Britain, there were a number of individuals in the United States to whom the "Kentucky giant" moniker was attached over the years. Among the potential identities of the great-uncle is Captain Smith Cook, from the same region as Cushing's patient, who was well-known in his time [11], appearing internationally at circuses; later in Kansas City, Missouri, he was the tallest policeman in the United States [12].

The clinical description and definition of acromegaly by Marie in 1886 led to a flood of cases being reported. By the early years of the 20th century, well over a thousand cases of acromegaly were in the medical literature . Among these one can find some of the first scientific descriptions of familial pituitary adenomas. In 1897 Maximillian Sternberg of Vienna reviewed the field of acromegaly thoroughly (his monograph was later translated into English in 1899 and met with great success) [13]. Sternberg noted the previous reports of familial cases of acromegaly from Bonardi [14] and Schwoner [15]. The latter involved a parent-sibling pair affected with acromegaly. In a later 1901 publication, Fraenkel et al described the case of Herr Gleiche a 50-year-old patient who had been seen by them for the first time in late 1898, due to physical features and symptoms of acromegaly [16]. They noted a very strong family history, with the patient's father, brother and sister all exhibiting similar acromegalic features (although a history of gastric cancer in affected individuals means that MEN1 cannot be outruled). In addition to noting the cases reported by Sternberg, Fraenkel et al describe a family reported by Bregmann that involved a 44-year-old patient with acromegaly, the patient's 42 year old sister and also a potentially affected child.

Given the typical features of acromegaly and their familiarity among the medical profession, it is not surprising that familial cases of acromegaly would come to light in advance of other pituitary tumors. A review of early literature shows no cases of familial pituitary tumors outside of acrogigantism [17], [18], even in the setting of Cushing's disease

which was also well publicized and is associated with a marked clinical phenotype. Indeed, even specific studies on the genealogy of acromegaly up to 1950 did not advance greatly the understanding of familial pituitary tumors [19]. It was not until the above-mentioned work of Wermer and related authors on what would become MEN1 that the understanding of the familial occurrence of pituitary tumors would begin to advance again.

## MEN1

MEN1 syndrome is an autosomal dominant condition that is associated with the occurrence of endocrine-active parathyroid, enteropancreatic and anterior pituitary tumors, among others [20]. Endocrine-inactive tumors, such as, lipomas, angiofibromas and collagenomas are a frequent finding in MEN1. In 1988, Larsson et al first linked the gene involved in MEN1 to a locus on chromosome 11q13 [21], and the *MEN1* gene was cloned by Chandrasekharappa et al in 1997 [22]. The *MEN1* gene has 10 exons of which exons 2 to 10 encode nuclear protein, menin [23]. The *MEN1* gene has a complex upstream promoter apparatus, elements of which are regulated by menin activity; this echoes the known interactions of menin itself with the transcription of endocrine gene promoters [24, 25, 26, 27]. Differential regulation of menin expression in different tissues via upstream genetic elements may explain in part how mutations of *MEN1* preferentially involve cells of the endocrine system, despite the fact that menin is also expressed in a variety of non-endocrine cells and tissues.

As of the end of 2007, Lemos and Thakker reported that 1336 mutations of the MEN1 gene have been described [28]. Most mutations occur in coding exons, but also among intronic sequences [29]. These include point mutations and small deletional or insertional mutations, which are thought to significantly alter the structure or biological function of menin. Over 70% of MEN1 mutations would be predicted to cause truncated forms of menin. Among the reported mutations, four have been reported to each account for 2.5-5% of cases [28]. In about 30% of cases that are clinically suggestive of MEN1, no MEN-1 mutation is found. These patients, sometimes termed as having "MEN1 phenocopy" can present sporadically [30] or as part of MEN1 kindreds [31]. Such patients often show an incomplete MEN-1 phenotype with less frequent enteropancreatic tumors and more frequent GH-secreting rather than prolactin-secreting pituitary tumors. In MEN1 mutation positive patients there is no correlation between genotype and tumoral phenotype.

The biological role of *MEN1* appears to be to act as a tumor suppressor gene, albeit one with an immensely elaborate series of interactions. Recent studies from the NIH group indicate that menin potentially interacts with the promoter regions of thousands of genes, indicating that it has a wide transcriptional regulatory role [32]. A subsequent study of menin-occupied chromatin regions found that menin binding regions are found within promoter regions, at 3' sites, within genes and one third of sites occurred outside of genes [33]. Menin binding to such "intragenic" areas is a novel finding which suggests a potential structural regulatory role for the protein.

Menin is a nuclear protein with a wide variety of molecular interactions, and the biological

significance of which are still undergoing elucidation [20, 34]. Menin interacts with JunD, leading to the formation of a growth-inhibiting complex, which can be disrupted by specific mutations in either component [35]. In recent years, menin has been shown to interact with nuclear factor κB, the Smad family, DNA, cell cycle regulators and a variety of other transcription factors, cell structural elements and regulators of apoptosis [36, 37, 38, 39, 40]. Little is known about how menin interacts with these molecules, and many structure-function relationships of menin remain to be clarified [41].

## Pituitary tumorigenesis in MEN1

Menin orthologs have been found in mice [42], which has allowed the development of knockout models to study the development of tumors [43, 44]. Mice that are homozygous knockouts for *Men1* (*Men1*$^{-/-}$) die in embryonic life and have multiple severe developmental defects [45]. Biondi et al and others used conditional homozygous inactivation via the Cre-recombinase *lox*P system to circumvent this lethality and create adult mice with a constitutional *Men1*$^{-/-}$ genotype restricted to the pituitary gland [46]. The pituitaries of these mice developed normally in the absence of menin, but prolactinomas were common. Also as in MEN1-affected humans pituitary tumorigenesis in *Men1*$^{-/-}$ mice lagged behind the development of adenomas in other tissues. Bertolino et al followed *Men1*$^{+/-}$ heterozygotic mice over a period of up to 26 months to assess the penetrance of various tumors over time [45]. Enlargement of the *pars distalis* of the mouse pituitary, which corresponds to the anterior pituitary in humans, was a common finding in mice aged over 13 months. Pituitary tumors were noted in 19% of mice at 13-18 months, rising to 36.6% at 19-26 months, whereas wild-type controls did not develop pituitary tumors. These tumors were more common in female mice, and over 50% of all pituitary tumors were carcinomas. Of 15 tumors that were characterized immunohistochemically, 14 were positive for either prolactin or GH. Complete or partial loss of the wild-type *Men1* allele occurred in all endocrine tumors (pituitary and elsewhere) in these heterozygotic mice [45]. These results are in keeping with the disease process and pathological features in tumor tissues from humans with MEN1 [47].

Menin over-expression has been shown to inhibit the activity of the prolactin gene promoter [26]. Prolactin expression in lactotrope cells is under the negative control of activin [48], this action is regulated by menin and the Smad pathway [37]. Lacerte et al reported that menin plays an important role in activin-TGFβ-induced regulation of prolactin expression and pituitary cell growth [49]. Some of these actions involving menin appear to be mediated via activin-induced down-regulation of the pituitary transcription factor, Pit-1. Inactivation of menin led to disruption of activin-induced repression of prolactin expression and pituitary cell growth[49].

Somatic mutations of the *MEN1* gene are not an important factor in the tumorigenesis of non-MEN1 sporadic pituitary adenomas [50, 51 52, 53, 54, 55, 56, 57]. In 35 sporadic pituitary adenomas of various secretory phenotypes, Poncin et al found only one tumor to exhibit homozygosity for a mutation close to the MEN1 promoter region [51]. Theodoropoulou et al used menin immuno-

histochemistry and immunofluoresence in 68 sporadic non-MEN1 pituitary tumors and found that menin was detectable in 67 cases although often at lower levels than in normal pituitary tissue [58].

## MEN1-related pituitary tumors

The prevalence of pituitary adenomas in patients with MEN1 is approximately 40%, the rates reported vary [59, 60, 61]. In a *Groupe d'Etude des Neoplasies Endocriennes Multiples* study of 324 MEN1 patients, the characteristics of pituitary disease in MEN1 were compared with those of 110 non-MEN1 patients with pituitary adenomas, who were matched for age, year of diagnosis and follow-up period [62] (Figure 1). Among MEN1 patients, 42% had pituitary tumors, which was the presenting tumor in 17% of cases. Presentation with MEN1 occurred seven years earlier in patients who presented with pituitary tumors as compared to patients presenting with enteropancreatic lesions. The mean delay in time to presentation with the next MEN1-related tumor was significantly longer in those with a pituitary tumor at initial diagnosis (9 ± 8.1 years) compared with those presenting initially with a parathyroid (5.2 ± 5.1 years) or an enteropancreatic tumor (4.1 ± 4.0 years). Among the familial MEN1 cases, pituitary disease was significantly more frequent compared with sporadic MEN1 cases (59% versus 34% respectively, p<0.0001). Only female sex was associated with an increased risk of having a pituitary tumor.

| | Pituitary adenoma | | |
|---|---|---|---|
| | MEN1 | Non-MEN1 | *P* |
| Age (yr) | 38.0 ± 15.3 | 36.2 ± 14.6 | NS |
| Mean follow-up (yr) | 11.1 ± 8.7 | 10.0 ± 6.3 | NS |
| Adenoma type | | | |
| Prolactinoma | 85 | 68 | NS |
| GH-secreting | 12 | 15 | NS |
| ACTH-secreting | 6 | 7 | NS |
| Co-secreting | 13 | 2 | NS |
| Non-secreting | 20 | 18 | NS |
| Tumor size | | | |
| Micro (n, %) | 19 (14%) | 64 (58%) | <0.001 |
| Macro (n, %) | 116 (85%) | 46 (42%) | |
| Clinical signs due to tumor size (n, %) | 39 (29%) | 15 (14%) | <0.01 |
| Normalization of pituitary hypersecretion (n, %) | 49 (42%) | 83 (90 %) | <0.001 |

**Table 1. Comparison of pituitary tumor characteristics between MEN-1 and non-MEN-1 patients. Micro = microadenomas, macro = macroadenoma. Adapted from reference 62.**

Pituitary adenomas are significantly more aggressive in MEN1 as compared with sporadic tumors, with macroadenomas being present in 85% of the former, compared with only 42% of the sporadic cases (*P*<0.001). MEN1-associated pituitary tumors were

significantly more likely to cause signs due to tumor size ($P$<0.001) and had a significantly lower rate of hormonal normalization than non-MEN1 pituitary tumors ($P$<0.001; Table 1). Prolactinomas predominate among both MEN1 associated and non-MEN1 pituitary adenomas, and the proportions of prolactinomas, GH-secreting, ACTH-secreting, non-secreting and co-secreting adenomas ware similar between the MEN1 and non-MEN1 patients. MEN1-related prolactinomas are predominantly macroadenomas (84%) and of these, 20 were invasive. The response of MEN1-related prolactinomas to dopamine agonists is relatively poor, with a normalization rate of only 44% of patients. Pituitary adenomas in MEN1 are characterized mainly by prolactinomas; pituitary tumors in MEN1 appear to be larger and more aggressive than in patients without MEN1[63].

There appears to be no relationship between the site or type of genetic mutation in the *MEN1* gene and the expressed MEN1 disease phenotype [64]. A variety of clusters can occur in individual families with MEN1, and the severity of disease expression can vary [65]. The best known of these is the so-called "prolactinoma variant" of MEN1, which includes MEN1$_{BURIN}$ and MEN1$_{TASMAN}$. MEN1$_{BURIN}$ was described in a family from the Burin peninsula of Newfoundland, Canada, who exhibited a syndrome of prolactinomas and carcinoid tumors, without pancreatic involvement [66, 67].

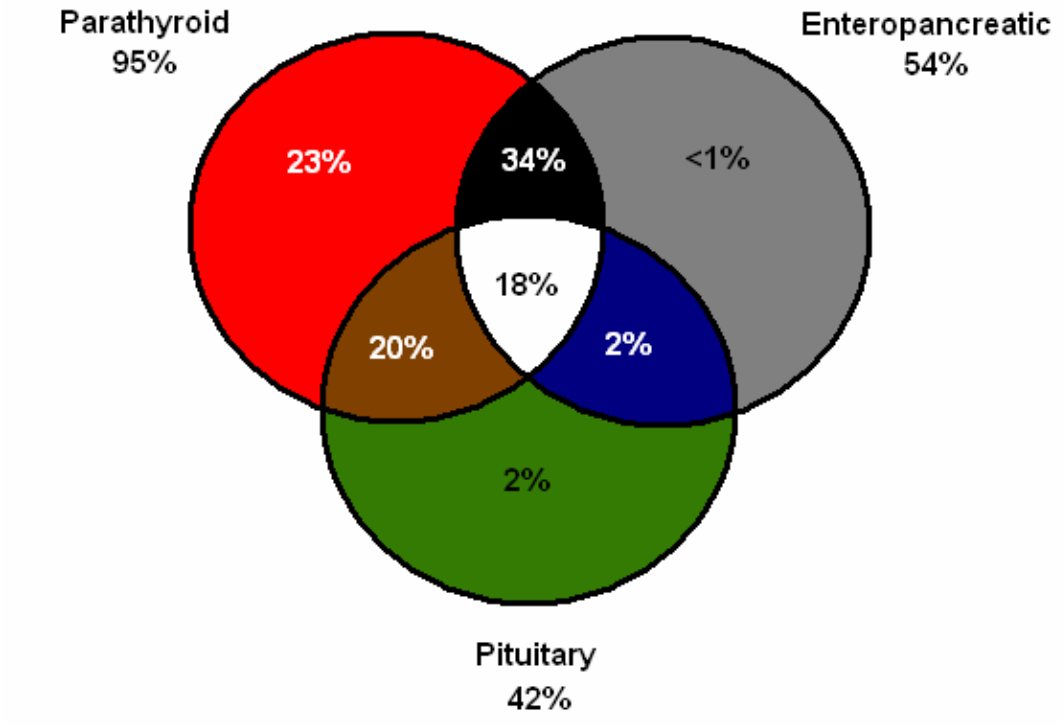


**Figure 1 . Distribution of the three main classes of endocrine lesions seen in 324 MEN1 patients. The percentages of the total number of MEN1 patients with each type or combination of tumors are shown. Adapted from reference 62.**

MEN1$_{TASMAN}$ was described in patients with prolactinomas and non-functioning adenomas [60]. Two prolactinoma variant MEN1 kindreds that underwent long-term scrutiny showed a similar phenotype but due to different *MEN1* mutations [68]. In the *GENEM* study, GH-secreting tumors were infrequent and had a similar mean age at diagnosis as sporadic cases. Half of these patients achieved hormonal normalization with multimodal therapy and 33% had persistent hypersecretion (17% had hypopituitarism). The features of other pituitary tumors in MEN-1 is also believed to be similar to sporadic adenomas.

## Carney Complex (CNC)

### Background

A complex of spotty skin pigmentation, myxomas, endocrine overactivity and schwannomas was described by Carney in the mid-1980's [4]. Carney complex (CNC) is rare, having been described in about 500 people in largest database [69]. CNC is familial in 70% of cases, occurs in all racial groups and has a slight female preponderance [70]. Two gene loci have been identified, one on chromosome 17q22-24 [71] and the other on chromosome 2p16 [72]. The former is associated with the gene encoding the Iα regulatory subunit of protein kinase A type I (*PRKAR1A*) and mutations have been identified in up to 60% of CNC patients [70, 73]. The 2p16 locus has not yet been localized to an individual gene [74]. Almost all *PRKARIA* mutations lead to mRNA instability, decreased or absent protein expression PRKAR1A haploinsufficiency in CNC tumors [70, 75]. Loss of heterozygosity (LOH) at 17q22-24 and allelic loss have been shown in CNC tumors [74, 75]. The loss of PRKAR1A function enhances intracellular response to cAMP in CNC tumors [74, 76].

Knockout mouse models demonstrate embryonic lethality of the homozygous *Prkar1a*$^{-/-}$ state [77,78]. In heterozygous *Prkar1a* $^{+/-}$ mice, no typical CNC features are encountered. A transgenic mouse with an antisense *PRKAR1A* exon 2 construct develops multiple endocrine abnormalities similar to CNC.

As in MEN1, sporadic pituitary tumors do not exhibit somatic mutations the *PRKAR1A* gene [79].

### CNC-related pituitary tumors

The main endocrinological abnormalities seen in CNC are primary pigmented nodular adrenocortical disease (PPNAD), thyroid tumors and nodules, testicular tumors (large cell calcifying Sertoli cell tumor, Leydig cell tumors) and acromegaly due to a pituitary adenoma [80]. Acromegaly itself is uncommon in CNC, but 75% of patients exhibit asymptomatic elevations in GH, IGF-1 and prolactin levels, or abnormal responses to dynamic pituitary testing [79]. A histologic analysis of eight CNC patients who underwent surgery for acromegaly, reported that all tumors were prolactin and GH positive, while a minority also stained for thyroid-stimulating hormone, luteinizing hormone or alpha-subunit [81]. A distinguishing feature of CNC-related acromegaly was the discovery of multifocal hyperplasia of somatomammotropic cells that included non-adenomatous pituitary tissue within the tumors of CNC patients. The zones of

hyperplasia were not well demarcated and exhibited increased cellularity and altered reticulin staining that merged with normal pituitary tissue. No consistent genetic abnormalities were seen on comparative genome hybridization. On electron microscopy, tumors from acromegalic patients with CNC demonstrate heterogeneous intra-cellular structure [82]. Acromegaly in CNC develops insidiously and may begin in apparently normal somatomammotrope tissue that undergoes multifocal hyperplasia to form GH/prolactin-secreting adenomas.

## Multiple Endocrine Neoplasia 4 (MEN4)

A MEN-like syndrome (MENX) that occurred spontaneously in the rat was reported by Fritz et al [83] and later expanded upon by Piotrowsksa et al from the same group [84]. In brief, the rat phenotype consisted of multiple neuroendocrine cancers that included pheochromocytoma, medullary thyroid cell neoplasia, parathyroid adenomas, paragangliomas, pancreatic hyperplasia and pituitary adenomas. These are preceded by the development of early cataracts within a few weeks of life. MENX was initially mapped to a chromosome 4 locus and was later revealed to occur due to a mutation in the *cyclin dependent kinase n1b (cdkn1b)* gene [85]. In humans the corresponding *CDKN1B* gene (which codes for $p27^{kip1}$) is on chromosome 12 and Pellagata et al identified a nonsense mutation in the *CDKN1B* gene in a German family exhibiting acromegaly, primary hyperparathyroidism, renal angiomyolipoma, and testicular cancer among various members. A Dutch patient with a pituitary adenoma (Cushing's disease), a cervical carcinoid tumor, and hyperparathyroidism and no *MEN1* mutation was recently identified as having a heterozygous 19-bp duplication in *CDKN1B* leading to a truncated protein product [86]. A study of a population from the NIH with parathyroid and pituitary tumors and no *MEN1* mutation noted no abnormalities in *CDKN1B* [87]. Although it appears to be a very rare syndrome, given the multiple endocrine neoplastic features of *CDKN1B* mutations in the human, it has been proposed to call this condition MEN4.

## Familial Acromegaly

As noted in the historical background to this chapter, familial acromegaly has been described for over a century. Isolated familial acromegaly or somatotropinomas (IFA/IFS) not associated with MEN1 or CNC is a clinical condition that is defined as ≥2 cases of acromegaly or gigantism in a family in the absence of MEN1 or CNC. About 50 familial acromegaly kindreds with over 120 individuals have been described in total [88, 89, 90]. Familial acromegaly may have a slight male preponderance although this is of arguable relevance. There is a younger age at onset in familial acromegaly (25 years) as compared with sporadic acromegaly; gigantism is a not infrequent feature of familial acromegaly kindreds. Tumors in these patients are almost without exception macroadenomas. Genetic linkage studies in familial acromegaly have been performed and pointed to a region of chromosome 11q13 [91, 92], with a specific area between microsatellite markers D11S956 and D11S527 on chromosome 11q13.1-

q13.3 [93]. The recent recognition of mutations in the aryl hydrocarbon receptor interacting protein gene (*AIP* ) in association with familial acromegaly kindreds has explained the pathophysiology in only a proportion of cases [94] and other genetic culprits are being sought.
The various endocrine syndromes associated with familial pituitary adenomas are summarized in Table 2. The clinical, genetic and pathological features of familial isolated pituitary adenomas (FIPA) are presented in full in later chapters related to the work undertaken for this thesis.

| Syndrome | Gene | Molecular Pathology | Pituitary Tumor |
|---|---|---|---|
| MEN1 | *MEN1* (Ch11q13) | Decreased menin expression/function | All pituitary tumor types (prolactinomas, non-secreting adenomas and GH-secreting adenomas most frequent) |
| MEN4 | *CDKN1B* (Chr 12p13) | Decreased p27 levels in tumor | Associated with only acromegaly and Cushing's disease in 2 patients to date |
| CNC | *PPKR1A* (Ch17q22-24)<br><br>? (Ch2p16) | Decreased protein kinase A regulatory subunit Ia expression/function | GH and GH/prolactin secreting adenomas |
| FIPA | *AIP* (Ch11q13.32) in 15% of cases (50% of familial acromegaly)<br><br>Other genes? | Decreased mRNA and protein in some mutated tissues. ?Altered regulation of AhR or phosphor-diesterase function | All pituitary adenoma subtypes involved; *AIP* mutation associated cases include somatotropinomas, prolactinomas, mixed GH/prolactin tumors, non-secreting adenomas. |

**Table 2. Familial pituitary tumor syndromes. AIP = aryl hydrocarbon receptor interacting protein; CNC = Carney complex, FIPA = Familial isolated pituitary adenoma; MEN1 = multiple endocrine neoplasia type 1, PRKAR1A = protein kinase A type I regulatory subunit Iα**

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# Chapter Five.

## The Epidemiology of Pituitary Adenomas

### Introduction

For nearly 80 years it has been recognized that pituitary adenomas are frequently found incidentally in autopsy specimens from individuals in the general population without known pituitary disease. This fact has been confirmed in recent years as the ready availability of radiological imaging using CT and MRI has allowed the recognition of a high frequency of pituitary tumors in asymptomatic subjects. These data were analyzed in depth by Ezzat and co-workers in 2004 and underline the high prevalence of unsuspected pituitary adenomas in the general population [1].

In contrast to these data showing a high prevalence of incidentally found pituitary adenomas, the data on clinically-relevant pituitary adenomas argues for the opposite. Data from cancer registries and the small number of population epidemiology studies characterize pituitary adenomas as being relatively rare. There are particular issues that are discussed below relating to the under-reporting of pituitary tumors in cancer databases that may explain this relative rarity as compared with autopsy/radiological data. However, one of the most important determinants of the difference between the two datasets is the fact that few, if any, well-designed, intensive epidemiological studies have been performed in the modern era.

### Autopsy and radiology series

Research into the epidemiology of pituitary adenomas began in earnest with the publication in 1936 of the results of an autopsy study from Russell Costello at the Mayo Clinic in the United States [2]. This study was designed to characterize the occurrence and features of what Costello described as "subclinical adenomas" of the anterior pituitary gland. As such, these pituitary adenomas were not associated with any clinical symptoms, and came from autopsy specimens of subjects that had died of other causes and had no features suggestive of pituitary disease. Costello reviewed the previous reports in the field dating back to 1903, which provided estimates of prevalence in the range of 8.4-10% in larger autopsy series. Costello's study was notable for its painstaking nature. In total he took a series of 1000 formalin-preserved pituitary glands. These glands were then sectioned by hand into slices of 1-1.5 mm in thickness, which provided the lower size limit for determining an adenoma in the study. Pituitary gland slices were embedded in paraffin and each block had two 1 micron sections taken, which were stained with hematoxylin and eosin. Overall, Costello reported that this method yielded 3-10 histological sections for each of the 1000 pituitary glands. These sections were then assessed by light microscopy for the presence of adenomas.

Of the 1000 pituitaries, 224 contained a total of 265 adenomas; one other gland contained 10 or more adenomas. The histological staining showed that 52.8% were chromophobic, 27.2% were basophilic, 12.4% were of mixed staining pattern and 7.5% were eosinophilic. The age at death of those with

pituitary adenomas was from 2 to 86 years; the highest incidence was seen in those aged 40-50 years at death. As compared with current times, the life expectancy was much lower in 1936 and the population contained relatively few individuals aged more than 70 years. The sex incidence was equal in males and females. Costello did not report the sizes of the adenomas, which is unsurprising as the advent of the modern concept of "microadenoma" and "macroadenoma" was still many decades off. He did note that in some cases the adenoma was large enough to nearly destroy the entire pituitary, although no clinical features had been apparent. This possibility of unrecognized pituitary macroadenomas occurring in the general population has been validated by later work in the autopsy and radiological realms as recently as late 2007 [3].

Multiple pathological studies have been performed by many groups since Costello's work, some in 2007 [4]. In general they confirm the high prevalence of pituitary adenomas in the unselected general population, although at a lower level than the 22.5% reported by Costello. These studies have been the subject of a systematic review by Ezzat and colleagues, which combined the findings from seven autopsy series (apart from Costello the other six studies were performed between 1981-1999) [1]. Among these studies, 3375 autopsied pituitaries were included. The reported prevalence rate varied from 0.015 to 0.84, with an overall prevalence of 14.4%. A subset of five studies also examined immunohistochemical staining of pituitary adenomas discovered at autopsy. Prolactin staining adenomas were the most frequent adenomas identified with 25-90% of available specimens showing positivity. No other hormone type (GH, ACTH, LH, TSH) was found in more than 18% of tumor specimens. It should be noted, however, that immunohistochemical evidence of prolactin in adenoma cells does not indicate that the adenoma was secreting excess prolactin. Since the publication of that systematic review, a very large series of subclinical pituitary adenomas that were identified postmortem was reported by Buurman and Saeger [5]. In their single series they studied 3048 autopsy cases from 1991-2004 and correlated the findings with immunohistochemical and other pathological analyses. They found a total of 334 adenomas in 316 pituitary glands (16 cases had two adenomas and one case had three adenomas). The mean maximum diameter of the adenomas identified was 1.97 mm (range: 0.1-20mm); only 22.7% of the adenomas were ≥3mm in size and a full 41.3% were 0.1mm in diameter or less. Three cases of macroadenomas were found. The range of tumor types seen according to histopathology is shown in Figure 1.

Widespread access to CT and MRI modalities for neuroradiology over the last 20 years has also had an impact on estimates of the prevalence of unsuspected pituitary adenomas. Older studies, particularly those using CT slice diameters greater than 5-10mm and not focussing on the pituitary specifically are of less value given the potential for missing microadenomas. Ezzat et al identified only three imaging studies that were of sufficient quality for inclusion; in contrast to the >3000 subjects included in the autopsy part of the analysis, only 202 subjects were included in the radiological studies. The prevalence of pituitary adenomas in radiological series ranged from 0.10

to 0.38, with a mean prevalence of 22.3%. A combined analysis that included both radiological and autopsy data yielded a final prevalence rate of unsuspected pituitary adenomas of 16.7%.

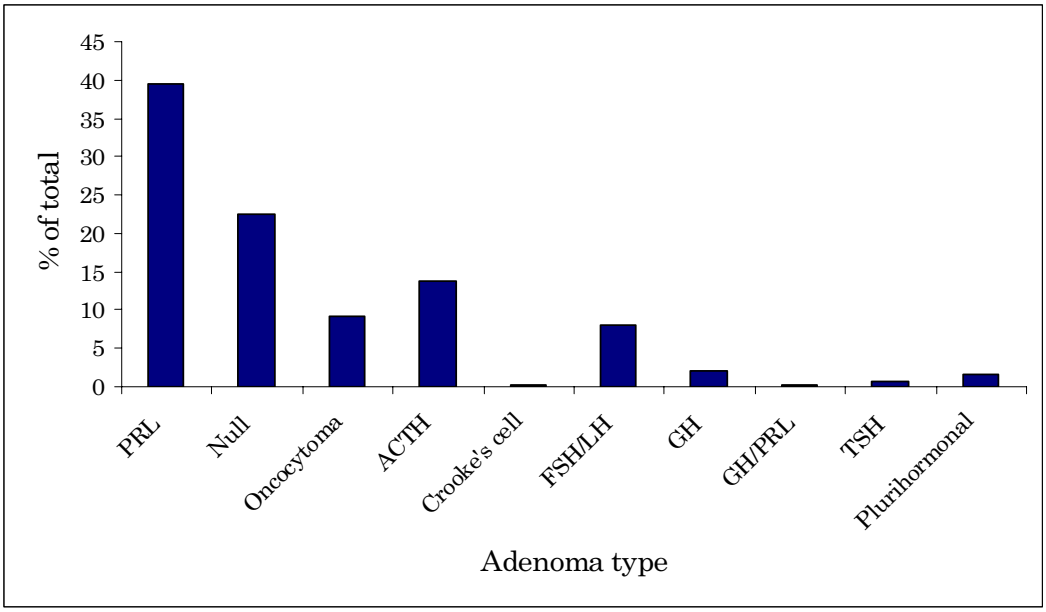


**Figure 1. Subclinical (incidental) pituitary adenomas noted in 316 pituitary glands from 3048 autopsy cases during the period 1991-2004 (adapted from data presented in Buurman H & Saeger W [5]).**

## Cancer registries

Data from cancer registries suggest that primary brain and central nervous system (CNS) tumors have a prevalence of approximately 130-230 cases/100,000 of the population [6, 7, 8]. Estimates for the prevalence of pituitary adenomas in larger databases in Europe and the United States suggest that they constitute 5–20% of primary CNS tumors, a relatively low population prevalence. Overall, pituitary tumors (including craniopharyngiomas) constitute 7.2% of brain CNS tumors by site [6].

In many jurisdictions pituitary adenomas are included as primary CNS tumors along with other benign tumors (meningiomas) and the more aggressive primary CNS malignancies. Registries, while they provide vital data, are highly dependent on having in place a comprehensive reporting system for all tumors. Without such rigor, it is difficult to ensure the accuracy of registry data and the subsequent assessments of the burden of CNS tumors in the populace. This is not a major problem in the case of primary CNS malignancies, as the reporting of such cancers by neurosurgeons, oncologists and neuropathologists is usually mandatory. In the case of benign CNS tumors, the case is less clear. For instance, in the United States, there was no legal obligation to report benign tumors to the Central Brain Tumor Registry of the United States (CTBRUS) until 2004. Therefore, previous estimates of the incidence of benign brain tumors are not entirely valid as they may be based on incomplete reporting. The case is particularly acute for pituitary adenomas, as they are managed medically not by

oncologists, but by endocrinologists. In cases where neurosurgery is not required (e.g. many micro-prolactinomas), opportunities for reporting such tumors usually do not exist or are not legally required. Registries use incidence and mortality data to derive calculations of CNS tumor prevalence. This adds another area of difficulty to the calculation of pituitary adenoma prevalence, as survival is much greater than in many other primary CNS tumors. Taken together the nature of the management of pituitary adenomas and the design of reporting mechanisms confounds prevalence assessments from these databases.

## Population studies

In contrast with the numerous autopsy, radiology and registry datasets available, very few population-based studies of the epidemiology of pituitary adenomas have been reported. This is due to the difficulty in undertaking such studies, which require close scrutiny of patients for inclusion and accurate definition of study populations. Use of data from larger tertiary referral centers may not be accurate as the catchment areas for such hospitals is often difficult to control and is usually not representative of a single geographically-defined population. Two population-based studies of pituitary adenoma prevalence were performed in the past. In the larger of the two, Clayton reported prevalence data for the Stoke-on-Trent area of the West Midlands of the United Kingdom [9]. This region had a population of approximately 1 million inhabitants at the time of their study, although this was not defined specifically in terms of geographic or political boundaries. Furthermore, a tertiary referral center was chosen as it was the only such center in the region; this did not exclude patients going outside of their region for treatment or entering the region from another geographical site. Patient populations in the study were based upon those seen by endocrinologists or neurosurgeons, which may have excluded individuals followed up by general practitioners or private specialists for stable micro-adenomas. Clayton reported an overall prevalence of 190–280 cases/million, of whom 31.6-35.7% had prolactinomas, 32.1-36.8% had non-secreting tumors, 21.1-21.4% had somatotropinomas and 10.5-10.7% had Cushing's disease. Ambrosi et al reported a similar study conducted in Italy in the early 1990's and reported an even lower prevalence of pituitary adenomas than that seen in the United Kingdom [10]. With this latter study the caveats raised regarding control of patient populations hold equally true.

In summary, therefore, the data on the prevalence of pituitary adenomas presents a major disconnect between the high frequency of subclinical adenomas in series from autopsy studies and the relative rarity in studies from cancer registries. This is further compounded by the virtual absence of rigorous population-based epidemiological studies of clinically-active pituitary adenomas. These precise aspects are addressed in one of the core studies in the following Chapters.

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# Personal Contribution

# Chapter Six

## High Prevalence of Pituitary Adenomas: A Cross-Sectional Study in the Province of Liège, Belgium.

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# High Prevalence of Pituitary Adenomas: A Cross-Sectional Study in the Province of Liège, Belgium

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**Context:** Prevalence data are important for assessing the burden of disease on the health care system; data on pituitary adenoma prevalence are very scarce.

**Objective:** The objective of the study was to measure the prevalence of clinically relevant pituitary adenomas in a well-defined population.

**Design:** This was a cross-sectional, intensive, case-finding study performed in three regions of the province of Liège, Belgium, to measure pituitary adenoma prevalence as of September 30, 2005.

**Setting:** The study was conducted in specialist and general medical practitioner patient populations, referral hospitals, and investigational centers.

**Methods:** Three demographically and geographically distinct districts of the province of Liège were delineated precisely using postal codes. Medical practitioners in these districts were recruited, and patients with pituitary adenomas under their care were identified. Diagnoses were confirmed after retrieval of clinical, hormonal, radiological, and pathological data; full demographic and therapeutic follow-up data were collected in all cases.

**Results:** Sixty-eight patients with clinically relevant pituitary adenomas were identified in a population of 71,972 individuals; the mean (± SD) prevalence was 94 ± 19.3 cases per 100,000 population (95% confidence interval, 72.2 to 115.8). The group was 67.6% female and had a mean age at diagnosis of 40.3 yr; 42.6% had macroadenomas and 55.9% underwent surgery. Prolactinomas comprised 66% of the group, with the rest having nonsecreting tumors (14.7%), somatotropinomas (13.2%), or Cushing's disease (5.9%); 20.6% had hypopituitarism.

**Conclusion:** The prevalence of pituitary adenomas in the study population (one case in 1064 individuals) was more than 3.5–5 times that previously reported. This increased prevalence may have important implications when prioritizing funding for research and treatment of pituitary adenomas. **(*J Clin Endocrinol Metab* 91: 4769–4775, 2006)**

TUMOR PREVALENCE DATA are important for the estimation of disease burden in populations and are often used to calculate health care resource distribution within and among clinical specialties. Existing data on the prevalence of pituitary adenomas are discordant. Estimates from cancer registries suggest that pituitary adenomas are uncommon, particularly as compared with solid tumors such as breast, lung, and colon cancers (1). In contrast, a comprehensive metaanalysis of data from autopsy and radiological studies indicates that pituitary tumors may be present in as many as one in every six people (2). The inclusion of a sizable number of small nonclinically relevant adenomas (incidentalomas) in autopsy/radiological series probably accounts for a proportion of the reported high prevalence, but as noted by Ezzat *et al.* (2), many tumors from autopsy series are immunohistochemically positive for pituitary hormones. Existing epidemiological data suggest that the incidence of pituitary adenomas is rising, although it is difficult to determine whether this is due to widespread access to magnetic resonance imaging (MRI) and accurate biochemical testing, leading to improved recognition of clinically relevant pituitary tumors (3). The uncertainty regarding the true prevalence of clinically active pituitary adenomas led us to undertake an intensive, cross-sectional epidemiological study of the current prevalence of pituitary adenomas in a tightly defined geographical area in Liège, Belgium.

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Abbreviations: CT, Computerized tomography; FIPA, familial isolated pituitary adenoma; MEN1, multiple endocrine neoplasia type 1; MRI, magnetic resonance imaging.

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## Patients and Methods

### *Study setting*

Three separate geographic areas within the province of Liège were chosen for the study. The definition of *prevalence* for this study was that generally used in cancer epidemiology: "prevalence is the number and/or proportion of people with a past or present diagnosis of a pituitary adenoma within a well-defined population at a fixed point in time" (4). To reflect the diverse characteristics of the Belgian population densities, the individual areas had specific demographic profiles: rural (Soiron), suburban (Oupeye), and urban (Ans-Alleur), and all had a similar number of inhabitants. To define the geographical boundaries of each study region precisely, Belgian post office code designations were used. Study district I, Soiron (postal codes 4860, 4861, 4870, 4877), consisted of a population of 21,024 inhabitants; study district II, Oupeye (postal codes 4680, 4681, 4682, 4683, 4684), had 23,598 inhabitants; and study district III, Ans-Alleur (postal codes 4430, 4431, 4432), had 27,350 inhabitants (Fig. 1). The total population for the study was 71,972. Only living individuals residing within the predetermined geographic boundaries on a specific day were deemed eligible for inclusion in the study. The defined date for validating whether patients were alive and were residing in one of the postal code-defined areas was September 30, 2005. The study protocol was approved by the Ethics Committee of the University of Liège (Liège, Belgium) and was performed under the tenets of the Declaration of Helsinki and its subsequent amendments.

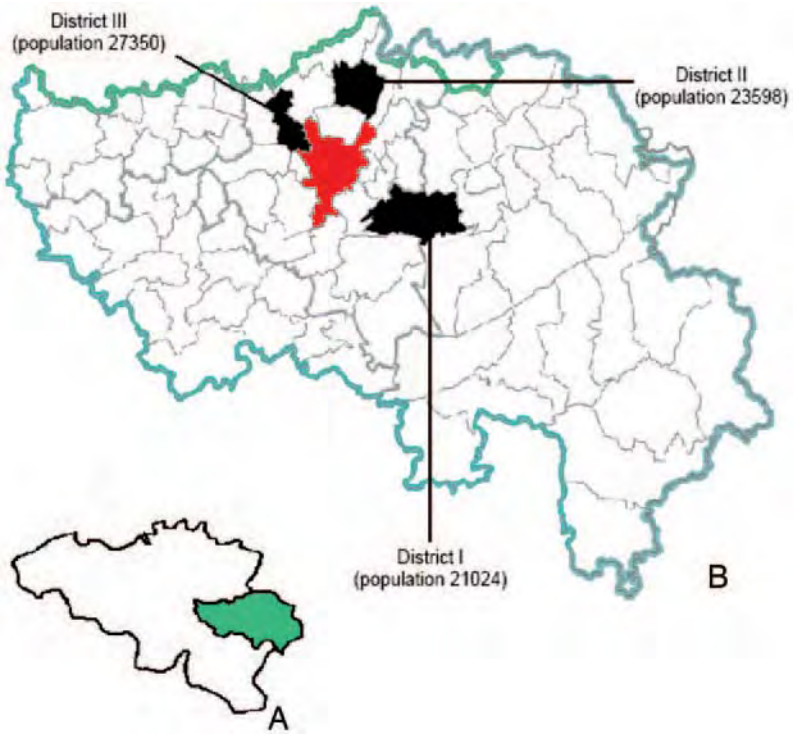


FIG. 1. Map of Belgium (A) with province of Liège outlined (*green*). Detailed view of districts in the province of Liège, including the three study districts (*black*) and the city of Liège (*red*). [Adapted with permission from the Institut Géographique National-Belgique (www.ign.be).]

### *Data gathering*

Within the three defined study areas, all general practitioners and relevant specialists (endocrinologists, gynecologists, neurosurgeons) working in public/private practice were identified. Subsequently, the identified medical practitioners were contacted directly to recruit them to the study, and each received a simple case report form containing headings for demographic and disease characteristic criteria. Educational meetings on the topic of the clinical recognition, investigation, and management of patients with pituitary tumors were organized for medical practitioners within each study area; attendees received information regarding the design and purpose of the present epidemiological study. The proportion of medical practitioners within the study sample sites that participated in the study was 70–80%. Participating medical practitioners analyzed their patient records to identify individuals with an established (past or current) diagnosis of a pituitary adenoma.

Patients were contacted to inform them of the study and the anonymous nature of data gathering and to confirm their eligibility (living and residing in one of the three study areas). Individual patient characteristics including data on demographics, residence, diagnosis, date of diagnosis, therapy, and site of hospital treatment were recorded on the case report form. Thereafter in each case further definitive information establishing the diagnosis of a pituitary adenoma was sought from hospital case files or other relevant clinical records. Patients with other pituitary conditions like craniopharyngioma or inflammatory lesions were excluded. In all cases, the primary clinical signs/symptoms at presentation (maximum of three), radiological imaging studies of the pituitary region, and hormonal profiles demonstrating relevant disordered secretion had to be available. In cases in which surgery was performed, operative findings and pathological reports were sought. Patient follow-up data (treatment and disease control) also had to be available in all cases; for the purpose of this study, patients were defined as having biochemically stable disease if their hormonal levels were controlled to a level at which hormonal hypersecretion symptoms were not evident. In the case of patients with acromegaly, IGF-I had to be controlled to within the normal ranges for age and sex. Furthermore, patients with hypopituitarism were required to have evidence of adequate dosing with hormonal replacement therapy before being assessed as biochemically stable. Tumor stability was assessed in all cases, either in terms of whole tumor size changes in nonoperated cases or tumor remnant behavior in cases with incomplete primary resection of the pituitary adenoma. Before being included in the final cohort, each patient's symptom and hormonal, radiological, pathological, and follow-up data were reviewed and verified separately by two of the authors (A.F.D. and A.B.).

### *Familial screening*

The study had its genesis in the investigation of a series of patients with pituitary adenomas in a valley area in one of the postal code regions of study district I (Soiron) involving less than 5000 people. During this initial work, the issue of family clustering was suggested; however, investigation of patients' family histories and genealogies revealed no familial cases. Given the relatively close geographic distance between the study sampling sites, in the current study, identified patients also underwent screening for familial links to assess for clustering due to pituitary tumor-associated syndromes such as multiple endocrine neoplasia-1 (MEN1) and familial isolated pituitary adenomas (FIPA) (5, 6). Medical practitioners and patients were questioned about their knowledge of other family members with diseases suggestive of MEN1 and for the presence of other family members with pituitary adenomas (Carney's complex, FIPA). Further assessments of patients' medical records were undertaken to rule out the presence of biochemical abnormalities typical of MEN1.

### *Data analysis*

Means and ranges were calculated for the following criteria for each tumor type and the total pituitary adenoma population: age, symptom duration before diagnosis, number of MRI/computerized tomography (CT) scans, and maximum tumor diameter. Data on sex, the main three symptoms at presentation, tumor characteristics (macro-/microadenoma, suprasellar extension, invasion), requirement for surgery, postoperative medical therapy, and disease control (biochemical, tumor) were collected, summarized, and tabulated for each tumor type and for the group as a whole. The prevalence of pituitary adenomas at each of the three sampling sites was calculated individually, and the overall prevalence in the study was expressed as the mean (± SD; 95% confidence interval) of the three individual values.

## Results

*Prevalence of pituitary adenomas*

A total of 68 living patients with clinically confirmed pituitary adenomas were resident in the study areas as of September 30, 2005. The mean (± SD) prevalence across the three study areas was 94 cases per 100,000 population (95% confidence interval, 72.2 to 115.8 cases). This translates into a mean of one case per 1064 individuals (95% confidence interval, 1:864 to 1:1385). A further 30 patients who were highlighted by the participating medical practitioners were excluded for the following reasons: deceased before cutoff date of September 30, 2005 (n = 10); mild hormonal abnormalities (predominantly hyperprolactinemia) without verifiable evidence of a tumor on MRI (n = 9); resident outside the geographical limits of the study sites (n = 7); craniopharyngioma (n = 2) and arachnoid cyst (n = 2).

*Demographics*

The summary details of the individual patients are shown in Table 1. The group of patients with verified pituitary adenomas consisted of 22 males and 46 females. Two patients were of North African origin; the rest were Caucasian. The mean age at diagnosis was 40.3 yr (range 12–86 yr), and patients on average had suffered symptoms attributable to their diagnosis for 45.3 months (range 1–300 months) before a diagnosis was made. Patients were not uniformly distributed by age at diagnosis: 0–9 yr (n = 0), 10–19 yr (n = 5), 20–29 yr (n = 13), 30–39 yr (n = 18), 40–49 yr (n = 13), 50–59 yr (n = 9), 60–69 yr (n = 7), 70–79 yr (n = 2), 80–89 yr (n = 1), older than 90 yr (n = 0).

*Disease characteristics*

Overall, prolactinomas were the most frequent tumor found (45 of 68; 66.2%), followed by nonsecreting tumors (10 of 68; 14.7%), somatotropinomas (nine of 68; 13.2%), and Cushing's disease (four of 68; 5.9%). No patient in the cohort had a tumor that secreted TSH alone, although one patient with acromegaly had a tumor that cosecreted GH, prolactin, and TSH, and the patient exhibited signs/symptoms of hyperthyroidism in addition to acromegaly. Familial links among patients were not found in this cohort, and only one patient (a female with a macroprolactinoma) had sporadic MEN1 that had been confirmed by genetic screening.

Radiological diagnosis and follow-up were performed using MRI of the pituitary (3-mm cuts) in 56 patients. The remaining 12 patients had tomography or CT at diagnosis, and nine of these 12 patients subsequently had their tumor characteristics confirmed during surgery. MRI was used for long-term follow-up in all patients originally diagnosed using tomograms and CT. The mean number of MRI and CT scans per patient during their diagnosis and follow-up was 4.9 (range 1 to 16 scans). All 68 patients had valid radiological results to determine the presence of a macroadenoma (n = 29) or a microadenoma (n = 39); the mean maximal tumor diameter was 12.9 mm (range: 2–50 mm) for the group overall. Suprasellar extension was noted in eight patients with prolactinomas (17.8%), seven patients with nonsecreting adenomas (70%), and four patients with acromegaly (44.4%). Tumor invasion was noted in eight (17.8%), four (40%), four (44.4%), and one patient (25%) in the prolactinoma, nonsecreting adenoma, acromegaly, and Cushing's disease groups, respectively (Table 1).

*Treatment and follow-up*

A total of 38 patients (55.9%) underwent surgery, and the approach was transsphenoidal in all but one patient (transnasal), whereas two patients underwent repeat surgery. Pathological results were available in 34 of 38 operated cases (89.5%), and in all cases tumors were benign adenomas. Only two patients received radiotherapy: the patient with MEN1 whose macroprolactinoma was resistant to surgery and dopamine agonists and a second patient with Cushing's disease and residual tumor postoperatively. Hypopituitarism was present at diagnosis in eight patients, all of whom had nonsecreting adenomas. Postoperatively, seven of these patients still had hypopituitarism, along with six patients with prolactinoma and one with acromegaly.

As noted above, prolactinomas were the most frequent tumors encountered in the current study (66.2%), and approximately 80% were microprolactinomas that occurred in females. The most frequent presenting symptoms in these cases were oligo or amenorrhea in two thirds of cases, followed by galactorrhea and headache in about 50% of cases each. As shown in Table 1, dopamine agonists were used in 39 of 45 patients with prolactinomas, 26 of whom did not have surgery. Of the 19 patients (12 female) who underwent surgery, nine patients had macroadenomas. Among prolactinomas, biochemical control was achieved in all but four cases, and tumor size remained stable during subsequent dopamine agonist therapy. Three of these patients had macroadenomas, two of whom were males with invasive tumors. Eight of the nine patients with acromegaly underwent surgery (one twice); long-term medical therapy with somatostatin analogs was used in four cases. Only one patient with acromegaly failed to achieve adequate long-term biochemical control; this patient was intolerant to both somatostatin analogs and pegvisomant postoperatively. Seven patients with nonsecreting adenomas underwent surgery. As noted above, one patient with Cushing's disease had persistent biochemically active disease despite surgery and therefore required radiotherapy; the patient remains hormonally controlled and without hypopituitarism at this time.

## Discussion

In this cross-sectional study, we found that verified, clinically relevant pituitary adenomas occurred with a prevalence of 1:1064 of the population, which is notably higher than previous data would suggest. This is the first cross-sectional study of pituitary adenomas to involve an intensive case-finding approach at a community level involving not only endocrinologists but also general practitioners and other medical specialists. This approach was intended to maximize the identification of relevant cases within the study districts irrespective of the site or manner in which they were followed up clinically.

Specific epidemiological studies regarding clinically active pituitary adenomas are relatively scarce. Most available in-

**TABLE 1.** Characteristics of patients with clinically active pituitary tumors in the study population

| Patient no. | District | Sex | Age at diagnosis (yr) | Duration prediagnosis (months) | Biochemical diagnosis | Max. tumor diameter (mm) |
|---|---|---|---|---|---|---|
| Prolactinomas | | | | | | |
| 1 | I | M | 19 | 12 | Increased PRL, +TRH test | 20 |
| 2 | I | F | 33 | 5 | Increased PRL, +TRH test | 3.5 |
| 3 | I | M | 61 | 36 | Increased PRL, +TRH test | 20 |
| 4 | I | F | 42 | 36 | Increased PRL, +TRH test | 15 |
| 5 | I | M | 48 | N/A | Increased PRL | 27 |
| 6 | I | F | 47 | 24 | Increased PRL, +TRH test | 6 |
| 7 | I | F | 21 | 24 | Increased PRL, ± TRH test | 6 |
| 8 | I | F | 50 | N/A | Increased PRL | 4 |
| 9 | II | M | 38 | 90 | Increased PRL, +TRH test | 7 |
| 10 | II | F | 33 | 18 | Increased PRL | N/A |
| 11 | II | F | 23 | 60 | Increased PRL, +TRH test | 8 |
| 12 | II | F | 24 | 54 | Increased PRL, +TRH test | 7.5 |
| 13 | II | F | 36 | 24 | Increased PRL, +TRH test | 5 |
| 14 | II | F | 28 | 42 | Increased PRL, +TRH test | 6 |
| 15 | II | F | 51 | 18 | Increased PRL, +TRH test | 9 |
| 16 | II | F | 24 | 36 | Increased PRL, +TRH test | 6 |
| 17 | II | M | 12 | 12 | Increased PRL, +TRH test | 8 |
| 18 | II | F | 32 | 6 | Increased PRL, +TRH test | 9 |
| 19 | II | F | 53 | N/A | Increased PRL, +TRH test | N/A |
| 20 | II | M | 31 | N/A | Increased PRL, +TRH test | 5 |
| 21 | II | F | 35 | 144 | Increased PRL, +TRH test | 10 |
| 22 | II | F | 28 | 12 | Increased PRL, +TRH test | 4 |
| 23 | II | M | 39 | 1 | Increased PRL, +TRH test | 20 |
| 24 | II | F | 54 | N/A | Increased PRL | 35 |
| 25 | II | F | 52 | 6 | Increased PRL, +TRH test | 25 |
| 26 | II | M | 54 | | Increased PRL | 5 |
| 27 | II | F | 42 | N/A | Increased PRL | N/A |
| 28 | II | F | 42 | N/A | Increased PRL | 4 |
| 29 | II | F | 21 | 12 | Increased PRL | 5 |
| 30 | II | F | 45 | N/A | Increased PRL | 4 |
| 31 | II | F | 40 | 12 | Increased PRL, +TRH test | 7 |
| 32 | III | F | 23 | 72 | Increased PRL, +TRH test | 20 |
| 33 | III | F | 26 | 12 | Increased PRL, +TRH test | 5 |
| 34 | III | F | 32 | 180 | Increased PRL, +TRH test | N/A |
| 35 | III | F | 27 | 6 | Increased PRL, +TRH test | 9 |
| 36 | III | F | 40 | 216 | Increased PRL, +TRH test | N/A |
| 37 | III | F | 26 | 1 | Increased PRL, +TRH test | 20 |
| 38 | III | F | 28 | 36 | Increased PRL, +TRH test | 5 |
| 39 | III | F | 45 | 120 | Increased PRL, +TRH test | 3 |
| 40 | III | F | 15 | N/A | Increased PRL, +TRH test | 5 |
| 41 | III | M | 30 | 12 | Increased PRL, +TRH test | 5 |
| 42 | III | F | 30 | 6 | Increased PRL, +TRH test | 4 |
| 43 | III | F | 37 | 18 | Increased PRL, +TRH test | 5 |
| 44 | III | F | 35 | N/A | Increased PRL | 3 |
| 45 | III | F | 25 | 12 | Increased PRL, +TRH test | 2 |
| Somatotropinomas | | | | | | |
| 46 | I | M | 35 | 12 | Increased IGF-I, GH, +OGTT | 11 |
| 47 | I | M | 47 | 180 | Increased GH, IGF-I, +OGTT | 13 |
| 48 | I | M | 60 | 300 | Increased GH, IGF-I | 36 |
| 49 | II | M | 19 | 60 | Increased GH, PRL, TSH, +OGTT | 15 |
| 50 | II | M | 32 | 72 | Increased GH, IGF-I, +OGTT | 30 |
| 51 | III | F | 63 | 20 | Increased GH, +OGTT | 14 |
| 52 | III | F | 17 | 36 | Increased GH, +OGTT | N/A |
| 53 | III | F | 56 | 60 | Increased GH, IGF-I, +OGTT | 28 |
| 54 | III | M | 65 | 48 | Increased GH, IGF-I, +OGTT | 15 |
| Nonsecreting adenomas | | | | | | |
| 55 | I | M | 50 | 12 | Low LH/FSH/GH, +ITT | 15 |
| 56 | I | F | 77 | 24 | Low LH/FSH, +TRH/LHRH test | 35 |
| 57 | I | M | 76 | 4 | Low LH, low Tes | 20 |
| 58 | II | F | 42 | 24 | No pituitary hormone abnormality | 5 |
| 59 | III | M | 49 | 4 | Panhypopituitarism, +TRH/LHRH test | 19 |
| 60 | III | M | 69 | 36 | High LH/FSH | 23 |
| 61 | III | M | 86 | 1 | Low LH, low IGF-I | 50 |
| 62 | III | M | 61 | 12 | Low Tes/IGF-I, +ITT | 35 |
| 63 | III | M | 62 | 24 | Low Tes | 14 |
| 64 | III | F | 41 | 120 | Low LH/FSH, low Est | 10 |
| Cushing's disease | | | | | | |
| 65 | I | F | 55 | 120 | Increased ACTH, + including 24-h dexamethasone suppression test | 5 |
| 66 | I | F | 30 | N/A | Increased ACTH, + 24 h urinary cortisol | 5 |
| 67 | II | F | 37 | 24 | Increased ACTH, + 24 h urinary cortisol | N/A |
| 68 | III | F | 36 | 24 | Increased cortisol, + 24 h urinary cortisol, + dexamethasone suppression test | 12 |

**TABLE 1.** *Continued*

| Micro/macro | Suprasellar extension | Invasion | Surgery | Radiotherapy medical therapy | Hormonal control | Tumor stable |
|---|---|---|---|---|---|---|
| Macro | Yes | Yes | TS | CAB, Tes, GH | No | Yes |
| Micro | No | No | No | CAB | Yes | Yes |
| Macro | Yes | Yes | TS | CAB, HC, Tes, GH | Yes | Yes |
| Macro | Yes | No | TS | CAB | Yes | Yes |
| Macro | No | Yes | TS | CAB | No | Yes |
| Micro | No | No | No | BR | Yes | Yes |
| Micro | No | No | TS | CAB | Yes | Yes |
| Micro | No | No | No | CAB | Yes | Yes |
| Micro | No | Yes | TS | CAB | Yes | Yes |
| Micro | No | No | No | CAB | Yes | Yes |
| Micro | No | No | TS | | Yes | Yes |
| Micro | No | No | TS | | Yes | Yes |
| Micro | No | No | TS | CAB | Yes | Yes |
| Micro | No | No | No | CAB | Yes | Yes |
| Micro | No | No | No | CAB | Yes | Yes |
| Micro | No | No | TS | | Yes | Yes |
| Micro | No | No | TS | | Yes | Yes |
| Micro | No | No | TS | CAB, Thy, HC | Yes | Yes |
| Micro | No | Yes | No | CAB | Yes | Yes |
| Micro | No | No | No | CAB | Yes | Yes |
| Macro | No | Yes | No | CAB | No | Yes |
| Micro | No | No | TS | | Yes | Yes |
| Macro | Yes | Yes | TS | CAB, Tes, GH | Yes | Yes |
| Macro | Yes | Yes | TS | Radiotherapy CAB, Thy, HC | Yes | Yes |
| Macro | Yes | No | TS | CAB | Yes | Yes |
| Micro | No | No | No | CAB | Yes | Yes |
| Micro | No | No | No | CAB | Yes | Yes |
| Micro | No | No | No | CAB | Yes | Yes |
| Micro | No | No | No | BR | Yes | Yes |
| Micro | No | No | No | CAB | Yes | Yes |
| Micro | No | No | No | CAB | Yes | Yes |
| Macro | Yes | No | No | CAB | Yes | Yes |
| Micro | No | No | TS | | Yes | Yes |
| Macro | No | No | TS | BR | Yes | Yes |
| Micro | No | No | No | CAB | Yes | Yes |
| Micro | No | No | No | BR | Yes | Yes |
| Macro | Yes | No | TS | CAB, Thy, HC, Est | Yes | Yes |
| Micro | No | No | No | CAB | Yes | Yes |
| Micro | No | No | No | CAB | Yes | Yes |
| Micro | No | No | No | CAB | Yes | Yes |
| Micro | No | No | No | CAB | Yes | Yes |
| Micro | No | No | No | BR | No | Yes |
| Micro | No | No | No | CAB | Yes | Yes |
| Micro | No | No | No | CAB | Yes | Yes |
| Micro | No | No | No | CAB | Yes | Yes |
| Macro | No | No | TS | | Yes | Yes |
| Macro | No | No | TS | LAN | Yes | Yes |
| Macro | No | Yes | TS | LAN | Yes | Yes |
| Macro | Yes | Yes | TS (× 2) | | Yes | Yes |
| Macro | Yes | Yes | No | LAN | Yes | Yes |
| Macro | No | No | TS | | No | |
| Micro | No | No | TS | Thy, Est, GH | Yes | Yes |
| Macro | Yes | Yes | TS | OCT | Yes | Yes |
| Macro | Yes | No | TS | | Yes | Yes |
| Macro | No | No | No | Thy, HC | Yes | Yes |
| Macro | Yes | Yes | TS | CAB, Thy, HC, | Yes | Yes |
| Macro | Yes | Yes | TS | | Yes | Yes |
| Micro | No | No | No | | Yes | Yes |
| Macro | Yes | No | TS | Thy, HC, Tes, GH | Yes | Yes |
| Macro | Yes | No | TS | | Yes | Yes |
| Macro | Yes | No | TS | Thy, HC | Yes | Yes |
| Macro | Yes | Yes | TS | Thy, HC, Tes, GH | Yes | Yes |
| Macro | No | Yes | No | Tes | Yes | Yes |
| Macro | Yes | No | TN | HC | Yes | Yes |
| Micro | No | No | TS | | Yes | Yes |
| Micro | No | No | TS | Radiotherapy | Yes | Yes |
| Micro | No | No | TS | | Yes | Yes |
| Macro | No | Yes | TS (× 2) | | Yes | Yes |

Data are divided as per tumor phenotype and then listed in order of study district. Biochemical control was defined as a hormonal level at which patients' symptoms were kept at bay; whereas in the case of acromegaly, patients had to have an IGF-I level in the normal range for age and sex to be considered controlled. N/A, Not available; BR, bromocriptine; CAB, cabergoline, Est, estrogen, HC, hydrocortisone, ITT, insulin tolerance test, LAN, lanreotide, OCT, octreotide, OGTT, oral glucose tolerance test, PRL, prolactin, Tes, testosterone; Thy, thyroxine; TN, transnasal; TS, transsphenoidal.

formation comes from larger cancer registries, in which data on pituitary adenomas are reported as a subgroup of all brain or central nervous system tumors. Such registry data reveal that pituitary adenomas comprise approximately 5–20% of primary central nervous system tumors, which would translate into a relatively low prevalence of pituitary adenomas (7). In contrast, data from autopsy series or MRI studies of unselected populations indicate that the presence of a pituitary tumor, irrespective of clinical correlates, is relatively common. In their recent metaanalysis, Ezzat *et al.* (2) reported that pituitary adenomas occurred with a frequency of 14.4% (range: 1–35%) and 22.5% (range: 1–40%) in pooled autopsy and radiological series, respectively. Of autopsy specimens that underwent immunohistochemical analysis, 25–41% of cells were prolactin positive, with much more infrequent staining for other pituitary hormones (0.7–4.9%).

These interesting data suggest that a proportion of cases found at autopsy may represent undiagnosed clinically relevant pituitary tumors. The metaanalytic data need to be balanced against the small size of the database from which prevalence data were derived; the autopsy population included 3375 patients, and the radiology series comprised 202 individuals. Autopsy/radiology estimates do not include clinical correlates, such as symptoms and hormonal data, whereas the current study included clinically relevant pituitary adenomas that had already been diagnosed. The study was not designed to screen for either occult pituitary adenomas with relevant, albeit undiagnosed, clinical effects or pituitary incidentalomas that lacked clinical correlates. Therefore, the current study may underestimate the true prevalence of pituitary adenomas in the general population. It remains practically difficult to estimate what proportion of incidentally discovered autopsy cases, particularly microadenomas, have objective hormonal abnormalities or significant symptomatology. We would suggest, however, that the inclusion of true incidentalomas into prevalence estimates does not aid the assessment of the clinical burden attributable to pituitary adenomas in the general population.

Current estimates of brain cancer epidemiology from the Central Brain Tumor Registry of the United States suggests a prevalence of 130.8 cases per 100,000 population (1, 8). Data from 2005 in Finland reported an even higher prevalence of primary brain tumors, with a prevalence rate of 228 cases per 100,000 (9). With respect to the Central Brain Tumor Registry of the United States data, Davis *et al.* (1) estimated that benign tumor cases constitute 97.5 cases per 100,000, a large majority of the total prevalence. These benign cases are comprised of meningiomas and other histological types in addition to pituitary adenomas, so a precise estimate of the prevalence of the latter alone is not readily feasible. The proportional incidence rates of pituitary tumors, 7.2% of primary brain tumors by site and 6.3% by histology, is not particularly helpful in estimating prevalence (8). The low associated mortality in pituitary adenomas would lead to a higher elevated prevalence rate during long-term follow-up as compared with other brain tumors that have a higher annual incidence rate but a concomitantly high 1- to 5-yr mortality rate.

As noted by Monson (10), the indolent nature of many endocrine tumors, the patterns of clinical care among various specialties, and the lack of a relationship between incidence and mortality may mitigate against obtaining accurate epidemiological data on endocrine tumors. These factors are particularly true in the case of pituitary adenomas. Historically, benign brain tumors, such as pituitary adenomas, have been underreported in cancer registries due to a lack of legally obligated reporting (1, 11). This will change in the future with greater emphasis being placed on nonmalignant tumors; in the United States, the passage of the Benign Brain Tumor Cancer Registries Amendment Act means that new cases of pituitary adenomas have been reportable since January 1, 2004 (12). It will therefore be some years before comprehensive data on pituitary adenoma incidence and prevalence are available from major cancer registries.

Few studies specifically examining the epidemiology of pituitary adenomas have been undertaken. In a study of the Stoke-on-Trent region in the United Kingdom between 1988 and 1998, Davis *et al.* (13) reported that pituitary adenomas occurred with a prevalence of 190–280 cases/million (1:3571 to 1:5263). In that study, patients investigated by an endocrinologist were included whether or not they had surgery. It is not clear, however, whether the study captured all patients with pituitary adenomas resident in the region that may have received treatment outside the geographical boundaries. Our study reported a prevalence rate of 3.4–5 times that of Davis *et al.*, and this may have been due, in part, to our being able to identify and verify patients with pituitary adenomas more completely in a more tightly controlled population. Nilsson *et al.* (3) studied incidence and mortality data in a Swedish Cancer Registry study. This study, which excluded patients with acromegaly and Cushing's disease, reported an incidence of 11 cases/million population per year during a period up to 1991. This constituted nearly a doubling in annual incidence in comparison with previous data from 1958. It is unknown whether this apparent rise in incidence was due to the advent of better diagnostic techniques or a true increase in incidence. Widespread access to both MRI and laboratory techniques may have had an important impact on the ability to diagnose pituitary adenomas that are associated with subtle signs and symptoms. Also, patients may be more likely than before to seek medical attention earlier for more insidious symptoms associated with pituitary adenomas, such as disorders of libido, sexual dysfunction, and infertility. Importantly, as therapies have improved, the life span of patients with pituitary tumors has also undoubtedly lengthened, which would tend to increase the prevalence of pituitary tumors in the population. We would suggest that the high prevalence of pituitary adenomas seen in the current study may be due to such a combination of these factors.

As compared with large cancer registries that assess data on millions of patients, the current study population may appear limited in size. However, the aim of the study was to identify pituitary tumors in alliance with community medical practitioners and report on only those with verifiable hormonal, radiological, and clinical profiles. We undertook an intensive process of identifying, recruiting, and informing the entirety of the medical population of the chosen study sites, followed by a similar process of identifying, validating, and recruiting potential patients. Given these requirements and the parallel process of data validation in all cases, a

population of approximately 72,000 approached the maximum feasible for an academic cross-sectional study. Further confirmation of these results will require international cooperative efforts using new or existing data-gathering and epidemiological tools.

We considered the question of clustering of cases within the study regions and the effect that might have on our estimates. Few or no data exist on the potential impact of race, socioeconomic status, age, and environmental factors on the development of pituitary adenomas. We did, however, verify that known inherited factors did not influence the data, using a combination of family history data and genetic studies (14). Only one patient, a female with a relatively treatment-resistant macroprolactinoma, had MEN1, and this was a sporadic case with no other relatives forming part of the study population. Carney's complex is very rare and was not a feature of the patients with somatotropinomas. Of potentially greater relevance is the syndrome of FIPA, which may be linked to mutations in the aryl hydrocarbon receptor interacting protein (6, 15). The patients included in the current study did not have known relatives with a diagnosis of pituitary tumors, making the influence of FIPA in this population unlikely. The role of specific environmental factors such as carcinogen exposure in the etiology of pituitary adenomas requires further assessment, particularly because the aryl hydrocarbon receptor, for which aryl hydrocarbon receptor interacting protein is a ligand, mediates cell responses to toxins such as dioxin (16).

In the current study, prolactinomas comprised 66% of the entire series, of which the majority were microadenomas in female patients (80%) that presented classically with either oligo/amenorrhea, galactorrhea, or headache. This is in keeping with previous data from surgical series and immunohistochemical studies of autopsy data (13, 17, 18). Despite the fact that the majority of prolactinomas were small, the attendant use of health care resources appears sizable, given the performance of multiple MRI/CT scans, dynamic pituitary function tests, and the frequent requirement for medical or surgical therapy. The management of other tumor types requires even greater resource use than for prolactinomas. High health care resource use in the setting of a much increased prevalence of pituitary adenomas represents an important issue for calculating medical and research budgets, although confirmation in formal pharmacoeconomic studies is required.

In conclusion, the current cross-sectional study indicates that clinically active pituitary adenomas occur relatively frequently in the general population. In contrast to autopsy and radiological studies, the current study included only patients that had a previous definitive diagnosis of a pituitary adenoma. The historical lack of mandatory reporting of benign brain tumors may have led to an underestimation of the prevalence of pituitary adenomas in large cancer registries. In the absence of registry data, larger cooperative studies using a similar intensive case finding approach to ours and involving diverse population samples from multiple centers could help to provide further information on the true prevalence of pituitary adenomas internationally.

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# Chapter Seven

## Clinical Characterization of Familial Isolated Pituitary Adenomas

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# Clinical Characterization of Familial Isolated Pituitary Adenomas

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**Context:** Familial pituitary adenomas occur rarely in the absence of multiple endocrine neoplasia type 1 (MEN1) and Carney complex (CNC).

**Objective:** Our objective was to characterize the clinical and genealogical features of non-MEN1/CNC familial isolated pituitary adenomas (FIPA).

**Design and Setting:** We conducted a retrospective study of clinical and genealogical characteristics of FIPA cases and performed a comparison with a sporadic population at 22 university hospitals in Belgium, Italy, France, and The Netherlands.

**Results:** Sixty-four FIPA families including 138 affected individuals were identified [55 prolactinomas, 47 somatotropinomas, 28 nonsecreting adenomas (NS), and eight ACTH-secreting tumors]. Cases were *MEN1/PRKAR1A*-mutation negative. First-degree relationships predominated (75.6%) among affected individuals. A single tumor phenotype occurred in 30 families (homogeneous), and heterogeneous phenotypes occurred in 34 families. FIPA cases were younger at diagnosis than sporadic cases ($P = 0.015$); tumors were diagnosed earlier in the first *vs.* the second generation of multigenerational families. Macroadenomas were more frequent in heterogeneous *vs.* homogeneous FIPA families ($P = 0.036$). Prolactinomas from heterogeneous families were larger and had more frequent suprasellar extension ($P = 0.004$) than sporadic cases. Somatotropinomas occurred as isolated familial somatotropinoma cases and within heterogeneous FIPA families; isolated familial somatotropinoma cases represented 18% of FIPA cases and were younger at diagnosis than patients with sporadic somatotropinomas. Familial NS cases were younger at diagnosis ($P = 0.03$) and had more frequently invasive tumors ($P = 0.024$) than sporadic cases.

**Conclusions:** Homogeneous and heterogeneous expression of prolactinomas, somatotropinomas, NS, and Cushing's disease can occur within families in the absence of MEN1/CNC. FIPA and sporadic cases have differing clinical characteristics. FIPA may represent a novel endocrine neoplasia classification that requires further genetic characterization. **(*J Clin Endocrinol Metab* 91: 3316–3323, 2006)**

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Abbreviations: CNC, Carney complex; CT, computed tomography; FIPA, familial isolated pituitary adenoma; IFS, isolated familial somatotropinoma; MEN1, multiple endocrine neoplasia type 1; MRI, magnetic resonance imaging; NS, clinically nonsecreting.

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PITUITARY ADENOMAS CAN occur in a familial setting in multiple endocrine neoplasia type 1 (MEN1) and Carney complex (CNC) (1). MEN1 is caused by an inactivating mutation in the *MEN1* gene on chromosome 11q13, which encodes the nuclear protein menin (2). The clinical presentation of MEN1 has been extensively characterized, and pituitary adenomas occur in about 40% of patients (3).All tumor phenotypes can occur, but prolactinomas predominate (3). Although more than 350 *MEN1* gene mutations

have been described, at least 10% of patients with clinical features of MEN1 do not have *MEN1* mutations (1). This suggests that other causes, such as mutations in the *MEN1* promoter region or in other regulatory genes, may be involved in the pathophysiology of MEN1. CNC is a rare condition that is linked in more than 50% of cases to an inactivating mutation in the gene encoding protein kinase A type 1A regulatory subunit (*PRKAR1A*) at 17q24; a second, as yet uncharacterized, locus at 2p16 has also been implicated (1, 4, 5). A key pathological abnormality in CNC pituitary disease is multifocal somatomammotropic cell hyperplasia (6). Hence, about 75% of patients with CNC exhibit subclinical increases in GH, IGF-I, and prolactin levels or abnormal responses to dynamic pituitary function tests, whereas clinical acromegaly occurs in less than 10% of patients (7, 8).

Isolated familial somatotropinoma (IFS) has been reported and is defined as the occurrence of at least two cases of acromegaly/gigantism in a single family in the absence of CNC or MEN1 (9); 108 affected members in 46 families have been described to date (10). To date, studies indicate that MEN1 and other candidate genes are unlikely to be directly implicated in the molecular pathogenesis of IFS (11–13). A disease locus for IFS appears to exist within a region of approximately 2.1 Mb on chromosome 11q13.3 (10, 14). Apart from IFS, a handful of reports of other isolated pituitary adenomas occurring in families have appeared in the literature (15–17). The scarcity of data regarding the characteristics of these families has limited our understanding of the clinical features and patterns of presentation of familial pituitary adenomas in patients without MEN1/CNC. To address these issues, we undertook an international, multicenter, retrospective study to identify non-MEN1/CNC families with familial isolated pituitary adenomas (FIPA). The aims of our study were to analyze the characteristics of FIPA and to describe their phenotypic presentation compared with a matched population of patients with sporadic pituitary tumors.

## Patients and Methods

### *Patient characteristics*

This retrospective study from 1970–2004 was undertaken to identify FIPA; this was defined as families with two or more confirmed members presenting with anterior pituitary tumors and no evidence of MEN1/CNC. In identified FIPA families, additional questioning was undertaken to search for other affected relatives. The study was performed at 22 centers in Belgium, France, Italy, and The Netherlands, and existing case records and databases were scrutinized for previously diagnosed familial pituitary tumor cases. Data from 15 patients have been reported previously (11, 13, 18–22). Informed consent for collection of personal and clinical data was obtained from all patients; data were anonymized before entry into a central database at the University of Liège, Belgium. Relevant demographic, genealogical, clinical, and radiological data were extracted from case records at individual study centers. Although the study period was from 1970–2004, families with patients who had been diagnosed with a pituitary tumor before 1970 were included.

Using available hormonal and clinical data, pituitary adenomas were classified as prolactinomas, GH-secreting, clinically nonsecreting (NS), ACTH-secreting, and TSH-secreting tumors, respectively. Gonadotropinomas with a high plasma FSH were included in the NS group. MEN1 was ruled out clinically by family history and the demonstration of a normal serum calcium and PTH in all cases, whereas in a subset of individuals. normal levels of gastrin, vasoactive intestinal polypeptide, and pancreatic polypeptide were also demonstrable. Patients with acromegaly underwent echocardiographic studies to exclude the presence of a cardiac myxoma related to CNC.

Neuroradiological studies consisted of a contrast-enhanced computerized tomography (CT) scan of the pituitary before 1986 and magnetic resonance imaging (MRI), predominantly with gadolinium enhancement, thereafter. Based on the maximal diameter, tumors were defined as microadenomas (≤10 mm), macroadenomas (>10 mm), and giant adenomas (>40 mm). Invasion of the cavernous or sphenoidal sinuses was assessed based on CT/MRI results and/or intraoperative findings.

### *Sporadic pituitary tumors*

We compared the demographic and tumor characteristics of FIPA cases with those of the corresponding sporadic non-MEN1, non-CNC phenotype. This series of patients with sporadic pituitary adenomas was obtained from registries of patients treated from 1970–2004 in Belgium (Liège) and Italy (L'Aquila, Rome), which comprised a total of 2600 patients. Each patient from the familial group was paired with two patients with the same tumor phenotype extracted randomly from the sporadic registries (Statview 5.1 software; SAS Institute, Cary, NC). A postextraction analysis was undertaken to ensure that the familial and sporadic groups were matched with respect to year at diagnosis for each tumor phenotype; this was done to exclude bias introduced by improvements in diagnostic methods over the study period.

### *Immunohistochemistry*

Among the group of patients that underwent surgery (n = 83), tumor tissue from 74 individuals was studied by immunohistochemistry for LH, FSH, TSH, GH, prolactin, ACTH, and α-subunit. GH-secreting adenomas were subclassified as pure GH-secreting, mixed GH/prolactin, or glycoprotein/GH adenomas, whereas NS adenomas were subclassified as null cell, gonadotroph-secreting, or silent adenomas. Silent adenomas were defined as tumors that were immunopositive for pituitary hormones in the absence of preoperative biochemical or clinical evidence of hormonal hypersecretion.

### *Genetic analysis*

Blood samples were collected in all available patients, and DNA was extracted from leukocytes. Germline mutations of the *MEN1* gene were excluded by direct sequencing of exons 1–10 in at least one affected member of each family. In addition, sequencing of the *PRKAR1A* gene was performed in one affected member of families with IFS. Informed consent for genetic studies was obtained in all cases.

### *Statistical analysis*

Unless otherwise specified, results are expressed in mean ± SD. Data were analyzed using Statview 5.1 software (SAS Institute). As noted above, to verify that the groups were correctly matched at time of diagnosis, a postextraction comparison of centile distributions of year at diagnosis in the familial and sporadic adenoma patient groups was performed. For patients with recurrent disease, only the characteristics at first presentation were retained for the study. Because different patterns of pituitary tumor phenotypes could present within the same kindred, families were divided into homogeneous (families presenting with a single tumor phenotype) and heterogeneous (at least two phenotypes per family) groups for subsequent analyses.

Intergroup analyses were performed to compare FIPA with sporadic adenomas and to distinguish between homogeneous and heterogeneous subgroups, whereas multiple comparisons were used for the comparison of homogeneous or heterogeneous tumors with their sporadic counterparts and for comparisons between tumor phenotypes (prolactinoma, somatotropinoma, NS adenoma, and Cushing's disease), respectively. The distributions of nominal data were compared using the $\chi^2$ test for single or multiple comparisons, whereas continuous variables were compared by the Mann-Whitney test for univariate analyses and by ANOVA followed by the Bonferroni/Dunn *post hoc* test for multivariate analyses. The analysis of parental transmission data was performed using $\chi^2$ to compare percentages of maternal/paternal transmission with the 50% theoretical value that would occur by chance; a $\chi^2$ test for multiple comparisons was used to analyze differences among tumor

phenotype subgroups. The level of statistical significance was $P < 0.05$ for the two-group analyses, whereas the $\alpha$-level was adjusted to compensate for multiple groups where necessary (*e.g.* $\alpha < 0.0167$ for three groups).

## Results

### *Demographics and disease characteristics*

A total of 64 families with isolated pituitary tumors were identified, which included 138 affected individuals (52 males, 86 females). Within the reference study centers, FIPA cases represented 1.9–3.2% of the total patient population with pituitary adenomas. The mean follow-up period for FIPA cases was 9.6 ± 8.0 yr (median, 7 yr; range, 1–44 yr). The sporadic group consisted of 288 patients (109 male, 179 female) with sporadic, nonfamilial, non-MEN1/CNC pituitary adenomas (Table 1). There was no difference between the FIPA and sporadic groups in terms of gender distribution, and the mean year at diagnosis in both groups was 1993. Prolactinomas and somatotropinomas were the most prevalent phenotypes among the familial group, accounting for nearly 75% of the entire series.

Fifty-five families had two affected members, eight families had three affected members, and one family had four affected members. First-degree relationships (parents, offspring, or siblings) predominated (103 of 138, 74.6%). The mean (± SD) total family size in the study was 15.4 ± 9.4 individuals, and the average degree of relatedness among the FIPA population was 0.62. When families were subdivided according to tumor phenotype, 30 families with 62 patients had homogeneous tumor expression; they consisted of 28 patients with prolactinoma in 14 families, 26 with somatotropinomas in 12 families, four with NS tumors in two families, and four patients with Cushing's disease in two families. In the 34 families (76 affected individuals) exhibiting heterogeneous tumor expression, up to three different tumor phenotypes were noted; every heterogeneous kindred had at least one prolactinoma or somatotropinoma.

### *Age at diagnosis*

The mean age at diagnosis was significantly lower in the familial group as compared with the sporadic group (38.4 ± 16.3 *vs.* 41.9 ± 15.1 yr, respectively; $P = 0.015$). This difference was predominantly because of the younger age of patients with IFS and familial NS adenomas compared with their sporadic counterparts (Table 1). Furthermore, the mean age at diagnosis in the homogeneous families was significantly lower than in the heterogeneous families ($P = 0.023$). In families distributed over two generations, tumors were diagnosed significantly earlier in the second generation compared with the first (Table 2; mean age at diagnosis, 29.0 ± 10.2 *vs.* 50.5 ± 14.2 yr, respectively; $P < 0.0001$). This generational effect was preserved after correction for homogeneous or heterogeneous in a multivariate analysis ($P < 0.0001$). Similarly, the second generation was diagnosed significantly earlier than the first generation in patients with prolactinomas, somatotropinomas, and NS adenomas occurring as part of FIPA families ($P \leq 0.02$). However, a generation effect independent of familial tumor status was seen only for prolactinomas ($P < 0.0001$).

### *Tumor characteristics*

There was no difference between FIPA and sporadic groups overall in terms of the frequency of micro- and macroadenomas, suprasellar extension, and invasiveness, although there was a trend toward a higher rate of cavernous sinus invasion in the FIPA group compared with the sporadic group ($P = 0.058$; Table 1). Macroadenomas were more frequent in heterogeneous than in homogeneous FIPA cases (71.5 *vs.* 52.5%; $P = 0.036$), perhaps related to the predominance of NS adenomas in the heterogeneous FIPA group and the low frequency of macroadenomas in the homogeneous prolactinoma group.

### *Individual tumor subtype characteristics*

The clinical characteristics of FIPA subgroups and comparison with their relative sporadic counterparts are summarized in Table 1.

### *Familial prolactinomas*

Prolactinomas were the most commonly observed tumor overall (39.9%), with 55 affected members in 40 FIPA families. The mean age at diagnosis was 32.6 ± 12.5 yr (range, 15–61 yr) with a female predominance (41 females and 14 males); the age and sex distributions of prolactinomas did not differ from those of sporadic prolactinomas. Prolactinomas were equally distributed between homogeneous families and heterogeneous families. Prolactinomas in homogeneous FIPA families were indistinguishable from sporadic prolactinomas, with 71.4% (20 of 28 patients) being females with microprolactinomas. All males (four of four) but only four of 24 females (16.7%) from homogeneous families had macroprolactinomas. In six of the 14 homogeneous prolactinoma families, mother and daughter were affected, and 83.3% of these had microprolactinomas.

Prolactinomas from heterogeneous FIPA families had more aggressive characteristics than their homogenous counterparts, with a larger maximal diameter ($P = 0.047$) and more frequent suprasellar extension ($P = 0.038$). Compared with their sporadic counterparts, heterogeneous prolactinomas were also significantly larger than their sporadic counterparts ($P = 0.0137$) and had a higher rate of suprasellar extension ($P = 0.004$). The percentage of males with prolactinomas tended to be higher in heterogeneous than in homogenous FIPA families (37 *vs.* 14.8%; $P = 0.053$); a male patient from a heterogeneous FIPA family developed a malignant prolactinoma, as described previously (22).

### *Familial somatotropinomas*

Familial somatotropinomas occurred in 47 patients divided among 31 families (34.1% of the series), were similarly distributed between homogeneous/IFS and heterogeneous FIPA families, and did not differ from sporadic cases in terms of demographic characteristics. Patients with IFS were more than 10 yr younger at diagnosis than those from either heterogeneous phenotype families ($P = 0.002$) or sporadic somatotropinoma cases ($P = 0.0023$); all five patients with gigantism belonged to IFS families. IFS patients also had more aggressive tumors, with extrasellar ($P = 0.023$) and

**TABLE 1.** Demographic and tumor characteristics in patients with FIPA (homogeneous and heterogeneous tumor presentation) and sporadic pituitary adenomas

| | Familial | Sporadic | Homogeneous familial | Heterogeneous familial | Familial *vs.* sporadic | Homogeneous *vs.* sporadic | Heterogeneous *vs.* sporadic | Homogeneous *vs.* heterogeneous |
|---|---|---|---|---|---|---|---|---|
| All tumors | | | | | | | | |
| n | 138 | 288 | 62 | 76 | | | | |
| Age at diagnosis (yr) | 38.4 ± 16.3 | 41.9 ± 15.1 | 34.2 ± 16.1 | 41.8 ± 15.8 | 0.015 | 0.0040 | NS | 0.023 |
| Sex | 52 M/86 F | 109 M/179 F | 21 M/41 F | 31 M/45 F | NS | NS | NS | NS |
| Macroadenomas (%) | 63.4 | 66.3 | 52.5 | 71.0 | NS | NS | NS | 0.036 |
| Maximal diameter (cm) (n) | 1.74 ± 1.23 (92) | 1.50 ± 1.6 (217) | 1.63 ± 1.27 (44) | 1.84 ± 1.20 (48) | NS | NS | NS | NS |
| Extrasellar extension (%) | 66/127 (53.4) | 141/281 (50.2) | 28/56 (50.0) | 38/71 (54.3) | NS | NS | NS | NS |
| Suprasellar extension (%) | 54/126 (42.9) | 113/277 (40.8) | 21/56 (37.5) | 33/70 (47.1) | NS | NS | NS | NS |
| Invasive (%) | 60/129 (46.5) | 111/282 (39.4) | 23/55 (41.8) | 37/74 (50.0) | NS | NS | NS | NS |
| Cavernous sinus invasion (%) | 42/118 (35.6) | 71/272 (26.1) | 20/54 (37.0) | 22/64 (34.4) | NS (0.058) | NS | NS | NS |
| Prolactinomas | | | | | | | | |
| n | 55 | 113 | 28 | 27 | | | | |
| Age at diagnosis (yr) | 32.6 ± 12.5 | 34.0 ± 13.2 | 32.4 ± 13.1 | 33.7 ± 13.2 | NS | NS | NS | NS |
| Sex | 14 M/41 F | 26 M/87 F | 4 M/24 F | 10 M/17 F | NS | NS | NS | NS (0.053) |
| Macroadenomas (%) | 45.5 | 47.8 | 35.7 | 55.5 | NS | NS | NS | NS |
| Maximal diameter (cm) (n) | 1.43 ± 1.12 (40) | 1.21 ± 0.91 (64) | 1.10 ± 0.69 (22) | 1.83 ± 1.50 (16) | NS | NS | 0.0137 | 0.047 |
| Extrasellar extension (%) | 24/54 (44.4) | 36/110 (32.7) | 11/28 (39.3) | 13/26 (50.0) | NS | NS | NS | NS |
| Suprasellar extension (%) | 16/53 (30.2) | 19/108 (17.6) | 5/28 (17.9) | 11/25 (44.0) | NS | NS | 0.004 | 0.038 |
| Invasive (%) | 22/55 (40.0) | 40/112 (36.4) | 9/19 (32.1) | 13/27 (48.1) | NS | NS | NS | NS |
| Cavernous sinus invasion (%) | 17/52 (32.7) | 19/85 (18.3) | 8/28 (28.6) | 9/24 (37.5) | 0.044 | NS | NS | NS |
| GH-secreting tumors | | | | | | | | |
| n | 47 | 97 | 26 | 21 | | | | |
| Age at diagnosis (yr) | 40.7 ± 19.2 | 44.1 ± 13.5 | 33.8 ± 18.9 | 49.3 ± 16.3 | NS | 0.0023 | NS | 0.002 |
| Sex | 24 M/23 F | 44 M/53 F | 13 M/13 F | 11 F/10 M | NS | NS | NS | NS |
| Macroadenomas (%) | 76.7 | 80.4 | 77.3 | 76.2 | NS | NS | NS | NS |
| Maximal diameter (cm) (n) | 1.80 ± 1.10 (26) | 1.58 ± 0.87 (64) | 2.16 ± 1.25 (10) | 1.23 ± 0.40 (16) | NS | NS | NS | NS |
| Extrasellar extension (%) | 17/38 (44.7) | 48/94 (51.0) | 13/20 (65.0) | 4/18 (22.2) | NS | NS | NS | 0.023 |
| Suprasellar extension | 16/38 (42.1) | 41/93 (44.0) | 12/20 (60.0) | 4/18 (22.2) | NS | NS | NS | 0.043 |
| Invasive (%) | 15/40 (37.5) | 36/94 (38.3) | 10/19 (52.6) | 5/21 (23.8) | NS | NS | NS | NS |
| Cavernous sinus invasion (%) | 10/34 (29.4) | 26/93 (27.9) | 8/18 (44.4) | 2/16 (12.5) | NS | NS | NS | NS |
| NS tumors | | | | | | | | |
| n | 28 | 59 | 4 | 24 | | | | |
| Age at diagnosis (yr) | 46.4 ± 15.3 | 54.0 ± 12.0 | 49.7 ± 13.3 | 45.8 ± 13.3 | 0.030 | NS | 0.0132 | NS |
| Sex | 13 M/15 F | 36 M/23 F | 2 M/2 F | 11 M/13 F | NS | NS | NS | NS |
| Macroadenomas (%) | 92.9 | 98.3 | 100 | 91.7 | NS | NS | NS | NS |
| Maximal diameter (cm) (n) | 2.50 ± 1.20 (20) | 2.16 ± 1.07 (40) | 3.9 ± 1.6 (3) | 2.28 ± 0.97 (17) | NS | NS | NS | NS |
| Extrasellar extension (%) | 24/27 (88.9) | 54/58 (93.1) | 4/4 (100) | 20/23 (86.9) | NS | NS | NS | NS |
| Suprasellar extension | 21/27 (77.8) | 52/57 (91.2) | 4/4 (100) | 17/23 (73.9) | NS | NS | NS | NS |
| Invasive (%) | 22/26 (84.6) | 34/57 (59.6) | 4/4 (100) | 18/22 (81.8) | 0.024 | NS | NS | NS |
| Cavernous sinus invasion (%) | 14/24 (58.3) | 23/56 (41.0) | 4/4 (100) | 10/20 (50.0) | NS | NS | NS | NS |
| ACTH-secreting tumors | | | | | | | | |
| n | 8 | 19 | 4 | 4 | | | | |
| Age at diagnosis (yr) | 33.9 ± 12.9 | 40.2 ± 14.2 | 33.7 ± 14.7 | 34.0 ± 13.1 | NS | NS | NS | NS |
| Sex | 2 M/6 F | 3 M/16 F | 2 M/2 F | 0 M/4 F | NS | NS | NS | NS |
| Macroadenomas (%) | 12.5 | 5.3 | 0 | 25.0 | NS | NS | NS | NS |
| Maximal diameter (cm) (n) | 0.9 ± 1.10 (6) | 0.54 ± 0.27 (18) | 0.40 ± 0.26 (3) | 1.4 ± 1.4 (3) | NS | NS | NS | NS |
| Extrasellar extension (%) | 1/8 (12.5) | 3/19 (15.6) | 0/4 (0) | 1/4 (25.0) | NS | NS | NS | NS |
| Suprasellar extension (%) | 1/8 (12.5) | 1/18 (5.5) | 0/4 (0) | 1/4 (25.0) | NS | NS | NS | NS |
| Invasive (%) | 1/8 (12.5) | 2/18 (11.0) | 0/4 (0) | 1/4 (25.0) | NS | NS | NS | NS |
| Cavernous sinus invasion (%) | 1/8 (12.5) | 1/18 (5.5) | 0/4 (0) | 1/ 4 (25.0) | NS | NS | NS | NS |

The denominator used to calculate the percentages in terms of tumor characteristics may differ from the overall number of tumors because of missing or irretrievable data in a minority of cases. The level of significance was set at $P < 0.05$ for comparisons of the entire familial group *vs.* the sporadic group and between homogeneous and heterogeneous familial groups and $P < 0.0167$ for comparisons of homogeneous or heterogeneous groups *vs.* the sporadic group. F, Female; M, male; NS, Not significant.

**TABLE 2.** Mean ages at diagnosis in the first and second generations of multigenerational families with FIPA according to tumor phenotype and pattern of presentation

| Tumor type | Age at diagnosis (first generation) | Age at diagnosis (second generation) | *P* value |
|---|---|---|---|
| Overall | | | |
| All phenotypes (n = 80 in 37 families) | 50.5 ± 14.2 | 29.0 ± 10.2 | <0.0001[a] |
| All homogeneous (n = 29 in 14 families) | 43.2 ± 12.8 | 24.4 ± 6.6 | |
| All heterogeneous (n = 51 in 23 families) | 54.4 ± 13.4 | 31.8 ± 11.0 | <0.0001[b] |
| Individual phenotypes | | | |
| All prolactinomas (n = 41 in 29 families) | 44.7 ± 8.3 | 26.7 ± 9.0 | <0.0001[a] |
| Homogeneous (n = 22 in 11 families) | 44.4 ± 9.2 | 23.3 ± 4.6 | |
| Heterogeneous (n = 19 in 18 families) | 45.7 ± 5.8 | 29.2 ± 10.7 | <0.0001[b] |
| All somatotropinomas (n = 20 in 16 families) | 53.3 ± 17.4 | 34.4 ± 12.3 | 0.02[a] |
| Homogeneous (n = 5 in 2 families) | 27.0 ± 18.4 | 26.0 ± 12.8 | |
| Heterogeneous (n = 15 in 14 families) | 58.1 ± 12.9 | 40.7 ± 8.2 | |
| All NS adenomas (n = 18 in 17 families) | 56.0 ± 15.0 | 32.1 ± 10.8 | 0.006[a] |
| Homogeneous (n = 2 in 1 family) | 63.0 | 32.0 | |
| Heterogeneous (n = 16 in 16 families) | 55.3 ± 15.6 | 32.2 ± 11.8 | |

The ages at diagnosis are expressed as mean ± SD.
[a] One-way comparison between the first and second generations.
[b] Significant difference in age after a two-way analysis after exclusion of a significant interaction between generation and familial subtype status.

suprasellar extension ($P = 0.043$) occurring more frequently than heterogeneous somatotropinoma families. Giant tumors (>40 mm maximal diameter; n = 2) occurred only in IFS kindreds.

*Familial NS adenomas*

Twenty-eight NS adenomas were observed in 26 families, including one case of a clinically active gonadotroph-secreting adenoma. Most NS adenomas (85.7%) occurred in heterogeneous families. NS adenomas were diagnosed nearly 8 yr earlier in the FIPA group as compared with the sporadic group ($P = 0.03$). NS adenomas in the FIPA group were more frequently invasive than sporadic cases (84.6 *vs.* 59.6%; $P = 0.024$).

*Familial ACTH-secreting adenomas*

Eight patients were affected by Cushing's disease in five FIPA families (homogeneous, four patients, including two siblings, in two families; heterogeneous, four patients in three families). The demographic and clinical characteristics of the familial and sporadic Cushing's disease groups did not differ significantly from one another.

*Immunohistochemistry*

The diagnosis of prolactinoma was confirmed by immunohistochemistry in all operated cases (n = 26). Immunohistochemical analysis of 40 available GH-secreting tumors demonstrated that 70% stained for GH only, 27.5% were mixed GH/prolactin staining, and 2.5% were mixed glycoprotein/GH-positive adenomas (2.5%). Among IFS families, tumors exhibiting immunopositivity for GH alone or for combinations of GH/prolactin and GH/glycoprotein hormones were found to occur. Immunohistochemistry of NS tumor tissue showed them to be null cell (n = 11), FSH/LH-positive (n = 7), GH-positive (n = 2) or β-endorphin/TSH β-subunit-positive (n = 1) adenomas. The two silent, GH-positive, NS adenomas were giant tumors from second-degree relatives in the same homogeneous NS phenotype family; the other homogeneous NS tumor family comprised a mother-son pair with silent gonadotroph-positive adenomas.

*Analysis of genealogical trees*

Pituitary adenomas occurred in one in seven individuals among the genealogies of the FIPA group overall, whereas in generations containing at least one affected member, pituitary tumors occurred at a rate of one in 2.8 individuals. A sizeable majority of patients in the FIPA group (103 of 138, 74.6%) were first-degree relatives of other affected members. Potential parental transmission was studied in 78 generations and was identified in 66 patients from 48 families. A total of 38 of 66 (57.6%) of cases indicated potential maternal transmission, which did not differ significantly from the 50% that would be expected by chance ($\chi^2 = 1.51$; $P$ value not significant). A significantly high level of potential maternal transmission was seen in 69.7% of the homogeneous FIPA group ($\chi^2 = 5.12$; $P < 0.05$). For all prolactinomas, potential maternal transmission occurred in 74.2% of cases ($\chi^2 = 7.26$; $P < 0.01$) because of frequent maternal transmission among homogeneous prolactinoma families (81.2%; $\chi^2 = 6.25$; $P < 0.02$). In IFS families, tumors occurred predominantly among siblings (65.4%), whereas data in FIPA families with NS tumors and Cushing's disease were insufficient for a robust assessment of parental transmission.

*Genetic studies*

Germline mutations in exons 1–10 of the *MEN1* gene were excluded in at least one affected member of each family (90 patients were tested); upstream and downstream elements related to *MEN1* were not assessed. Mutations in the

*PRKRA1A* gene were found to be negative in at least one member from 11 of 12 families with a homogeneous acromegaly phenotype. One homogeneous, two-member IFS family was screened for *MEN1* but was not available for *PRKRA1A* gene screening. However, the subjects had no cardiac, cutaneous, or endocrine abnormalities that were suggestive of CNC.

## Discussion

Pituitary tumors that occur in a familial setting due to MEN1, CNC, or IFS account for a minority of pituitary tumors overall. Scheithauer *et al.* (23) estimated that 2.7% of pituitary adenomas were due to MEN1, whereas acromegaly due to CNC or IFS together account for no more than a few hundred cases worldwide (1, 8, 9). The current study indicates that FIPA may account for a similar proportion (2.5%) of pituitary adenomas to MEN1, suggesting that hereditary tumor syndromes may play a role in the clinical presentation of about 5% of pituitary tumors (24).

Familial prolactinoma unrelated to MEN1 was first described by Berezin and Karasik (16). In this study, we have characterized familial prolactinoma further in a large number of patients (n = 55). In line with the epidemiology of sporadic pituitary tumors (25), prolactinoma was the most frequently encountered tumor in FIPA families (39.9% of cases). Prolactinomas in the heterogeneous FIPA group were larger and had more frequent suprasellar extension than the homogeneous group. This may have been because of the presence of relatively more males in the heterogeneous prolactinoma group, because the clinical course of prolactinoma is thought to be generally more aggressive in males (26). Somatotropinomas accounted for about one third of FIPA cases. Although IFS has been characterized previously, in this study we noted that acromegaly cases could also occur in conjunction with prolactinomas, NS adenomas, or Cushing's disease in the same family. Among the IFS group (18% of FIPA cases), six families with 14 affected individuals (two three-member families and four two-member families) have not been reported previously, increasing the reported number of IFS cases to 122 overall. Patients with IFS were diagnosed more than a decade before those with sporadic somatotropinomas or somatotropinomas occurring in heterogeneous FIPA families, whereas all five cases of gigantism occurred in families with IFS. These results are in keeping with the previously reported early median age at diagnosis (26 yr) in patients with IFS (27). Similarly, suprasellar/extrasellar extension was more frequent in IFS than in the heterogeneous FIPA groups, mirroring previous reports of acromegaly being more aggressive in younger subjects (28). In contrast to previous reports of male predominance in IFS, however, equal sex distribution of somatotropinomas was seen in our series. NS adenomas occurred predominantly as part of heterogeneous FIPA families and were diagnosed earlier and were more frequently invasive than sporadic NS adenomas. No particular characteristics could be observed in the eight patients with familial Cushing's disease.

All *MEN1* gene mutation studies performed in the patient population were negative, which strongly suggests that FIPA, including patients characterized as IFS, represents an entity/entities unrelated to MEN1. In support of this, serum calcium and PTH were normal in all cases, and no abnormalities developed throughout follow-up; normal gastrin, vasoactive intestinal polypeptide, and pancreatic polypeptide levels were observed in the subgroup tested. A pituitary-restricted form of CNC was largely ruled out by normal *PRKAR1A* sequences in IFS families, whereas CNC-related multifocal somatomammotropic hyperplasia (6) was not reported in pituitary tumor samples. Echocardiography was also negative for CNC-related atrial myxomas in patients with somatotropinomas. Although these data do not entirely exclude the role of a potential disease locus on chromosome 2p16, CNC in somatotropinoma patients in this series is unlikely.

How do the characteristics of FIPA compare with the respective characteristics of pituitary tumors that occur in the setting of MEN1? Vergès *et al.* (3) described the characteristics of 136 MEN1 patients with pituitary adenomas from a group of 324 patients with demonstrated *MEN1* mutations. Approximately 75% of pituitary adenomas were diagnosed before the age of 51 yr in FIPA, which is older than the corresponding age (46 yr) in MEN1 (3). Macroadenomas predominated in MEN1-related pituitary adenoma cases (85%), and invasion occurred in one third of tumors. These results are not mirrored by FIPA, because tumor size and invasion did not differ significantly between the overall FIPA group and the sporadic cases. Both FIPA and MEN1 pituitary adenomas have a female preponderance, with prolactinomas being the most frequent phenotype encountered. In MEN1, the percentage of prolactinomas (62.5%) is markedly higher than in FIPA (39.9%) (3). Somatotropinomas, on the other hand, accounted for 34.1% of tumors in FIPA, compared with only 8.8% of tumors in MEN1 (3).

The epidemiology of sporadic pituitary adenomas and aspects of the genealogical data in this series indicate that the occurrence of uncommon pituitary tumors within multiple members of individual families, as seen in FIPA (Fig. 1), is more likely to occur because of inherited factors rather than by chance. This has been noted previously with respect to IFS (27). The prevalence of pituitary tumors within FIPA family trees is higher (one in seven individuals) than the historical prevalence of clinically active pituitary adenomas in the general population (190–280 per million) (29, 30). Although a recent meta-analysis of MRI and autopsy data suggested a high frequency of pituitary adenomas, many of these tumors were detected incidentally (31). Analysis of genealogical trees suggests autosomal dominant inheritance with variable penetrance as a general model, as has been hypothesized previously for IFS (27). Additional epidemiological studies will be required to assess the frequency of clinically active pituitary tumors in the modern diagnostic era; such data would help to determine accurately disease risk ratios and familiality in FIPA. This point is relevant to the FIPA population overall, with 74.6% of cases occurring in first-degree relatives, and chance is even less likely in families with more than two affected members. The period of follow-up (34 yr) may not have been sufficient, however, to identify patterns of disease across many generations, which could bias reporting toward first-degree relatives. Maternal transmission

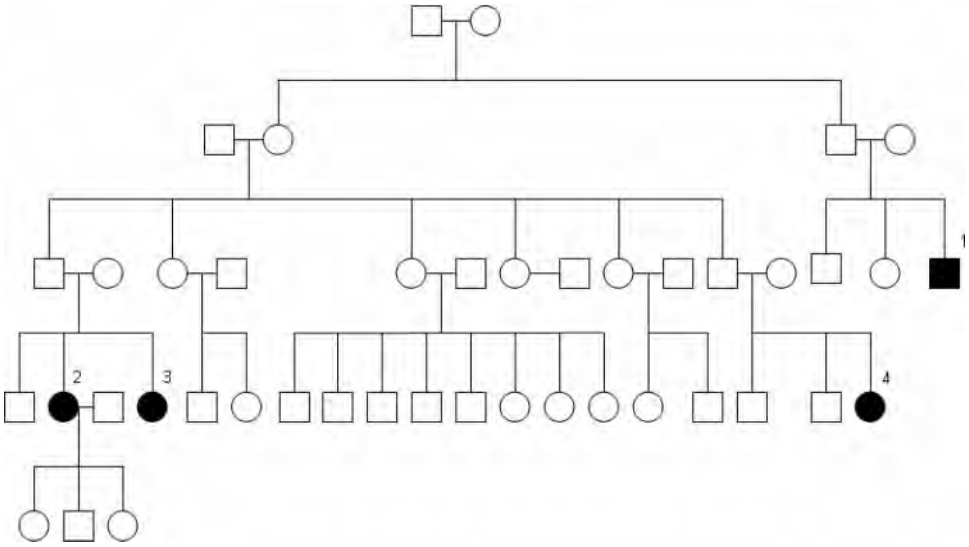


FIG. 1. The pedigree of a family exhibiting heterogeneous expression of pituitary tumors in four affected members across two generations. Affected members numbered 1 and 4 had acromegaly, whereas siblings 2 and 3 had a prolactinoma and Cushing's disease, respectively.

was significantly in excess of 50% in homogeneous prolactinoma families. The finding that patients from the second generation were diagnosed significantly earlier than the first generation in multigenerational FIPA families is intriguing and is suggestive of genetic anticipation, although a significant generational effect independent of familial tumor status could be documented reliably only for prolactinomas. Alternatively, other factors unrelated to the disease process itself may be involved, including increased awareness of symptoms within the family or improvements in diagnostic methods.

In conclusion, this multicenter, retrospective study indicates that FIPA may represent a new clinical entity/entities that includes IFS, and is unlikely to be related to MEN1 or CNC. Heterogeneous or homogeneous tumor phenotypes can occur within FIPA, which may indicate shared molecular pathophysiological mechanisms.

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# Chapter Eight

## *Aryl Hydrocarbon Receptor-Interacting Protein* Gene Mutations in Familial Isolated Pituitary Adenomas: Analysis in 73 Families

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# Aryl Hydrocarbon Receptor-Interacting Protein Gene Mutations in Familial Isolated Pituitary Adenomas: Analysis in 73 Families

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**Context:** An association between germline aryl hydrocarbon receptor-interacting protein (*AIP*) gene mutations and pituitary adenomas was recently shown.

**Objective:** The objective of the study was to assess the frequency of *AIP* gene mutations in a large cohort of patients with familial isolated pituitary adenoma (FIPA).

**Design:** This was a multicenter, international, collaborative study.

**Setting:** The study was conducted in 34 university endocrinology and genetics departments in nine countries.

**Patients:** Affected members from each FIPA family were studied. Relatives of patients with *AIP* mutations underwent *AIP* sequence analysis.

**Main Outcome Measures:** Presence/absence and description of *AIP* gene mutations were the main outcome measures.

**Intervention:** There was no intervention.

**Results:** Seventy-three FIPA families were identified, with 156 patients with pituitary adenomas; the FIPA cohort was evenly divided between families with homogeneous and heterogeneous tumor expression. Eleven FIPA families had 10 germline *AIP* mutations. Nine mutations, R16H, G47_R54del, Q142X, E174frameshift, Q217X, Q239X, K241E, R271W, and Q285frameshift, have not been described previously. Tumors were significantly larger ($P = 0.0005$) and diagnosed at a younger age ($P = 0.0006$) in *AIP* mutation-positive *vs.* mutation-negative subjects. Somatotropinomas predominated among FIPA families with *AIP* mutations, but mixed GH/prolactin-secreting tumors, prolactinomas, and nonsecreting adenomas were also noted. Approximately 85% of the FIPA cohort and 50% of those with familial somatotropinomas were negative for *AIP* mutations.

**Conclusions:** *AIP* mutations, of which nine new mutations have been described here, occur in approximately 15% of FIPA families. Although pituitary tumors occurring in association with *AIP* mutations are predominantly somatotropinomas, other tumor types are also seen. Further study of the impact of *AIP* mutations on protein expression and activity is necessary to elucidate their role in pituitary tumorigenesis in FIPA. (***J Clin Endocrinol Metab* 92: 1891–1896, 2007**)

PITUITARY ADENOMAS OCCUR relatively frequently based on autopsy and radiological series, while recent clinical data suggest a prevalence of approximately one case per thousand of the population (1, 2). Tumorigenesis of sporadic adenomas has been attributed to genetic and molecular abnormalities involving *gsp*, pituitary tumor transforming gene, and a pituitary derived truncated form of fibroblast growth factor receptor-4 (3–6). Pituitary adenomas due to hereditary causes are uncommon and can occur in the setting of multiple endocrine neoplasia-1 (MEN1) and Carney complex (CNC), due to mutations in the genes encoding menin (*MEN1*) and the R1a regulatory subunit of protein kinase A (*PRKAR1A*), respectively (7–9). However, *MEN1* and *PRKAR1A* mutations are an infrequent cause of sporadic pituitary tumors (10). Interest has also focused on isolated familial somatotropinomas (IFSs), which were thought to be linked to a locus close to that of *MEN1* on chromosome 11q13 (11). Vierimaa *et al.* (12) recently reported that inactivating mutations of the gene encoding aryl hydrocarbon receptor interacting protein (*AIP*) on chromosome 11q13.3 occurred in patients with pituitary tumors (mainly acromegaly) in the familial and sporadic settings. Recently, we described familial isolated pituitary adenomas (FIPA) in 64 families with two or more pituitary tumors in patients without *MEN1* or *PRKAR1A* mutations or clinical/biochemical features of MEN1/CNC that included a broader tumor phenotype than IFS (13). To address the potential role of *AIP* mutations in families having the FIPA phenotype, we undertook a genetic screening program involving both the original FIPA cohort and newly identified families.

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Abbreviations: AhR, Aryl hydrocarbon receptor; AIP, AhR-interacting protein; CNC, Carney complex; FIPA, familial isolated pituitary adenoma; FKBP-PPI, FK506-type binding protein type peptidyl-prolyl cis-trans isomerase; hsp90, heat shock protein 90; IFS, isolated familial somatotropinoma; MEN1, multiple endocrine neoplasia type 1; PRKAR1A, R1a regulatory subunit of protein kinase A; TPR, tetratricopeptide repeat.

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## Patients and Methods

This was an international study of *AIP* mutations in families having the FIPA phenotype performed across nine countries (Belgium, France, Italy, United States, Spain, Brazil, Argentina, The Netherlands, and Czech Republic). The clinical characteristics of the original FIPA cohort, involving 64 families (138 affected individuals), have been described previously (13). Clinical, biochemical, and genetic studies excluded MEN1 and CNC in all cases. Families with affected individuals that had the same tumor type throughout were termed "homogeneous," and the remaining families had different or "heterogeneous" pituitary tumors among affected subjects.

From the original FIPA cohort, 51 families took part in the current study of *AIP* mutations. In addition, 22 new, previously undescribed FIPA families without MEN1 or CNC were identified and included in the study. Relevant data on demographics and clinical characteristics were collected for each affected member of each family, including age at diagnosis, tumor size, and if available, pituitary hormone immunohistochemistry. Age at diagnosis and mean maximum tumor diameter in the FIPA group overall and for AIP mutation-affected subjects only were calculated as means, medians, 95% confidence intervals, and SD. In families in which a mutation in *AIP* was noted, genetic analysis for this mutation was offered in other affected and unaffected family members; clinical, hormonal, and radiological (magnetic resonance imaging) assessment of individuals that were positive for an *AIP* mutation was also offered. The study was conducted in accordance with the guidelines in The Declaration of Helsinki, approved by the Ethics Committee of the University of Liège, and all subjects provided informed written consent in their own language for the genetic analyses performed during the study.

### *Statistical analyses*

Statistical analyses were performed using GraphPad Instat for Macintosh (GraphPad Software, San Diego, CA). The Mann-Whitney test for univariate analyses, with a two-sided $P$ value, compared data from continuous variables (*e.g.* age at diagnosis and maximum tumor diameter) from subgroups of patients with and without *AIP* mutations. Sex distribution and the proportions of patients with microadenomas and macroadenomas in the *AIP* mutation-positive and negative groups were analyzed using the Fisher's exact test, with a two-sided $P$ value. A $P$ value of $<0.05$ was considered significant for all analyses.

### *AIP genetic analysis*

Genomic DNA was isolated from blood samples from at least one affected member of each FIPA family. The structure of *AIP* was based on Ensembl sequences ENST00000279146, ENSG00000110711, and ENSP00000279146. The primers used for the analysis of the *AIP* exonic and flanking intronic sequences are as reported by Vierimaa *et al.* (12). Each 25 $\mu$l PCR reaction contained 150 ng genomic DNA, 1 $\mu$M each primer, 1.5 mM $MgCl_2$, 10 mM Tris-HCl buffer (pH 8.3), 200 $\mu$M dNTPs, and 1.25 U FastStart *Taq* polymerase (Roche, Vilvoorde, Belgium). PCR conditions were 95 C for 10 min, followed by 30 cycles of 30 sec at 95 C, 30 sec at 68 C, and 20 sec at 72 C. PCR products were sequenced using ABI3100 and BigDye Terminator v3.1 technology (Applied Biosystems, Foster City, CA). A total of 100 blood samples from non-FIPA subjects in Belgium and France were analyzed to assess for polymorphisms in the *AIP* sequence.

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### *Accession numbers*

The accession numbers in GenBank for the novel *AIP* mutations reported in this study are: EF066502 (R271W), EF066503 (E174frameshift), EF066504 (delG47-R54), EF066505 (K241E), EF066506 (Q142X), EF066507 (Q217X), EF066508 (Q239X), EF066509 (Q285frameshift), and EF066510 (R16H).

## Results

### *Genetic screening*

A total of 156 subjects were identified among 73 families with the FIPA phenotype (see supplemental Table 1, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org). Eleven of 73 (15.1%) FIPA families were found to have 10 different germline mutations in the *AIP* gene (Fig. 1). Of these, nine *AIP* mutations in 10 families have not been reported to date. The characteristics of FIPA families that had *AIP* mutations are detailed in Table 1. There were three novel mutations that led to premature stop codons: Q142X (c.424C>T), Q217X (c.649C>T), and Q239X (c.715C>T). In addition, one three-member family that had a previously described R304X mutation (12) was identified (c.910C>T). Three missense mutations, R16H (c.47G>A), R271W (c.811C>T), and K241E (c.721A>G), were identified in four FIPA families; R271W was found in two two-member families (Table 1). One two-member family had an in-frame G47_R54del (c.138_161del24) mutation. A frameshift deletion, E174frameshift (c.517_521delGAAGA), that led to a stop codon after 21 incorrect amino acids was identified in a family with three affected members. A second frameshift mutation in a two-member family, Q285frameshift (c.854_857delAGGC), was followed by a stop codon after 17 incorrect amino acids.

### *Characteristics of FIPA cohort*

Demographic details and the phenotypic patterns of tumors seen are outlined in Tables 2 and 3, respectively. Briefly, families were divided equally (n = 78 each) among homogeneous and heterogeneous FIPA patterns; two-member homogeneous prolactinoma (n = 18) and somatotropinoma (n = 14) families were the most frequent. All but one heterogeneous FIPA family had at least one member with a prolactinoma or a somatotropinoma. Mean age at diagnosis was significantly lower in subjects with *AIP* mutations (n = 26 subjects) as compared with those without *AIP* mutations (n = 130 subjects) (25.7 ± 11.3 *vs.* 38.8 ± 16.8 yr, respectively; $P = 0.0006$). Mean maximum tumor diameter was significantly larger in the group with *AIP* mutations (24.6 ± 10.7 mm) than those without (14.5 ± 10.1 mm; $P = 0.0005$). Although the proportion of patients with macroadenomas was higher in the *AIP* mutation-positive group (88.5%) as compared with the *AIP* mutation-negative group (71.2%), this difference did not reach statistical significance.

### *AIP mutation screening in FIPA families*

Family members of subjects with pituitary adenomas and *AIP* mutations were contacted whenever possible and underwent genetic screening. Subjects that were positive for an *AIP* mutation were offered clinical assessment and hormonal screening. A total of 45 apparently unaffected relatives were screened, and nine individuals (mean age 39.7 yr; range 16–71) from five different families were found to be positive for mutations in *AIP*. These asymptomatic subjects did not have signs or symptoms suggestive of pituitary tumors, while hormonal and radiological screening was unremarkable.

## Discussion

This study involving an extensive cohort of 73 families having the FIPA phenotype has identified a total of 11 families having 10 mutations in the *AIP* gene; nine of these mutations were previously undescribed. The current study extends our

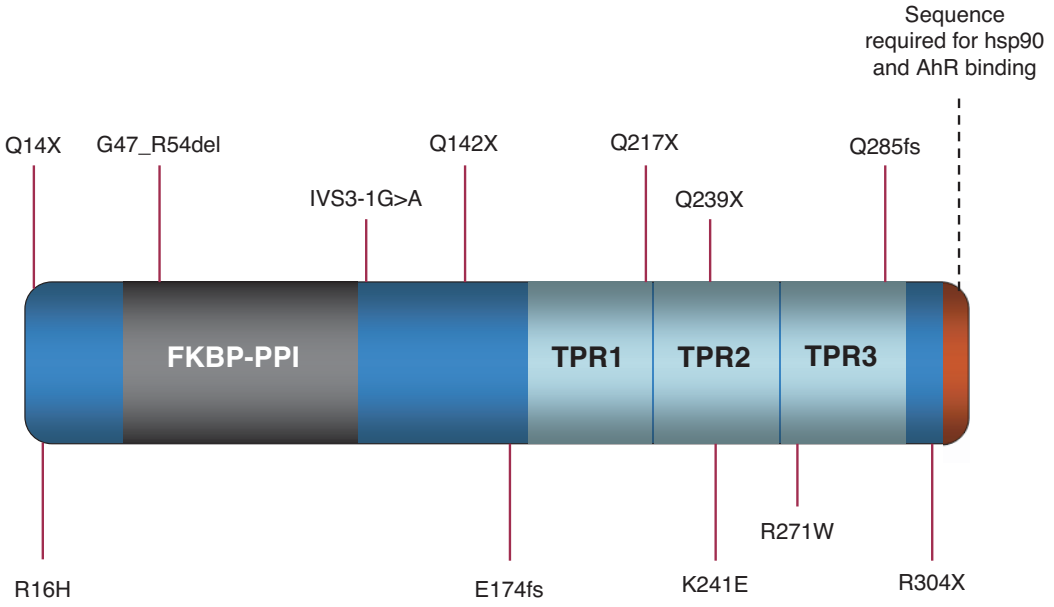


FIG. 1. Representation of *AIP* protein sequence with the position of gene mutations noted in the FIPA cohort and other studies indicated. The FKBP-PPI domain (amino acids 29–121) is shown in *gray*, and the three TPR domains (amino acids 189–296) are in *light blue*. The final carboxy-terminal amino acids that are necessary for interactions of *AIP* with hsp90 and AhR are shown in *orange*.

**TABLE 1.** Disease characteristics in 11 FIPA families having *AIP* mutations

| *AIP* mutation | No. of affected members | Relation between members | Disease type | Age at diagnosis (yr) | Tumor characteristics | Preoperative hormonal profile | Immunohistochemistry |
|---|---|---|---|---|---|---|---|
| R16H | 2 | a. First cousin | Acromegaly | 46 | Micro | ⇑GH, ⇑PRL | Not operated |
| | | b. First cousin | Acromegaly | N/A | Micro | ⇑GH/IGF-I | Not operated |
| G47_R54del | 2 | a. Brother | Acromegaly | 28 | Macro, invasive | ⇑GH/IGF-I | N/A |
| | | b. Brother | Acromegaly | 25 | Macro, invasive | ⇑GH/IGF-I | N/A |
| Q142X | 4 | a. Brother | Gigantism | 17 | Macro | ⇑GH | N/A |
| | | b. Brother | Acromegaly | 29 | Macro | ⇑GH | +GH |
| | | c. Sister | Acromegaly | 17 | Macro | ⇑GH | N/A |
| | | d. Daughter of b. | Prolactinoma | N/A | Micro | ⇑PRL | Not operated |
| E174fs | 3 | a. Brother | Acromegaly | 17 | Macro, invasive | ⇑GH/IGF-I/⇑PRL | +GH/+PRL |
| | | b. Sister | Acromegaly | 25 | Macro, invasive | ⇑PRL/slight ⇑IGF-I | Not operated |
| | | c. Aunt | Acromegaly | 35 | Macro, invasive | ⇑GH/IGF-I/⇑PRL | Not operated |
| Q217X | 2 | a. Brother | Acromegaly | 29 | Macro, invasive | ⇑GH/IGF-I, ⇑PRL | +GH, +PRL |
| | | b. Sister | Acromegaly | 24 | Macro | ⇑GH/IGF-I, ⇑PRL | +GH |
| Q239X | 2 | a. Father | Gigantism | 14 | Macro | ⇑GH | N/A |
| | | b. Son | Gigantism | 15 | Macro | ⇑GH/IGF-I | +GH |
| K241E | 2 | a. Brother | Prolactinoma | 40 | Macro, invasive | ⇑PRL | +PRL |
| | | b. Sister | Nonsecreting | 53 | Macro, invasive | Hypopituitarism | +LH/αSU |
| R271W | 2 | a. Father | Acromegaly | 42 | Macro | ⇑GH | N/A |
| | | b. Son | Acromegaly | 29 | Macro | ⇑GH/IGF-I | +GH |
| R271W | 2 | a. Mother | Acromegaly | 22 | Macro | ⇑GH | +GH, −PRL |
| | | b. Son | Prolactinoma | 10 | Macro | ⇑PRL | N/A |
| Q285fs | 2 | a. Brother | Acromegaly | 32 | Macro, invasive | ⇑GH/IGF-I, ⇑PRL | +GH, +FSH |
| | | b. Brother | Gigantism | 20 | Macro, invasive | ⇑GH | +GH, +PRL |
| R304X | 3 | a. Sister | Acromegaly | 19 | Macro, invasive | ⇑GH/IGF-I, ⇑PRL | +GH |
| | | b. Sister | Acromegaly | 21 | Macro, invasive | ⇑GH/IGF-I | +GH/PRL |
| | | c. Nephew of a. | Incipient gigantism | 9 | Macro | ⇑GH/IGF-I | Not operated |

N/A, Not applicable; PRL, prolactin; αSU, α-subunit.

understanding of the type of tumors associated with *AIP* mutations in the familial setting and increases the number of known *AIP* mutations associated with FIPA from three to 12. The clinical characteristics of this larger FIPA cohort are in line with our previous data indicating a relative predominance of prolactinomas and somatotropinomas in FIPA, and an early age at diagnosis, particularly in subjects with somatotropinomas (13). Furthermore, in this study we note that tumors in patients with *AIP* mutations have a significantly larger mean diameter than those in *AIP* mutation-negative patients, which could reflect a more aggressive disease profile. In the study by Vierimaa *et al*. (12) the pituitary tumors seen in families with *AIP* mutations were somatotropinomas or mixed GH/prolactin-secreting tumors. We found that while the majority of FIPA families with *AIP* mutations had somatotropinomas or mixed GH/prolactin-secreting tumors, one family included a subject with a nonsecreting tumor. This nonsecreting tumor was immunohistochemically negative for both GH and prolactin, and occurred in conjunction with a prolactinoma in the other affected family member. Hormonal patterns at diagnosis in "somatotropinoma" subjects with *AIP* mutations in FIPA families showed that 13 had GH hypersecretion, and eight had elevated GH and prolactin. The three subjects with prolactinomas had only hyperprolactinemia at diagnosis, while the subject with the nonsecreting tumor had hypopituitarism. An identical mutation (R271W) was associated with somatotropinomas in two adults in one family, and with a somatotropinoma (prolactin immunohistochemistry negative) and a macroprolactinoma (in a 10-yr-old child) in another family. Some heterogeneity in immunohistochemical patterns was also evident, with tumors from seven somatotropinoma patients having GH positivity only, four showing GH and prolactin staining, and one stained for GH and FSH.

Vierimaa *et al*. (12) undertook an extensive and detailed study of multiple genes to assess linkage to pituitary adenomas occurring in a familial setting, finally identifying *AIP* as being associated with pituitary adenomas in large, well-described kindreds in Finland. In that study an *AIP* mutation was identified in one family from Italy, but two other families with IFS from Germany and Turkey had normal *AIP* sequences (12). Our

**TABLE 2.** Demographic description of the FIPA cohort and the subgroup having *AIP* mutations

| | *AIP* mutation positive | *AIP* mutation negative | *P* value |
|---|---|---|---|
| No. of families | 11 | 62 | |
| No. of subjects | 26 | 130 | |
| Sex | | | |
| Males, n (%) | 15 (57.7) | 57 (43.8) | NS |
| Females, n (%) | 11 (42.3) | 73 (56.2) | NS |
| Median age (yr) at diagnosis (mean ± SD) | 24.5 (25.7 ± 11.3) | 36.0 (38.8 ± 16.8) | 0.0006 |
| Median maximum tumor diameter (mm) (mean ± SD) | 24.0 (24.6 ± 10.7) | 10.0 (14.5 ± 10.1)[a] | 0.0005 |
| Macroadenomas, n (%) | 23 (88.5) | 89 (71.2)[a] | NS |

NS, Clinically nonsecreting adenoma.
[a] Tumor size classification was not present for five individuals in the *AIP* mutation negative group.

**TABLE 3.** Tumor types in homogeneous and heterogeneous FIPA families overall and in the subgroup of families with *AIP* mutations

| Tumor type | Affected members per family | No. of families in FIPA cohort | No. of affected subjects in FIPA cohort | No. of families with *AIP* mutations | No. of affected subjects with *AIP* mutations |
|---|---|---|---|---|---|
| Homogeneous FIPA families | | | | | |
| Prolactinoma | 2 | 18 | 36 | 0 | 0 |
| Somatotropinoma | 2 | 14 | 28 | 6 | 12 |
| | 3 | 2 | 6 | 2 | 6 |
| Cushing's disease | 2 | 2 | 4 | 0 | 0 |
| NS-adenoma | 2 | 1 | 2 | 0 | 0 |
| Gonadotropinoma | 2 | 1 | 2 | 0 | 0 |
| **Homogeneous FIPA total** | | **38** | **78** | **8** | **18** |
| Heterogeneous FIPA families | | | | | |
| Prolactinoma-somatotropinoma | 2 | 8 | 16 | 1 | 2 |
| | 4 | 1 | 4 | 1 | 4 |
| Prolactinoma-NS-adenoma | 2 | 8 | 16 | 1 | 2 |
| | 3 | 1 | 3 | 0 | 0 |
| Somatotropinoma-NS-adenoma | 2 | 6 | 12 | 0 | 0 |
| Prolactinoma-somatotropinoma-NS-adenoma | 3 | 2 | 6 | 0 | 0 |
| Prolactinoma-gonadotropinoma | 2 | 3 | 6 | 0 | 0 |
| Somatotropinoma-prolactinoma-Cushing's disease | 4 | 1 | 4 | 0 | 0 |
| Somatotropinoma-prolactinoma-gonadotropinoma | 3 | 1 | 3 | 0 | 0 |
| Somatotropinoma-gonadotropinoma | 2 | 1 | 2 | 0 | 0 |
| Somatotropinoma-thyrotropinoma | 2 | 1 | 2 | 0 | 0 |
| Prolactinoma-Cushing's disease | 2 | 1 | 2 | 0 | 0 |
| NS-adenoma-Cushing's disease | 2 | 1 | 2 | 0 | 0 |
| **Heterogeneous FIPA total** | | **35** | **78** | **3** | **8** |

NS, Clinically nonsecreting adenoma.

data from screening a large, diverse population indicate that *AIP* mutations occur in about 15% of families in the FIPA cohort. The majority of FIPA families had normal germline *AIP* sequences, even those with three or four affected subjects. In particular, of the 16 FIPA families with homogeneous presentation of acromegaly (IFS), half were negative for *AIP* mutations, indicating that this gene does not readily explain IFS in its entirety. Other, as yet unidentified, genetic mutations may be involved in producing the FIPA clinical phenotype. The evidence to date suggests that mutations in *AIP* may be linked to the expression of a variety of tumor types. Although somatotropinomas predominate among FIPA families with *AIP* mutations, both pure GH and mixed GH-prolactin secretion and immunohistochemical staining occur commonly, even within the same family. Heterogeneous expression of tumors in FIPA tumor, including prolactinomas or nonsecreting adenomas, can occur in association with *AIP* mutations. The FIPA cohort contained few patients with less common pituitary tumors such as Cushing's disease and only one patient with a TSH-secreting adenoma; these were negative for *AIP* mutations. Therefore, it remains to be seen whether *AIP* mutations can also occur in families with Cushing's disease or TSH-secreting adenomas.

A Q14X mutation was the one most frequently seen in the Finnish patients studied, and both familial and sporadic cases were associated with this germline mutation; tumor analysis indicated loss of heterozygosity at the *AIP* locus (12). One other mutation, IVS3–1G>A, was reported in a sporadic case of acromegaly. In the current study these mutations were not identified in our international series of FIPA families. This, allied with the recent report of the absence of these mutations in sporadic pituitary tumor patients treated in the United States, suggests that these mutations may be characteristic of the Finnish population (14). This would not be unusual in terms of clinical genetics because Finland is known to be relatively genetically homogeneous and subject to founder effects (15). The role of extensive genealogic analysis such as that undertaken by Vierimaa *et al.* (12) to identify distant links among various affected families is important. We describe an Italian FIPA family with an R304X mutation (c.910C>T), the same mutation reported in an apparently unrelated family elsewhere in Italy (12). Further studies may highlight whether specific patterns of *AIP* mutations occur among specific geographical or cultural groups.

The impact of reported mutations in *AIP* on protein expression and function remains to be determined. Data on the structural components of AIP from *in vitro* studies provide some indicators in that regard. AIP is a protein of 330 amino acids in length, and contains conserved domains that include three tetratricopeptide repeat (TPR) domains and a FK506 binding protein-type peptidyl-prolyl cis-trans isomerase (FKBP-PPI) domain that is analogous to a related domain found in immunophilin proteins. Although the function of the FKBP-PPI domain remains to be determined fully, the importance of the "carboxy half" of AIP (residues 154–330) has been well established (16). Mutation studies of the third TPR domain have revealed that it is necessary for interactions with both heat shock protein 90 (hsp90) and the aryl hydrocarbon receptor (AhR) (17). Point mutations of the third TPR domain in murine AIP, including Y268A, G272D, G272E, A284T, and F288A, lead to an AIP that cannot coimmunoprecipitate hsp90; of these, Y268A and G272D cannot coimmunoprecipitate AhR (18). A further mutation, K266A, also abrogated hsp90 binding but retained AhR binding, albeit at a decreased level (19). Other studies that removed the last 32 amino acids from the C-terminal of AIP also prevented hsp90 binding, while the removal of the last 17 amino acids at the C-terminal led to rapid AIP turnover within COS-1 cells (20). Alanine replacement of any of the final four amino acids or

deletion of the final five amino acids at the C terminus of AIP prevents AhR binding (19).

In families with mutations that led directly to stop codons (Q142X, Q217X, Q239X, and R304X), the mutated gene would not encode the third TPR domain, the carboxy terminal amino acids, or both correctly (see supplemental Fig. 1, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org). Two other frameshift mutations (Q285fs and E174fs) also led to premature stop codons 17 and 21 amino acids downstream, respectively, and the loss of the sequences coding for the hsp90 and AhR interaction sites on AIP. The G47_R54del mutation, which read in-frame thereafter, would be expected to delete a series of amino acids within the FKBP-PPI domain, which could interfere with the enzymatic function of this region. In FIPA families with missense mutations of *AIP*, the functional impact is somewhat more difficult to predict. R271W, K241E, and R16H were not found in 100 non-FIPA individuals screened for *AIP* polymorphisms. Two unrelated FIPA families had an R271W mutation in *AIP*. This arginine is highly conserved across species, including the mouse, and forms part of the critical third TPR domain. As noted previously, mutation studies in this region in the mouse are known to abrogate hsp90 or AhR binding, or both (17). Given the sequence identity between the human being and mouse in this important region, it appears reasonable to suggest that R271W could interfere with the interaction of AIP and hsp90/AhR in these subjects. Both K241 and R16 are conserved amino acids across a variety of species; however, the impact of such mutations on the structural and functional status of AIP remains to be determined.

In conclusion, the current study shows that *AIP* mutations occur in 15% of families with the FIPA phenotype. *AIP* mutations that may abrogate expression or function of *AIP* protein could impact subsequent AhR responses to cellular and environmental signals, although *AIP* modulates a variety of other cellular signals (*e.g.* phosphodiesterases, cAMP) that may be involved in tumorigenesis. Experimental studies to assess AIP protein expression, receptor interactions, and xenobiotic responses will be useful in determining the precise effect on pituitary tumorigenesis of the multiple *AIP* mutations now identified.

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# Supplemental Tables and Figures

## Legends

Supplemental Table 1 - Description of affected members from 73 families exhibiting the FIPA phenotype. hom = homogeneous tumor pattern within a family; het = heterogeneous tumor pattern within a family

Supplemental Figure 1 - Sequencing chromatographs of the novel AIP mutations: R16H, Q217X, K241E, Q285frameshift, Q142X, Q239X, R271W, E173frameshift, and G47_R54del.

| Family/Patient Number | | Sex | Relation to Index Case | Tumor Type | Family Type |
|---|---|---|---|---|---|
| | | | | | |
| 1 | a | f | | prolactinoma | hom |
| | b | f | daughter | prolactinoma | hom |
| 2 | a | f | | non secreting | het |
| | b | f | sister | prolactinoma | het |
| 3 | a | f | | non secreting | het |
| | b | m | uncle | prolactinoma | het |
| | c | m | uncle | somatotropinoma | het |
| 4 | a | f | | prolactinoma | hom |
| | b | f | daughter | prolactinoma | hom |
| 5 | a | f | | somatolactotrope | hom |
| | b | f | cousin | somatotropinoma | hom |
| 6 | a | m | | somatolactotrope | hom |
| | b | f | sister | somatotropinoma | hom |
| 7 | a | f | | prolactinoma | het |
| | b | m | father | non secreting | het |
| 8 | a | m | | non secreting | het |
| | b | m | brother | prolactinoma | het |
| 9 | a | m | | prolactinoma | hom |
| | b | f | cousin | prolactinoma | hom |
| 10 | a | f | | prolactinoma | hom |
| | b | f | aunt | prolactinoma | hom |
| 11 | a | f | | prolactinoma | hom |
| | b | m | nephew | prolactinoma | hom |
| 12 | a | f | | somatolactotrope | het |
| | b | f | daughter | prolactinoma | het |
| 13 | a | f | | prolactinoma | hom |
| | b | f | half sister | prolactinoma | hom |
| 14 | a | m | | prolactinoma | het |
| | b | f | sister | non secreting | het |
| 15 | a | f | | somatotropinoma | hom |
| | b | m | brother | somatotropinoma | hom |
| 16 | a | m | | somatotropinoma | hom |
| | b | m | brother | somatotropinoma | hom |

| Family/Patient Number | | Sex | Relation to Index Case | Tumor Type | Family Type |
|---|---|---|---|---|---|
| 17 | a | f | | somatotropinoma | hom |
| | b | f | cousin | somatotropinoma | hom |
| | c | m | nephew | somatotropinoma | hom |
| 18 | a | f | | somatotropinoma | het |
| | b | m | brother | somatotropinoma | het |
| | c | m | brother | somatotropinoma | het |
| | d | f | niece | prolactinoma | het |
| 19 | a | f | | non secreting | het |
| | b | f | aunt | prolactinoma | het |
| 20 | a | m | | non secreting | het |
| | b | f | cousin | somatotropinoma | het |
| 21 | a | f | | prolactinoma | het |
| | b | f | sister | Cushing's disease | het |
| | c | f | cousin | somatolactotrope | het |
| | d | m | cousin | somatotropinoma | het |
| 22 | a | f | | somatolactotrope | het |
| | b | f | daughter | prolactinoma | het |
| 23 | a | m | | prolactinoma | hom |
| | b | f | aunt | prolactinoma | hom |
| 24 | a | f | | prolactinoma | hom |
| | b | f | daughter | prolactinoma | hom |
| 25 | a | f | | somatotropinoma | hom |
| | b | f | cousin | somatotropinoma | hom |
| 26 | a | f | | prolactinoma | hom |
| | b | f | sister | prolactinoma | hom |
| 27 | a | m | | prolactinoma | hom |
| | b | m | grandson | prolactinoma | hom |
| 28 | a | m | | somatotropinoma | hom |
| | b | m | son | somatotropinoma | hom |
| 29 | a | f | | somatolactotrope | het |
| | b | f | daughter | thyrotropinoma | het |
| 30 | a | m | | prolactinoma | het |
| | b | m | son | somatolactotrope | het |
| 31 | a | m | | gonadotropinoma | het |

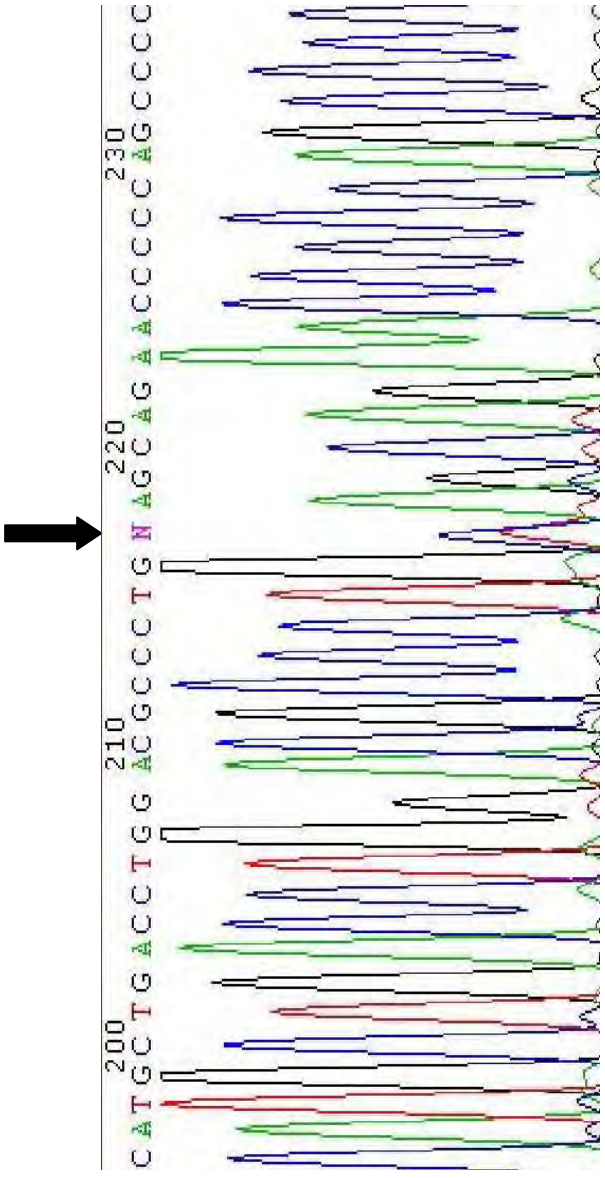
| Family/Patient Number | | Sex | Relation to Index Case | Tumor Type | Family Type |
|---|---|---|---|---|---|
| | b | f | sister | prolactinoma | het |
| 32 | a | m | | Cushing's disease | hom |
| | b | m | brother | Cushing's disease | hom |
| 33 | a | m | | somatotropinoma | het |
| | b | m | son | prolactinoma | het |
| 34 | a | m | | somatotropinoma | het |
| | b | f | sister | non secreting | het |
| 35 | a | f | | prolactinoma | hom |
| | b | f | daughter | prolactinoma | hom |
| 36 | a | f | | somatotropinoma | het |
| | b | f | daughter | non secreting | het |
| 37 | a | m | | prolactinoma | het |
| | b | m | son | somatotropinoma | het |
| | c | m | son | gonadotropinoma | het |
| 38 | a | m | | somatotropinoma | hom |
| | b | m | son | somatotropinoma | hom |
| 39 | a | f | | somatotropinoma | hom |
| | b | m | brother | somatotropinoma | hom |
| 40 | a | m | | prolactinoma | hom |
| | b | m | son | prolactinoma | hom |
| 41 | a | m | | somatotropinoma | het |
| | b | f | sister | prolactinoma | het |
| 42 | a | f | | somatotropinoma | het |
| | b | m | son | non secreting | het |
| 43 | a | f | | non secreting | het |
| | b | m | son | prolactinoma | het |
| 44 | a | m | | somatotropinoma | het |
| | b | m | son | somatotropinoma | het |
| 45 | a | f | | somatotropinoma | het |
| | b | m | father | non secreting | het |
| | c | m | brother | prolactinoma | het |
| 46 | a | m | | somatolactotrope | hom |
| | b | f | sister | somatolactotrope | hom |
| | c | f | aunt | somatotropinoma | hom |

| Family/Patient Number | | Sex | Relation to Index Case | Tumor Type | Family Type |
|---|---|---|---|---|---|
| 47 | a | m | | somatotropinoma | het |
| | b | m | brother | non secreting | het |
| 48 | a | m | | somatotropinoma | hom |
| | b | f | daughter | somatotropinoma | hom |
| 49 | a | m | | somatolactotrope | het |
| | b | f | daughter | prolactinoma | het |
| 50 | a | m | | somatotropinoma | hom |
| | b | m | brother | somatotropinoma | hom |
| 51 | a | f | | somatotropinoma | het |
| | b | f | daughter | prolactinoma | het |
| 52 | a | f | | prolactinoma | het |
| | b | f | mother | non secreting | het |
| 53 | a | f | | Cushing's disease | het |
| | b | f | daughter | prolactinoma | het |
| 54 | a | f | | non secreting | het |
| | b | m | brother | prolactinoma | het |
| | c | m | son | prolactinoma | het |
| 55 | a | m | | non secreting | het |
| | b | f | cousin | somatotropinoma | het |
| 56 | a | f | | somatotropinoma | hom |
| | b | m | son | somatolactotrope | hom |
| 57 | a | m | | non secreting | hom |
| | b | m | son | non secreting | hom |
| 58 | a | f | | prolactinoma | hom |
| | b | f | sister | prolactinoma | hom |
| 59 | a | m | | gonadotropinoma | het |
| | b | m | brother | somatotropinoma | het |
| 60 | a | m | | prolactinoma | hom |
| | b | f | daughter | prolactinoma | hom |
| 61 | a | f | | Cushing's disease | het |
| | b | m | cousin | non secreting | het |
| 62 | a | m | | somatotropinoma | hom |
| | b | f | daughter | prolactinoma | het |
| 63 | a | f | | prolactinoma | hom |

| Family/Patient Number | | Sex | Relation to Index Case | Tumor Type | Family Type |
|---|---|---|---|---|---|
| | b | f | sister | prolactinoma | hom |
| 64 | a | m | | somatotropinoma | hom |
| | b | m | son | somatotropinoma | hom |
| 65 | a | f | | prolactinoma | het |
| | b | f | daughter | non secreting | het |
| 66 | a | f | | gonadotropinoma | hom |
| | b | m | son | gonadotropinoma | hom |
| 67 | a | f | | gonadotropinoma | het |
| | b | f | daughter | prolactinoma | het |
| 68 | a | m | | prolactinoma | hom |
| | b | f | daughter | prolactinoma | hom |
| 69 | a | f | | Cushing's disease | hom |
| | b | f | cousin | Cushing's disease | hom |
| 70 | a | m | | gonadotropinoma | het |
| | b | m | son | prolactinoma | het |
| 71 | a | m | | somatotrope | hom |
| | b | m | cousin | somatotrope | hom |
| 72 | a | m | | prolactinoma | hom |
| | b | f | cousin | prolactinoma | hom |
| 73 | a | f | | prolactinoma | hom |
| | b | m | cousin | prolactinoma | hom |

Table 1

Q142X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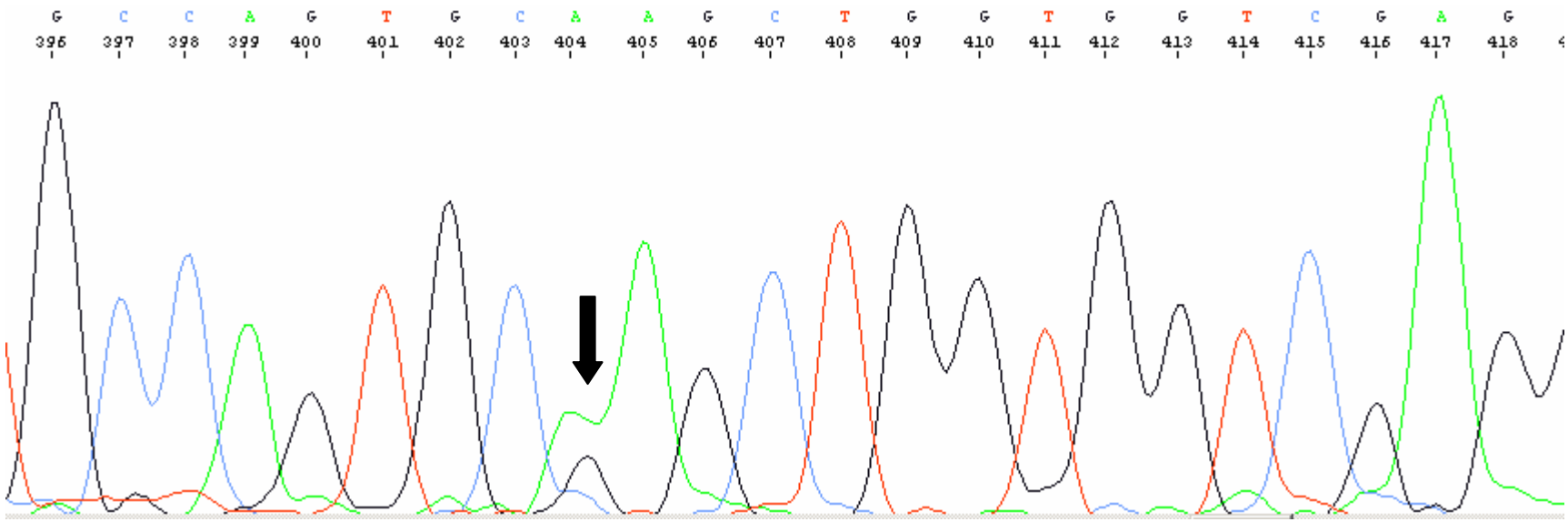
K241E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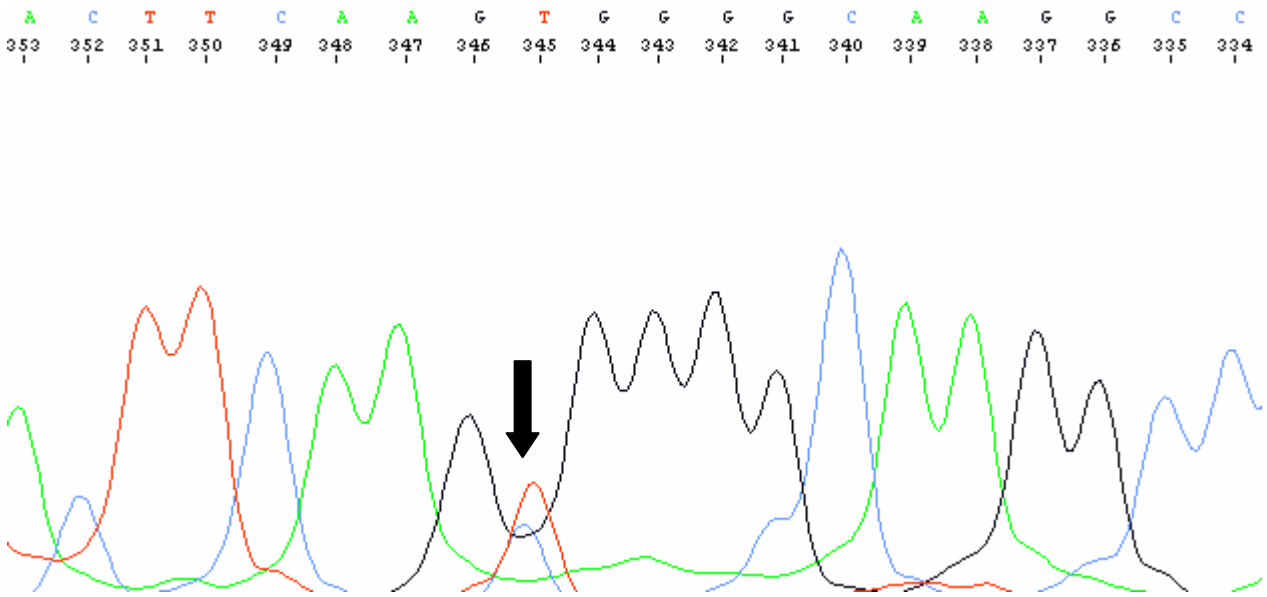
R271W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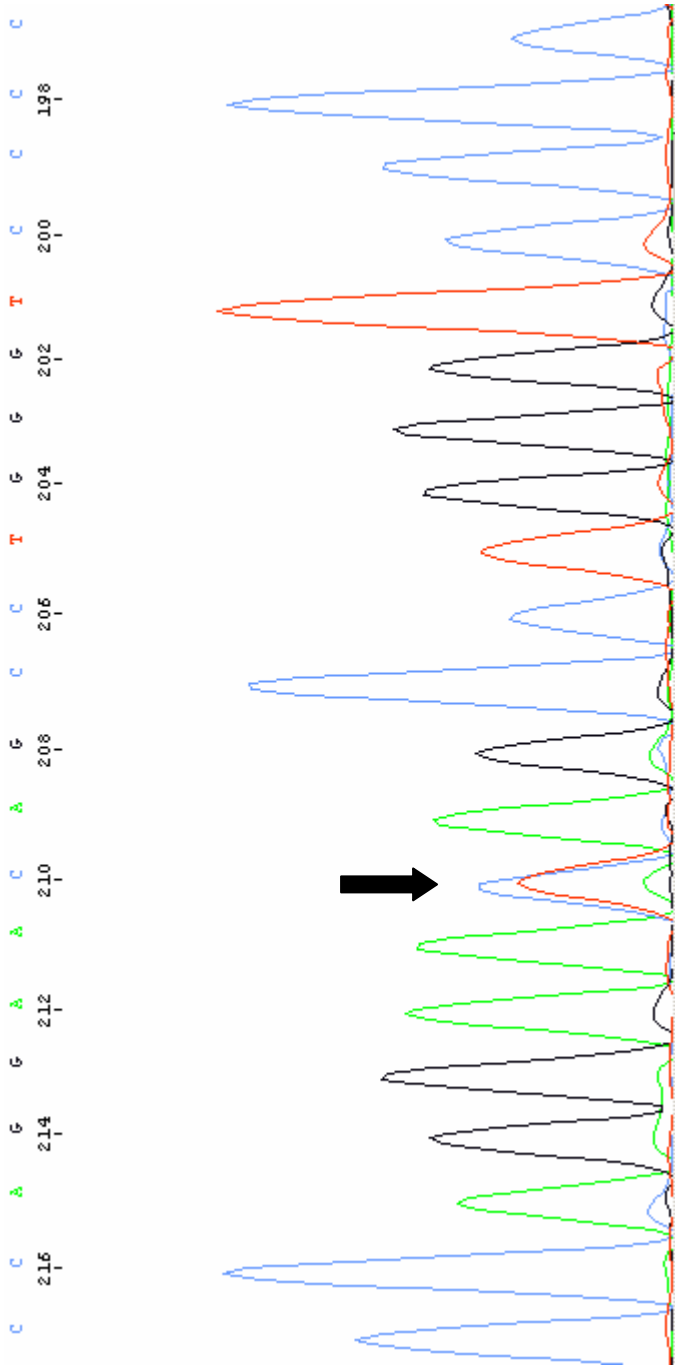
Q217X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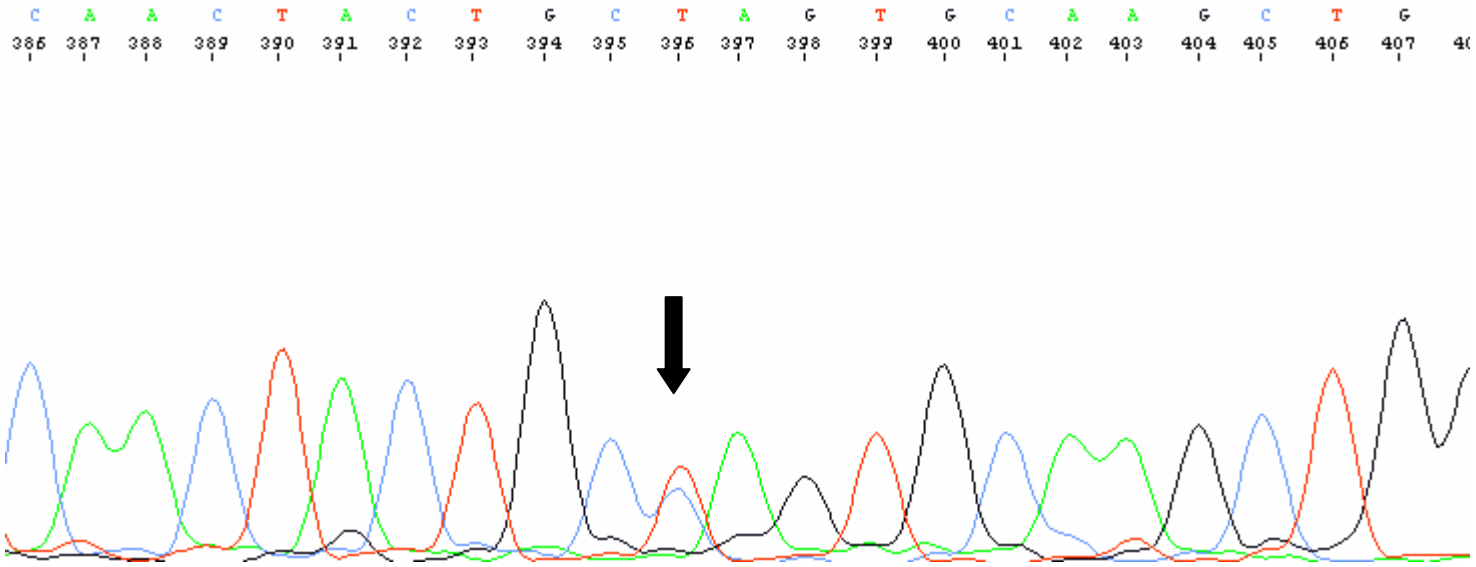
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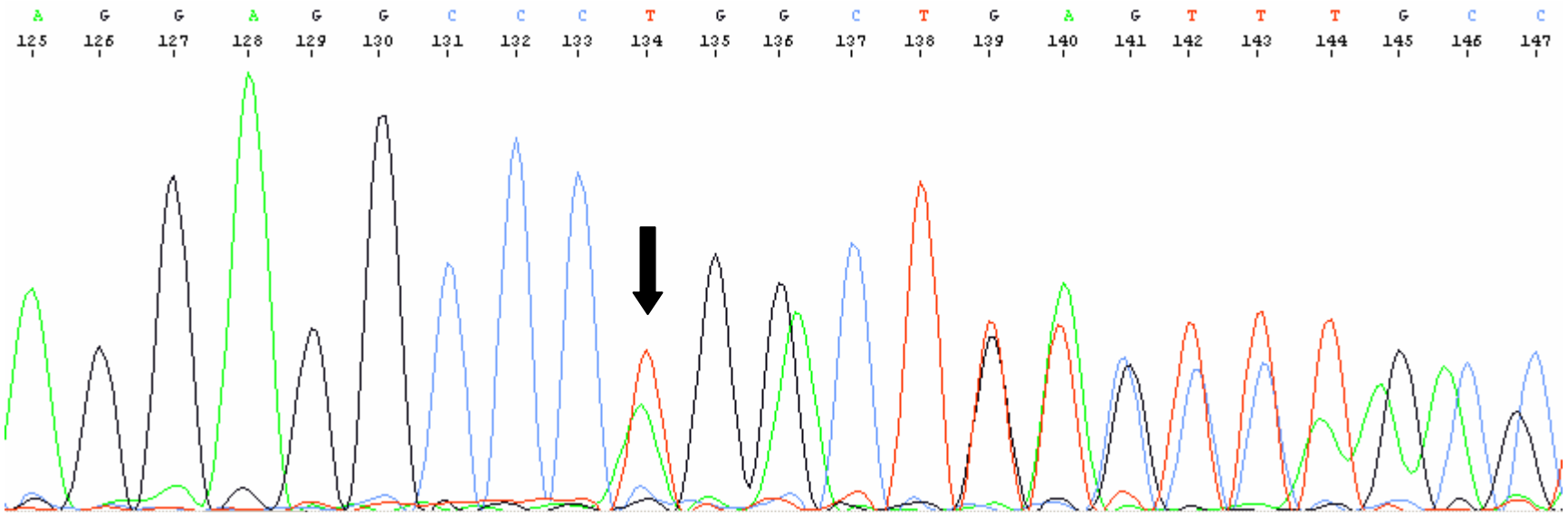

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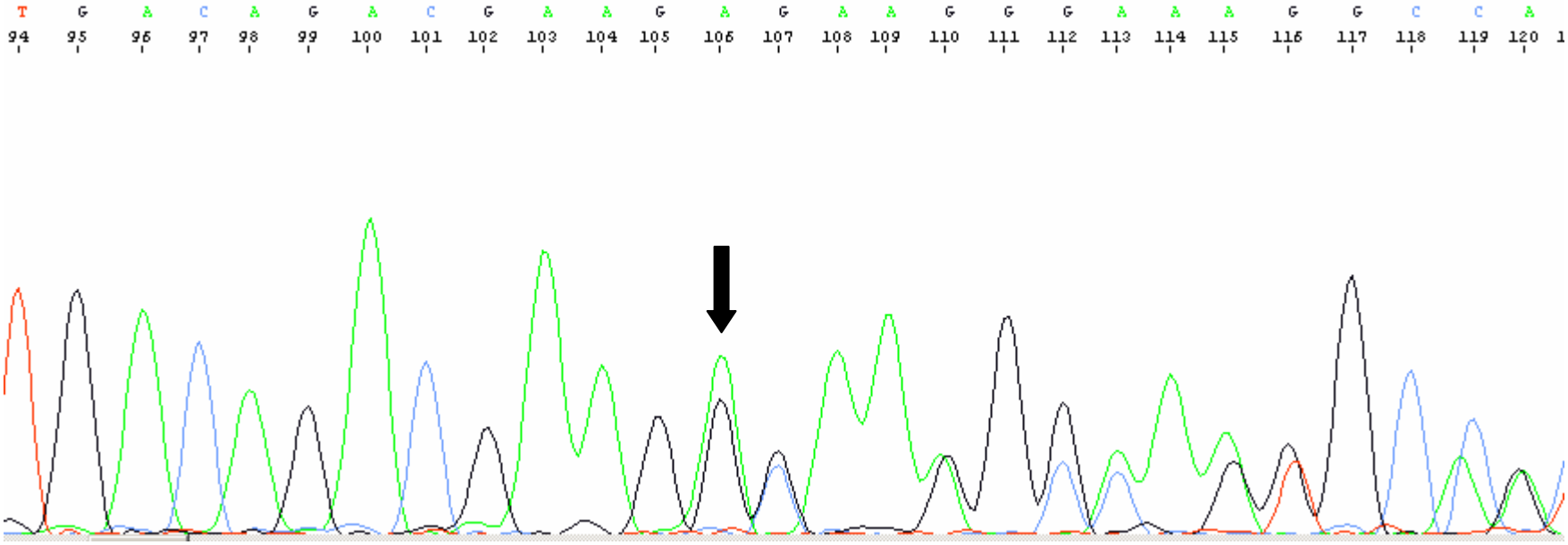

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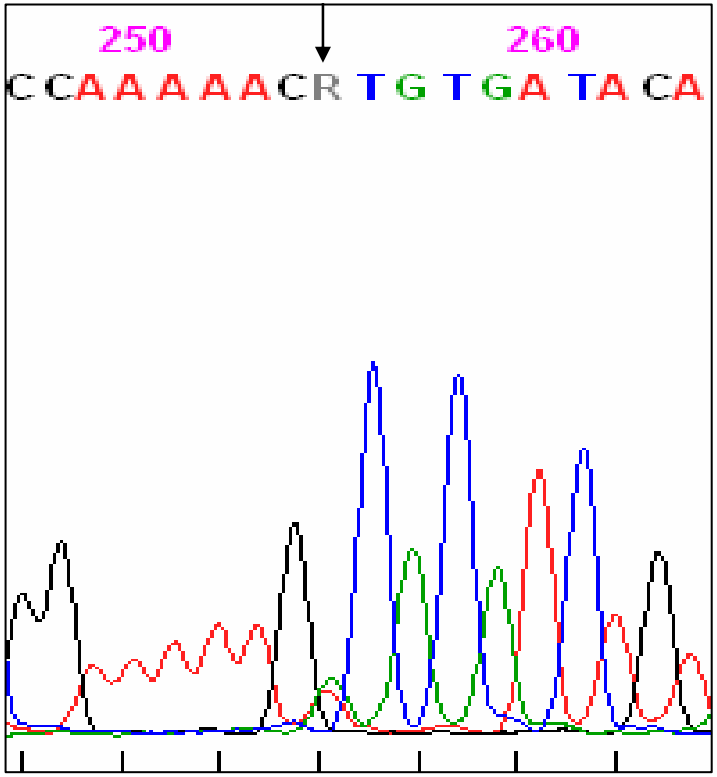

# Chapter Nine

## Variable Pathological and Clinical Features of a Large Brazilian Family Harboring a Mutation in the *Aryl Hydrocarbon Receptor Interacting Protein* Gene

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CLINICAL STUDY

# Variable pathological and clinical features of a large Brazilian family harboring a mutation in the aryl hydrocarbon receptor-interacting protein gene

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## Abstract

*Background*: Germline aryl hydrocarbon receptor-interacting protein (*AIP*) mutations occur in 15% of familial isolated pituitary adenoma (FIPA) cases. To date, studies have focused on the identification of such mutations in large international cohorts. Detailed genetic and clinical studies within *AIP* mutation-positive families have been limited.

*Aim*: To undertake a comprehensive study of a large Brazilian FIPA kindred with an E174 frameshift (E174fs) *AIP* mutation to assess clinical, hormonal, and radiological features in mutation carriers.

*Methods*: The kindred included 122 subjects across six generations; all underwent clinical examination. Genetic studies were performed to identify E174fs mutation carriers. E174fs-positive subjects underwent magnetic resonance imaging (MRI) and hormonal assessments.

*Results*: Of the ten germline *AIP* mutation carriers, three had pituitary tumors, while seven were asymptomatic carriers. Three patients with pituitary tumors showed variability in terms of tumor phenotype (two with acromegaly, one with prolactinoma, or mixed prolactin/GH-secreting tumor) and age at diagnosis; both patients with acromegaly had poor responses to octreotide. Tumor AIP immunohistochemistry from the operated patient showed decreased expression when compared with normal tissue. Two adult subjects with normal MRI had elevated IGF-I in the absence of other causes. A 2-year-old child with the E174fs mutation and a normal MRI had premature thelarche, ovarian development, and advanced bone age in the absence of other underlying causes.

*Conclusions*: The penetrance of pituitary tumors in *AIP* mutation-positive adult subjects was 33.3%, while clinical/hormonal features were variable. The features noted in *AIP*-mutation carriers in this kindred suggest that clinical characteristics of such carriers may extend beyond pituitary tumors.

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## Introduction

Pituitary adenomas comprise ~10% of intracranial tumors and recent estimates suggest that clinically apparent pituitary adenomas have a prevalence of ~1 in 1000 individuals (1, 2). The pathophysiology of pituitary adenomas is complex and a large number of genetic and molecular defects have been identified. The most relevant among these include mutations in the *Gsα* and *PTTG* genes (3, 4). In general, pituitary adenomas occur sporadically and the occurrence of pituitary adenomas in a familial or hereditary setting is rare; it is currently estimated that about 5% of pituitary adenomas are familial as reviewed by Beckers & Daly in this issue (5). Multiple endocrine neoplasia-I (MEN-I), caused by mutations in the *MEN-I* gene on chromosome 11q13 and Carney complex (CNC), due to mutations in the *PRKAR1A* gene on chromosome 17q22, are well recognized to cause familial pituitary adenomas (6, 7). Isolated familial somatotropinoma (IFS) is a rare condition separate from MEN-I and CNC that has been reported in over 50 families (8, 9). Familial isolated pituitary adenoma (FIPA), which comprises pituitary adenomas of all phenotypes occurring in a family setting, has been characterized in more than 90 families over the past decade (9). Vierimaa *et al*. reported that mutations in the aryl hydrocarbon receptor (AhR)-interacting protein (*AIP*) gene were associated with non-MEN-I, non-CNC familial pituitary adenomas (10). Recently, Daly *et al*. reported that *AIP* mutations occur



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in 15% of FIPA families (50% of those with IFS), while tumors in patients with *AIP* mutations are larger and are diagnosed at a significantly younger age than in FIPA patients without *AIP* mutations or in sporadic tumors (11). Current evidence suggests that the pituitary tumor phenotype of affected patients is heterogeneous within and among families (10–12). While somatotropinomas are the predominant finding in association with *AIP* mutations, somatolactotrope tumors, prolactinomas, and non-secreting adenomas have all been described in the FIPA setting (11); a patient with Cushing's disease was also reported to have an *AIP* mutation (12).

The studies mentioned above have focused on identifying mutations in *AIP* in large international cohorts of patients with sporadic or familial pituitary tumors. While data on *AIP* status have been reported retrospectively in previously described IFS families (13, 14), neither these studies nor the international cohort reports have presented detailed clinical, genetic, hormonal, and pathological analysis of disease status in families with *AIP* mutations. To this end, we undertook a comprehensive study of 122 members of a Brazilian FIPA kindred with an *AIP* mutation in order to determine the clinical status of mutation carriers, tumor characteristics (including immunohistochemistry), and disease penetrance.

## Methods

### Molecular genetic studies

Genomic DNA was isolated from the peripheral blood of participating subjects. We used ensembl sequences ENST00000279146, ENSG00000110711, and ENSP00000279146 to determine the sequence and structure of *AIP*. The primers used for the analysis of the *AIP* exonic and flanking intronic sequences are as reported by Vierimaa *et al.* (10). For the PCR, each 25 µl mixture contained 150 ng genomic DNA, 1 µM of each primer, 1.5 mM $MgCl_2$, 10 mM Tris–HCl buffer (pH 8.3), 200 µM dNTPs, and 1.25 U FastStart Taq polymerase (Roche). PCR conditions were as follows: 95 °C for 10 min followed by 30 cycles of 30 s at 95 °C, 30 s at 68 °C, and 20 s at 72 °C. PCR products were sequenced using ABI3100 and BigDye Terminator v3.1 technology (Applied Biosystems, Lennik, Belgium).

An E174 frameshift (E174fsi; c.517_521delGAAGA; GenBank accession number EF066503) *AIP* mutation was noted in three members of the family with pituitary adenomas; we have previously showed that the E174fs mutation is not present in DNA from screened normal volunteers (11).

The purpose of the study was explained and a comprehensive series of interviews with members of the kindred at various sites across Brazil were conducted to construct a complete genealogical tree of 122 members across six generations. The kindred was non-consanguineous. All consenting subjects provided a full medical history and underwent a clinical examination; these results were scrutinized for disease expression or patterns of disease. Beginning with the oldest living generations of the kindred, genetic studies were performed (after obtaining informed consent) to assess carrier status for the E174fs *AIP* mutation. In mutation carriers, all relatives underwent molecular genetic studies for *AIP* carrier status. Genetic studies were performed only in those subjects that were possible carriers of the E174fs *AIP* mutation based on the genetic status of their parents. *AIP* mutation carriers underwent further study involving non-enhanced magnetic resonance imaging (MRI) of the brain (2 mm cuts), static hormonal tests of the thyroid axis, cortisol, growth hormone (GH), insulin-like growth factor-I (IGF-I), and prolactin (PRL). All consenting *AIP* mutation carriers underwent a 2-h oral glucose tolerance test (OGTT) with measurement of GH.

### Immunohistochemistry

For determining the AIP immunohistochemistry of pituitary tumor tissue from one operated patient, paraffinized pituitary tumor sections were dewaxed in xylene and rehydrated using a descending ethanol series. Antigen retrieval was performed by microwave boiling in citrate buffer pH 6.0 for two periods of 5 min and one period of 3 min. Immunohistochemistry was performed using a mouse anti-human AIP mAb at a 1:500 dilution (Novus Biological, DBA Italia s.r.l, Segrate, Italy) and a multilink biotinylated antibody and the avidin–biotin peroxidase system according to the manufacturer's instructions (LSAB+ kit, DAKO Cytomation, Milan, Italy). For hormonal and tumor marker immunohistochemistry using the streptavidin–biotin system, the following dilutions of antibodies were employed, anti-GH (polyclonal 1:2000), anti-PRL (polyclonal 1:2000), p-53 (DO7 1:100), Ki-67 (Mib 1 1:100), and c-erb B2 (oncoprotein C 1:400). Reactions were developed with diaminobenzidine and counterstained with hematoxylin.

The study was conducted in accordance with the guidelines of the Declaration of Helsinki and the genetic analysis was approved by the Ethics Committee of the University of Liège. All subjects or guardians provided informed written consent for the investigations performed.

## Results

### Clinical features

***Case 1*** The index case, a male presented in 1997 at 17 years of age with a 3-year history of excessive linear growth (188 cm at presentation; mother's height

154 cm, father's height 160 cm), arthralgia, hyperhidrosis, weakness, headaches, and visual field impairment. On examination, the patient exhibited physical features typical of acromegaly, with soft tissue swelling, prognathism, and enlarged extremities (shoe size 52). MRI showed a pituitary macroadenoma measuring 36×45×36 mm with compression of the optic chiasma and bilateral cavernous sinus invasion (Fig. 1a). Basal GH was 51 ng/ml and no suppression was seen during an OGTT; the associated IGF-I level was 778 ng/dl (Table 1). The patient underwent transsphenoidal surgery in 1998, which reduced hormonal hypersecretion and improved vision. GH and IGF-I were elevated postoperatively and radiotherapy was performed in late 1998. Subcutaneous intermittent octreotide (300 μg/day) was initiated but tumor regrowth occurred by 2000. Despite increasing medical therapy to octreotide LAR 30 mg/month and cabergoline 2.0 g/week, the disease remained active and the patient gained 5 cm in height from 1998 to 2003. Late radiotherapy effects were seen from 2003 onwards with a decrease in IGF-I (although they remain elevated), and hypopituitarism (thyrotrope, corticotrope, and gonadotrope axes) was diagnosed in 2006. MRI follow-up in 2006 showed an empty sella.

***Case 2*** In 2002, the 25-year-old sister of the index case presented with secondary amenorrhea, galactorrhea, and headaches. On physical examination, there was mild soft-tissue swelling of the face and hands, and no visual field impairment. Hormonal assessment showed elevated levels of PRL (148.5 ng/ml) and an increased IGF-I (489 mg/dl). Basal GH secretion was 0.68 ng/ml, which decreased but did not fully suppress (nadir level 0.38 ng/ml) during an OGTT. A 24-h GH profile showed a mean GH concentration of 1.84 ng/ml. An MRI revealed a pituitary adenoma of 9×8 mm in maximum diameter (Fig. 1b). The patient was treated with dopamine agonists, with adequate hormonal control being achieved with a cabergoline dose of 2 g/week. Subsequently, menses returned and headaches lessened, but the patient has remained infertile despite normal gonadal function. During follow-up, IGF-I levels were noted to be intermittently above the normal range for age and sex. The lack of absolute GH suppression

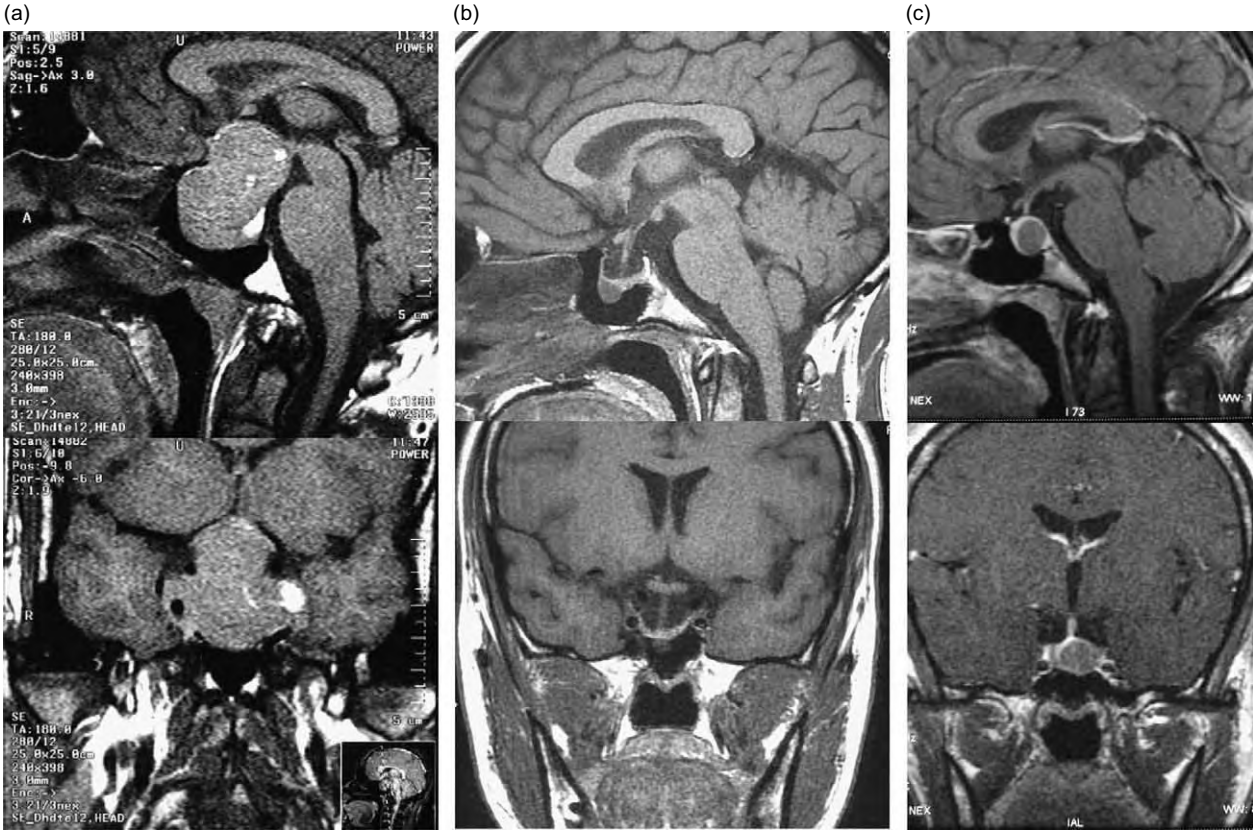


**Figure 1** Coronal and sagittal T1-weighted MRI taken at diagnosis in three family members with pituitary tumors in association with an E174 frameshift *AIP* mutation. (a) The index case presented at 17 years of age with a large invasive pituitary macroadenoma, (b) his sister presented aged 25 with a 9×8 mm pituitary adenoma, and (c) the aunt of the two other patients presented at age 35 with a non-invasive pituitary macroadenoma. For (a), 3 mm cuts were used and for (b) and (c), 2 mm cuts were used.

**Table 1** Characteristics of subjects with an E174 frameshift aryl hydrocarbon receptor-interacting protein (*AIP*) mutation.

| Patient | Sex | Age | Age at diagnosis | IGF-1 (normal range; ng/ml) | GH basal (ng/ml; normal 0–3) | GH nadir (ng/ml) | Prolactin (normal range; ng/ml) | Tumor dimensions (mm) | Clinical features |
|---|---|---|---|---|---|---|---|---|---|
| Case 1 | M | | 17 | 778 (100–474) | 51 | 49.2 | 50.0 (1–27) | 36×45×36 | Acromegaly, excessive height, delayed puberty, and dyslipidemia |
| Case 2 | F | | 25 | 489 (116–358) | 0.68 | 0.38 | 148.5 (1–27) | 9×8 | Infertility, mild soft-tissue swelling |
| Case 3 | F | | 35 | 610 (100–303) | 9.14 | 8.67 | 26.1 (1–27) | 10×9×11 | Acromegaly, three late miscarriages (22–32 weeks gestation) |
| IIa | M | 71 | N/A | 458 (60–100) | 0.7 | 0.08 | 10.0 (1–27) | No tumor | Hypertension |
| IIIa | F | 45 | N/A | 416 (101–267) | 0.43 | 0.18 | 20.0 (1–27) | No tumor | Hypothyroidism |
| IIIb | M | 48 | N/A | 220 (116–358) | 0.54 | 0.10 | 12.0 (1–27) | No tumor | None |
| IVa | M | 27 | N/A | 155 (117–329) | 0.40 | N/A | 15.0 (1–27) | No tumor | None |
| IVb | M | 22 | N/A | 164 (116–358) | 0.20 | N/A | 11.0 (1–27) | No tumor | None |
| IVc | M | 21 | N/A | 143 (116–358) | 0.13 | N/A | 16.0 (1–27) | No tumor | Hypertension and headache |
| Va | F | 2 | N/A | 170 (51–303) | 0.50 | N/A | 8.5 (1–27) | No tumor | Premature thelarche (Tanner 3), bone age 3 years at 21 months of age. Right ovary 1.8 $cm^3$ in volume. FSH, 1.69 mU/ml; LH, 0.1 mU/ml; and estradiol, 11.9 pg/ml |

GH, growth hormone; IGF-I, insulin-like growth factor-I; m, male; f, female.

combined with the marked IGF-I elevation and hyperprolactinemia suggest that a somatolactotrope adenoma may be present in this case.

***Case 3*** The 35-year-old maternal aunt of cases 1 and 2 was first evaluated in 2005 on the advice of her nephew (case 1). She had a 10-year history of worsening arthralgia, hyperhidrosis, weakness, and headaches. Her past medical history was relevant for three pregnancies that all ended in late miscarriages at 20, 25, and 30 weeks' gestation; no relevant structural or pathological obstetric causes were found. Clinical examination revealed typical facial features of acromegaly with prognathism and pronounced soft-tissue swelling of the lips and nose. No visual abnormalities were found and the patient was of normal stature. Her basal GH was elevated at 9.14 ng/ml, which failed to suppress during an OGTT (nadir GH level 8.67 ng/ml). Her IGF-I was also elevated at 610 ng/ml and her PRL was at 26.1 ng/ml. No other hormonal abnormalities were present at diagnosis. An MRI revealed a pituitary macroadenoma measuring 10×9×11 mm without evidence of extrasellar extension or invasion (Fig. 1c). Following 7 months of treatment with octreotide LAR at a dose of 30 mg/month, IGF-I decreased but remained elevated at 378 ng/ml and no tumor shrinkage was seen on MRI.

## *Characteristics of mutation carriers*

The genealogical tree for the family comprised 122 individuals and genetic studies identified seven additional asymptomatic carriers of the E174fs mutation across six generations; the mutation was traced to the maternal grandfather of the index case (Fig. 2). In the three cases with pituitary tumors, an E174fs mutation in *AIP* was noted. The penetrance of pituitary tumors among adult mutation carriers ($n=9$) in this FIPA family was 33.3%.

In the seven E174fs mutation carriers, clinical, hormonal, and MRI studies were performed. No pituitary adenomas were diagnosed in these seven individuals. Four mutation carriers were entirely hormonally and clinically normal. Two asymptomatic mutation carriers were found to have IGF-I levels above the normal range for their age and sex. In subject IIa, the 71-year-old grandfather of the index case, an IGF-I of 458 ng/ml (normal range for age and sex: 60–100 ng/ml) was noted, while subject IIIa, the 45-year-old mother of the cases 1 and 2, had an IGF-I of 416 ng/ml (normal range: 101–267 ng/ml). The elevated IGF-I levels were not associated with clinical signs/symptoms and no concomitant conditions associated with elevated IGF-I were present. In both cases an OGTT was normal (Table 1).

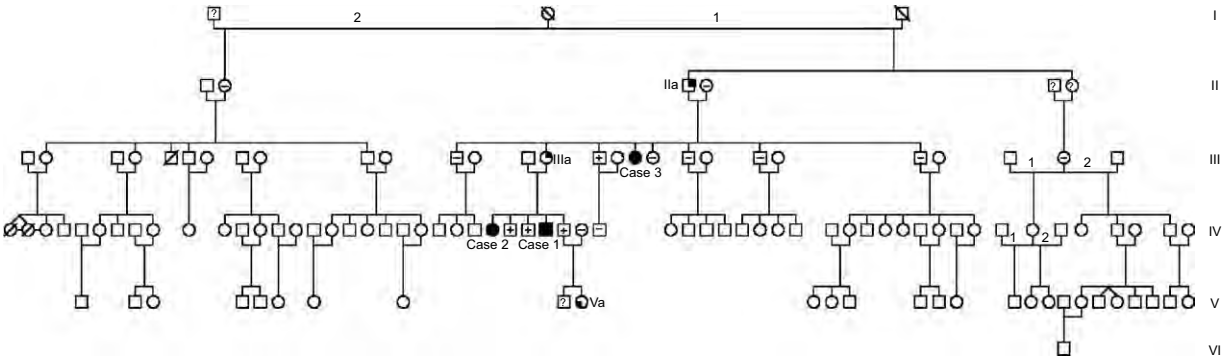


**Figure 2** Genealogical tree of six generations of a family with an E174 frameshift *AIP* mutation. All members of the kindred underwent medical assessments for signs and symptoms of pituitary or other diseases and the full genealogy was mapped. Consanguinity was ruled out. Potential routes of inheritance of the mutated *AIP* were traced and molecular genetic studies were undertaken in targeted individuals to assess whether the mutation had been passed to later generations. *AIP* mutation carriers underwent hormonal and radiological studies, and all relatives of carriers were genotyped. Subjects marked with filled (black) symbols were the mutation-positive individuals with pituitary tumors (cases 1, 2, and 3). Subjects marked with (+) were mutation carriers without clinical, hormonal, or radiological evidence of disease. Subjects marked with (−) were genotyped as being wild-type homozygotes for *AIP*. Subjects marked with (?) declined genetic analysis but were free from clinical evidence of pituitary or other illnesses. AIP mutation carriers with elevated IGF-I levels (subjects IIa and IIIa) are shown (filled (black) upper right quadrant symbol); an infant girl with the E174fs *AIP* mutation and premature thelarche, ovarian enlargement, and advanced bone age (subject Va) is shown (filled lower left quadrant symbol). Multiple partners/couplings are numbered separately. Apart from the subjects noted above, clinical history and examination were normal in all other members of the kindred.

A female infant (Va), a mutation carrier, was noted to have premature thelarche at 1 year of age. After 1 year of follow-up, breast development continued and reached Tanner stage 3. At 23 months of age, she had a bone age of 3 years. Pelvic ultrasound showed an enlarged right ovary (1.8 $cm^3$) with five visible follicles, while the left ovary had a normal volume (0.7 $cm^3$) and contained three follicles; the uterus was normal in appearance and volume (0.8 $cm^3$). Hormonal evaluations showed a follicle-stimulating hormone level of 1.3 mU/ml (normal $<$1.6 mU/ml), a luteinizing hormone level of 0.1 mU/ml (normal $<$0.6 mU/ml), an estradiol level of 11.9 pg/ml (normal $<$8 pg/ml), and a PRL level of 8.5 ng/ml (normal range 0.33–27.3 ng/ml). Gonadotrophin-releasing hormone and OGTT tests were declined by the parents at this time. An MRI revealed no pituitary, hypothalamic, or other abnormalities. Clinical examination showed no features associated with known causes of precocious puberty (e.g. no visible *café au lait* spots suggestive of McCune-Albright syndrome) at last follow-up in June 2007.

Further clinical evaluation of all ten individuals bearing E174fs mutations revealed no symptomatology or features suggestive of other potential *AIP* mutation-associated disease in other tissues.

## *Tumor immunohistochemistry*

Only one of the three subjects with pituitary tumors underwent surgery. Immunohistochemistry of the tumor tissue from this patient showed strongly positive staining ($>$50% of positive cells) for GH and PRL. Interestingly, strong c-erb staining was also seen (Fig. 3a), while the tumor was negative for Ki-67 and p53. AIP immunohistochemistry results are shown in Fig. 3b. Staining for

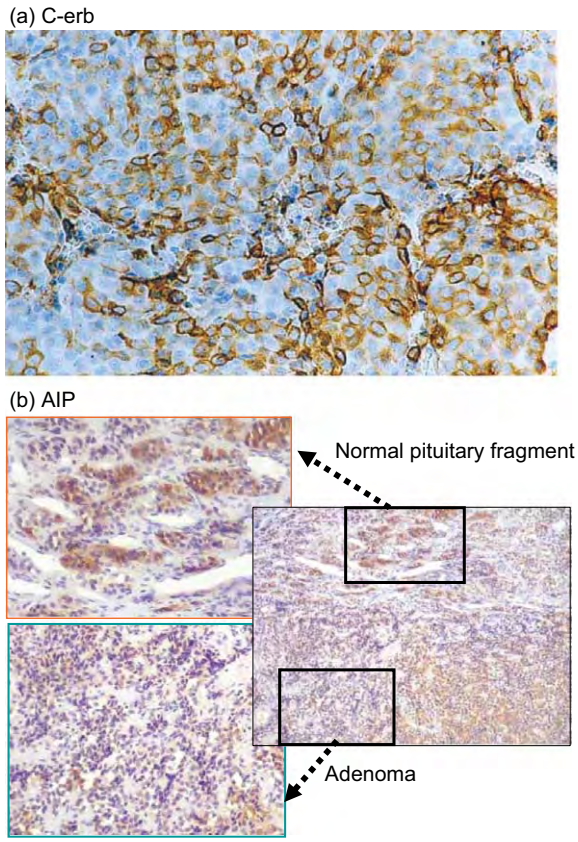


**Figure 3** Immunohistochemistry of pituitary tumor from index case. (a) The tumor, which stained strongly positive for growth hormone and prolactin (not shown) also had strong positive staining for c-erb. (b) Immunohistochemistry of the pituitary tumor for AIP, showing the reduced AIP staining seen in a region of tumor when compared with a section of normal pituitary.

AIP in adenomatous tissue was heterogeneous but markedly lower than in the adjacent normal pituitary fragment, where intense cytoplasmic staining was noted in most cells. Faint nuclear staining was observed only in a minority of normal pituitary cells.

## Discussion

The occurrence of pituitary tumors in a familial setting accounts for 5% of pituitary tumors (5); most are due to MEN-I, while CNC occurs very rarely (15). Recently, acromegaly and MEN-I-like features were reported in one family with a *CDKN1B* mutation, although such mutations appear very rare (16–18). Germline mutations of *AIP* occur in familial and sporadic pituitary adenomas, although the latter are very infrequent. In FIPA, *AIP* mutations account for half of all cases of IFS, while 85% of families are negative for *AIP* mutations indicating that other genes may be involved in its pathogenesis (11).

The current report concerns a family that has not been described in detail in the literature to date. With the advent of molecular genetic testing for *AIP*, it was possible to undertake an extensive prospective assessment of the frequency of the occurrence of the E174fs mutation across multiple generations. Overall, we found that ten individuals bore the E174fs mutation, of which three had a diagnosis of an isolated pituitary adenoma. As one of the mutation carriers was an infant and it is usual to express penetrance at a given age, we have elected to calculate penetrance of pituitary tumors among adult mutation carriers in this family. Thus, the penetrance of pituitary tumors in this FIPA family was 33.3% (3/9). This penetrance figure falls within the range of 25 to >85% seen in other FIPA families with *AIP* mutations (Beckers & Daly unpublished data). A similarly wide range for penetrance has also been reported recently by another international collaborative group (20). Taken together, these data suggest that the occurrence of pituitary tumors in patients with *AIP* mutations is not a low-penetrance condition.

The phenotypic characteristics of the patients with tumors in this family are varied, which appears to be a feature of FIPA in general and of those with *AIP* mutations in particular. On clinical findings, one patient had prolactinoma and two had acromegaly. However, hormonal data indicate that the patient with the prolactinoma also had intermittently elevated IGF-I secretion, suggesting the presence of a somatolactotrope tumor. The index case with acromegaly also had combined GH and PRL hypersecretion and his tumor stained strongly positive for both hormones. Although both cases 1 and 3 had acromegaly, they differed significantly from each other. Case 1 had much more aggressive disease in terms of an early age at onset (17 vs 35 years), a larger tumor size and much higher basal levels of GH secretion. Case 3 decided not to undergo surgery at this time; therefore, it was not possible to investigate pathological (immunohistochemical) differences between the GH-secreting tumors in both cases. Notably, both patients with acromegaly had poor responses to octreotide therapy and required combination treatment with a somatostatin analog and a dopamine agonist. The underlying reason for this relative octreotide resistance is unexplained, as is the more general issue of why the same *AIP* mutation would cause different pituitary tumor phenotypes in three closely related individuals.

AIP immunohistochemistry has been previously applied to pituitary tumor samples, although there are few data regarding patterns of staining in normal tissue and in different types of pituitary tumors. In the present study, we used an mAb against human AIP, which revealed cytoplasmic staining in only 30% of tumor cells when compared with stronger and diffuse expression in the adjacent normal pituitary fragment. This is in keeping with the potential loss of expression of *AIP* protein in the tumor tissue; however, results on loss of heterozygosity for *AIP* in the paraffin tumor sample were equivocal. Indeed, it is not known precisely how the E174fs mutation affects AIP protein expression. The mutation would predict a series of incorrect amino acids after position 174, followed by a premature stop codon. Such a mutation would abrogate the expression of the third tetratricopeptide repeat domain (TPR) and carboxy-terminal residues that are known to be crucial for interactions of AIP with the AhR and the co-chaperone molecule heat shock protein 90 (hsp90) (11). Indeed, many of the mutations reported to date predict protein changes that would affect the third TPR domain and the carboxy terminal of AIP (10–14, 19). *In vitro* expression studies will be useful in helping to determine whether the E174fs mutation is expressed as protein or targeted for destruction via nonsense-mediated mRNA decay or other pathways.

Detailed clinical and laboratory studies of the family revealed no consistent features that were indicative of potential *AIP*-related disease in other tissues, although some endocrine findings are worth highlighting. In two mutation carriers with normal MRI scans, elevated levels of IGF-I were seen in the absence of abnormal GH secretion on basal or strict dynamic testing. Additionally, case 2 showed intermittent elevations in IGF-I without abnormal GH secretion over the course of the follow-up of her prolactinoma. Other causes of increased IGF-I were ruled out in these cases, which suggests that such elevations might be related to their *AIP* mutation status, although a mechanistic explanation remains difficult. It is not known whether hormonal abnormalities could occur due to non-tumoral pituitary hyperplasia in the setting of *AIP* mutations, as frequently occurs in the pituitaries of patients with CNC and McCune–Albright syndrome (21, 22). Taken together with the differences in pituitary tumor characteristics among the three cases in this family, this clinical picture suggests wide variability in disease expression among *AIP* mutation

carriers. An explanation for this is lacking at this time, although the effects of additional random somatic mutations in the pituitary or elsewhere or the modulating actions of other genes (normal or mutated) must be considered.

Environmental factors are another important area of great relevance to discussions of AIP and its potential role in endocrine pathology. Current understanding of the role played by AIP in pituitary cell function is still relatively scanty. AIP has been studied extensively in toxicology, where the effects of AIP in modulating the activity of its receptor, AhR – the dioxin receptor – have received a great deal of scrutiny. However, other effects of AIP on cellular activity have been noted that may be equally relevant to tumorigenic potential. The phosphodiesterases, PDE4A5 and PDE2A, surviving, and translocase of outer mitochondrial membrane 20 (TOM20) are all defined targets for AIP could undergo dysregulation in the setting of *AIP* mutations, potentially leading to enhanced cell growth and survival (23–27). Fundamental work on the role of AIP mutations in abnormally modulating cell signaling remains to be performed, and the pathway by which pituitary tumors occur in patients with germline *AIP* mutations remains speculative.

In terms of the potential involvement of mutated *AIP* in the pathogenesis of the premature thelarche and abnormal ovarian/bone development in subject Va, AhR-related pathways are known to be involved in gonadotropic axis development (28, 29). AhR-mediated gene transcription is modulated via direct recruitment of estrogen receptor-$\alpha$, which links estradiol activity to AhR–dioxin-mediated signaling (30, 31). This and other associated pathways (e.g. via hsp70 (32, 33)) are thought to play an important role in the physiology of sexual development at a cellular level in the pituitary. Exposure to dioxin and other environmental toxins during embryonic development can have estrogenic effects, leading to alterations in cellular signaling, biochemical activity, and reproductive function (34–36). Alterations in the AhR pathway are associated with reproductive disorders in animal models (37) and even in humans (38, 39). Intriguingly, animal data suggest that a connection may exist among environmental estrogenic disruptors, AhR pathway signaling and expression of GH, and PRL mRNA in the pituitary, an effect that may be modulated at the level of Pit1 (40–42). While no definitive link between *AIP* status and the clinical phenotype of subject Va can be made, it is worth considering given the overlaps between AhR pathways, early life hormonal exposure, and subsequent reproductive physiology (43). There was no known history of abnormal estrogenic exposure in the child while *in utero* or subsequently and no pathological changes of the pituitary or hypothalamus were apparent on MRI. Ongoing follow-up will assess whether the breast, ovarian, and bone changes progress and if abnormalities of the gonadotropic axis become apparent.

In conclusion, this is the first study to examine in depth the clinical, hormonal, and radiological features of a large FIPA kindred with three patients with pituitary adenomas (acromegaly and prolactinoma) associated with mutations in *AIP*. Among seven asymptomatic mutation carriers, elevations in IGF-I were seen in two adult individuals, while an infant girl presented a progressing pattern of sexual precocity. These results suggest that the same *AIP* mutation can be associated with a variable phenotype of pituitary tumors among related family members (as suggested previously (11)) and raises the possibility of the involvement of non-tumoral pituitary pathology in mutation carriers. The field of AIP research in the setting of pituitary endocrinology is at an early stage and fundamental data on mutated AIP protein expression and function from *in vitro* and animal studies is lacking at this time. Before firm conclusions can be drawn regarding clinical disease characteristics related to *AIP* mutations, the analysis of data from larger cohorts of affected patients will be required.

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## Chapter Ten

# Mutations in the *Aryl Hydrocarbon Receptor Interacting Protein* Gene Are Not Highly Prevalent among Subjects with Sporadic Pituitary Adenomas

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# BRIEF REPORT

# Mutations in the *Aryl Hydrocarbon Receptor Interacting Protein* Gene Are Not Highly Prevalent among Subjects with Sporadic Pituitary Adenomas

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**Context:** Limited screening suggests that three germline mutations in the *aryl hydrocarbon receptor interacting protein* (*AIP*) gene are not involved in sporadic pituitary tumorigenesis. Multiple novel mutations of this gene have since been identified in familial isolated pituitary adenoma cohorts.

**Objective:** The objective of the study was to undertake full *AIP* coding sequence screening to assess for the presence of germline and somatic mutations in European Union subjects with sporadic pituitary tumors.

**Design:** The study design was the analysis of DNA from peripheral blood lymphocytes and analysis of exons 1–6 and paraexonic intron sequences of *AIP*. Multiplex ligation-dependent probe amplification was used to screen separate sporadic pituitary tumor tissue samples for discrete and extensive deletions or mutations of the *AIP* gene.

**Setting:** The study was conducted in university tertiary referral Clinical Genetics, Molecular Biology, and Endocrinology Departments.

**Results:** In 107 patients [prolactinomas (n =49), nonfunctioning tumors (n = 29), somatotropinomas (n = 26), ACTH-secreting tumors (n = 2), TSH-secreting tumors (n = 1)], no germline mutations of *AIP* were demonstrated. Among a group of 41 tumor samples from other subjects, a novel *AIP* mutation (R22X) was found in one sample in which the corresponding allele was deleted; follow-up screening of the patient demonstrated a germline R22X *AIP* mutation.

**Conclusions:** *AIP* mutations do not appear to play a prominent role in sporadic pituitary tumorigenesis in this population of European subjects. **(*J Clin Endocrinol Metab* 92: 1952–1955, 2007)**

MOLECULAR GENETIC STUDY of pituitary tumors has identified a variety of mutations or abnormal expression pattern that may play a role in tumorigenesis. Well-recognized examples include mutations in the *Gsα* gene, *PTTG* overexpression, and decreased *MEG3* expression (1–4). *MEN1* and *PRKAR1A* gene mutations play a critical role in endocrine tumorigenesis in multiple endocrine neoplasia-1 (MEN1) and Carney complex (CNC) (5, 6) but are of limited significance in sporadic pituitary tumors (7, 8). Thus, interest remains high in the identification of new molecular mechanisms to explain sporadic adenoma formation (9).

Aryl hydrocarbon receptor interacting protein (AIP), a 330-amino-acid protein present in the pituitary, interacts with the aryl hydrocarbon receptor and heat shock protein 90 dimer; AIP is involved in mediating cellular responses to environmental toxins like dioxin (10, 11). Mutations that remove conserved tetratricopeptide repeat domains at the carboxy terminal of AIP abrogate proper interaction with its receptor and heat shock protein 90, thereby disrupting nuclear localization (11). Vierimaa *et al.* (12) first reported the involvement of three *AIP* mutations in pituitary adenomas in non-MEN1, non-CNC families from Finland and Italy. Among the Finnish cohort, two *AIP* mutations were noted in germline and somatic DNA from sporadic pituitary ade-

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* A.Ba. and J.-F.V. contributed equally to this study.

Abbreviations: AGI, Applera Genomics Initiative; AIP, aryl hydrocarbon receptor interacting protein; CEPH, Centre d'Etude du Polymorphisme Humain; CNC, Carney complex; FIPA, familial isolated pituitary adenoma; LOH, loss of heterozygosity; MEN1, multiple endocrine neoplasia type 1; MLPA, multiplex ligation-dependent probe amplification; NFPA, nonfunctioning pituitary adenoma.

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noma patients (12). Recently Daly *et al.* (13, 14) described the clinical and genetic features of families exhibiting familial isolated pituitary adenomas (FIPAs). Among 73 FIPA families, 10 *AIP* mutations were present among 11 families; nine of these mutations were novel (13). Yu *et al.* (15) demonstrated that the three germline mutations described by Vierimaa *et al.* were not prevalent among U.S. patients with sporadic pituitary adenomas. We undertook a study of 107 subjects with sporadic pituitary adenomas and 41 separate sporadic pituitary tumor samples to screen for germline and somatic mutations in the full coding sequence of *AIP*.

## Subjects and Methods

### *Subjects*

The study was performed in 107 patients with sporadic pituitary adenomas from university hospital centers in France (Marseilles), Belgium (Liège), and Italy (L'Aquila). There were 49 subjects with prolactinomas [17 males, 32 females; mean (±SD) age at diagnosis: 33.9 ± 13.0 yr; 70.2% macroadenomas, 52.5% cavernous sinus invasion], 26 with somatotropinomas [17 males, nine females, mean (±SD) age at diagnosis: 44.4 ± 13.3 yr; 86.4% macroadenomas, 45.0% cavernous sinus invasion], 29 with nonfunctioning pituitary adenomas [NFPAs; 15 males, 14 females; mean (±SD) age at diagnosis: 50.6 ± 13.1 yr; 100% macroadenomas, 58.8% cavernous sinus invasion], two females with noninvasive ACTH-secreting microadenomas [mean (±SD) age at diagnosis: 32.5 ± 4.9 yr], and one male subject with a noninvasive TSH-secreting microadenoma diagnosed at age 48 yr.

None of the subjects had a history of MEN1, CNC, or FIPA. Twelve subjects (11.2%) had other endocrine tumors: parathyroid adenoma (n = 10), pancreatic endocrine tumor (n = 1), and thymoma (n = 1); MEN1 gene mutations were outruled by gene sequencing. All subjects gave their written informed consent to participate in the study, which was approved by the local ethics committee.

### *Pituitary tumor samples*

Forty-one frozen tissue samples from patients with sporadic pituitary tumors (different from the patients above) were collected as previously described (16) and studied for *AIP* gene mutations. These tumors, classified by immunohistochemical staining for pituitary hormones, included 23 somatotropinomas [13 males, 10 females; mean (±SD) age at diagnosis: 42 ± 13.9 yr] and 18 gonadotropinomas [nine males, nine females; mean (±SD) age at diagnosis: 45 ± 13.7 yr] and were clear of residual normal pituitary tissue before genetic analysis. *Gsα* sequence was performed in all somatotropinoma samples, as described previously (16); seven tumors had point mutations in *Gsα*, indicating the presence of the gsp oncogene.

### *Genomic analysis of AIP and MEN1*

Using DNA isolated from peripheral blood, exons of the *AIP* and *MEN1* genes were amplified using exon flanking primers (12, 17). Apart from exons 4 and 5 of *AIP*, the same primers were used for gene sequencing using a CEQ 8000 sequencer (Beckman Coulter, Fullerton, CA). The primers for *AIP* exons 4 and 5 were: GACGCAGCTGTGGTGTCC (AIP4F) and CTGAGGGGGAGGATGGAT (AIP5F). The primers used for sequencing *AIP* exons 1, 2, 3, and 6 and the *MEN1* gene are available on request.

*AIP* single-nucleotide polymorphism patterns were studied using a reference population of 86 normal individuals from Belgium (n = 31), France (n = 25), and Italy (n = 30). In addition, an international panel of reference populations were analyzed as follows: exons 2 and 5 [Applera Genomics Initiative (AGI) Caucasian and African American samples, Coriell Cell Repositories], exon 4 (CEU; Utah residents of northern/western European ancestry), and exon 6 [Centre d'Etude du Polymorphisme Humain (CEPH)].

### *Multiplex ligation-dependent probe amplification (MLPA) analysis*

Loss of heterozygosity (LOH) within the *AIP* gene and along larger segments of chromosome 11q was assessed in tumor samples using MLPA. MLPA probe design has been described previously (18). Control probe-pairs that were specific to unrelated genes on chromosomes 7 and 17 were also designed. All probes had amplification products from 87 to 136 bp in length and had an annealing temperature higher than 70 C as per the RAW Probe program (MRC-Holland, Amsterdam, The Netherlands). PCR products were analyzed on an ABI3100 capillary electrophoresis apparatus (Applied Biosystems, Lennik, Belgium). Copy number quantification involved normalization of the peak area of the *AIP*-specific MLPA probe by dividing it by the combined areas of the control probes. This ratio was compared with the similar ratio obtained from control DNA. LOH was observed when the wild-type signal was reduced by 35–60% for each AIP-specific probe.

## Results

### *AIP analysis in genomic DNA*

Analysis of the coding sequence of *AIP* in genomic DNA from 107 subjects with sporadic pituitary adenomas revealed no mutations. The genotype frequencies of polymorphisms were similar to those of the reference populations (Table 1).

### *AIP analysis in pituitary tumor DNA*

MLPA analysis revealed deletions at the *AIP* locus in two of 41 frozen pituitary tumor fragments [somatotropinoma (n = 2)]. In one somatotropinoma, a nonsense mutation p.Arg22X (CGA>TGA) at exon 1 of *AIP* was found, indicating a hemizygotic state with the loss of wild-type allele (GenBank accession no. EF158450). This male subject presented with acromegaly at a young age (24 yr) due to a macroadenoma that secreted high levels of GH and was resistant to somatostatin agonist therapy. Radiotherapy was required 1 yr postoperatively due to activity of the residual tumor. Analysis of peripheral blood DNA in the patient also revealed a germline p.Arg22X mutation. There was no family history of pituitary adenomas in this case. The other somatotropinoma sample with a large deletion that included the *AIP* and *MEN1* loci came from a young male with an invasive tumor. However, the corresponding *AIP* or *MEN1* genes were intact in the other allele.

The genotype frequencies of all *AIP* polymorphisms were similar to those of the reference populations (Table 1).

## Discussion

The involvement of *AIP* mutations in pituitary tumorigenesis was first suggested by Vierimaa *et al.* (12), who described three mutations (Q14X, R304X, and IVS3–1G>A) in subjects with somatotropinomas and prolactinomas. Daly *et al.* (13) recently described a further nine *AIP* mutations (R16H, G47_R54del, Q142X, E174 frameshift, Q217X, Q239X, K241E, R271W, and Q285 frameshift) in a cohort of 73 families with FIPA. Among these 12 described mutations, the majority lead to early stop codons and protein truncation, which would remove the third tetratricopeptide repeat domain and the last five carboxy-terminal amino acids that are necessary for the biological activity of AIP (10). Q14X and IVS3–1G>A occurred in Finnish individuals with sporadic pituitary tumors; LOH at the *AIP* locus was shown in all

**TABLE 1.** Frequencies of *AIP* gene polymorphisms in subjects with sporadic pituitary adenomas

| Exon | Sequence variation | Genotype | Controls (n = 86) | | Genomic (n = 107) | | Somatic (n = 41) | |
|---|---|---|---|---|---|---|---|---|
| | | | Frequency | Count | Frequency | Count | Frequency | Count |
| 2 | p.Asp44Asp (rs11822907) c.132C>T | C/C | 1 | 86 | 1 | 107 | 1 | 41 |
| | | C/T | 0 | 0 | 0 | 0 | 0 | 0 |
| | | T/T | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | p.Asp172Asp (rs2276020) c.516C>T | C/C | 0.98 | 84 | 0.97 | 104 | 0.98 | 40 |
| | | C/T | 0.02 | 2 | 0.03 | 3 | 0.02 | 1 |
| | | T/T | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | p.Gln228Lys (rs641081) c.682C>A | C/C | 0 | 0 | 0.01 | 1 | 0 | 0 |
| | | C/A | 0 | 0 | 0.04 | 4 | 0 | 0 |
| | | A/A | 1 | 86 | 0.95 | 102 | 1 | 41 |
| 6 | p.Ile327Ile (rs1049565) c.981C>T | C/C | 1 | 86 | 1 | 107 | 1 | 41 |
| | | C/T | 0 | 0 | 0 | 0 | 0 | 0 |
| | | T/T | 0 | 0 | 0 | 0 | 0 | 0 |

The control population comprised normal subjects from Belgium (n = 31), France (n = 25), and Italy (n = 30), and the polymorphism frequencies were similar to those seen in the genomic and somatic populations. An additional 92 control samples from international sources were also studied (data not shown). These included AGI for single-nucleotide polymorphisms located in exons 2 and 5, CEU for exon 4, and CEPH in exon 6. The populations included: AGI (Caucasian and African-American samples from Coriell Cell Repositories Collection); CEU [CEPH (Utah residents with ancestry from northern and western Europe)]; CEPH [the genomic DNA was comprised of U.S. (Utah; 93%), French (4%), and Venezuelan (3%) samples from Coriell Cell Repository, which were pooled in equimolar amounts for use]. Polymorphism frequencies were similar to those seen in the Belgian-French-Italian control population and the genomic and somatic DNA samples from patients with sporadic pituitary adenomas.

tumor samples analyzed. Subsequently Yu *et al.* (15) analyzed genomic DNA from 66 individuals with sporadic pituitary adenomas treated in a single tertiary referral center in the United States; they looked exclusively for the three mutations described previously by Vierimaa *et al.* (16). Because none of the patients had any of these three *AIP* mutations, the authors concluded that *AIP* was unlikely to be involved in the pathogenesis of sporadic pituitary tumors in a predominantly Californian group.

The results of the current study confirm the findings of Yu *et al.* (15) and expand our understanding of this issue in a number of ways. We undertook sequencing of the entire coding sequence of *AIP*. In that way we can conclude with certainty that *AIP* mutations did not play an important role in the pathogenesis of sporadic pituitary adenomas in our cohort. In the U.S. cohort, three of the 66 individuals (4.5%) had a family history of pituitary adenomas (two subjects with prolactinomas and one with a NFPA). These patients, negative for MEN1, may have comprised FIPA families. In FIPA, *AIP* mutations occur in about 15% of families, and tumors in such families can include somatotropinomas, mixed GH/prolactin-secreting tumors, prolactinomas, and NFPA (13). The majority of FIPA families, including 50% of those with familial somatotropinomas, are, however, negative for *AIP* mutations, indicating that other unidentified pathogenic factors may be involved. We also chose to screen DNA from subjects from three separate countries (France, Belgium, and Italy); absence of *AIP* mutations in genomic DNA from this geographically dispersed group supports the contention that founder effects may be involved in the frequent occurrence of the Q14X mutation in the Finnish cohort (12, 15).

Mutations in *AIP* are not, however, altogether absent among patients with sporadic pituitary adenomas. Our screening of tumor tissue revealed a p.Arg22X mutation in one *AIP* allele and a deletion of the other allele. Subsequently a germline *AIP* was also demonstrated in the same patient. The clinical characteristics (young age, large/aggressive tumor) of the patient are similar to those of other patients with *AIP* mutations recently described (13). This patient had no family history of pituitary adenomas or other endocrine disease. A tumor sample from another patient with a somatotropinoma had a deletion that also included both *AIP* and *MEN1*, but both genes in the other allele were wild type. This raises the possibility that a mutation in another gene in this region of 11q13 could be involved in pituitary tumorigenesis in such patients.

This study confirms that germline mutations of *AIP* are infrequently found in patients with sporadic pituitary adenomas in continental Europe. Founder effects should be considered when extrapolating from *AIP* mutation prevalence among genetically delimited populations. Given that at least 13 *AIP* mutations have been described in association with pituitary adenomas, full *AIP* sequencing is needed to outrule *AIP* involvement in sporadic pituitary tumorigenesis among other populations worldwide.

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# Chapter Eleven

## Discussion and General Conclusions

### Epidemiology

The initial aim of the work described was to design and implement the first comprehensive, cross-sectional study of the epidemiology of pituitary adenomas in a tightly defined region in a developed European country in order to determine the true prevalence of pituitary adenomas.

Following the study described in Chapter 6, it can now be estimated that prevalence of clinically relevant pituitary adenomas is approximately 1 case per 1000 of the general population. This study in a population of nearly 72,000 demonstrated that clinically apparent pituitary adenomas had a prevalence that was 3.5 to 5 times higher than that estimated previously. These results from the Province of Liège appear to be of relevance to countries with a similarly developed social and health system. The study did not identify inheritance as a major cause of pituitary adenomas, with only one case in the study having an identifiable genetic cause of a pituitary adenoma (MEN1). A family history of pituitary adenomas was not present in the cases identified. The study as designed is unlikely to have over-estimated the prevalence of clinically apparent pituitary adenomas, as it was performed in a sample that was generally representative of the geo-political composition of the Province. In fact, the study likely represents somewhat of an under-estimation of the number of clinically-active pituitary adenomas, as it was not a screening study but a cross-sectional case finding study. However, screening studies are not possible in a population of the magnitude of this one (>70,000 people) and the identification of commonly occurring sub-clinical incidentalomas is of questionable relevance from an endocrine of public health perspective.

A prevalence of about one pituitary adenoma per thousand people is a finding that could have many practical implications. Firstly, increased tumor prevalence can occur due to increased incidence, increased survival or both. Evidence suggests that the underlying rate of occurrence of adenomas today is unchanged as compared with older studies [1]. Hence, increased prevalence is likely to be due to a combination of more thorough recognition of cases once they have occurred and greater life expectancy in affected patients. No data are available to support the contention that pituitary adenoma patients are living longer in general. A greater understanding of the impact of disease control on mortality in acromegaly and Cushing's disease, allied with improved neurosurgical and medical care are likely to have had a beneficial effect on survival and, in turn, prevalence. However, probably the most likely reason for the increased prevalence of pituitary adenomas seen in the Liège study was the nature of the methodology. The intensive, case-finding approach in concert with local medical practitioners increased the yield of relevant cases for inclusion beyond that obtained in only referral hospital-based studies.

Disease prevalence rates are required to assess the "burden of

disease" in a given population. The increased prevalence of pituitary adenomas noted in Chapter 6 could have health economic implications. When calculations of health care or research budget allocations are made, these are usually done with some consideration of the frequency of the disease in the community. Pituitary adenomas, although almost never malignant, can have important attendant costs. The effects of untreated pituitary adenomas are also significant in terms of added morbidity due to hormonal hyper- or hypo-secretion. Pituitary microadenomas are more likely to benefit from curative neurosurgery than larger adenomas, making earlier diagnosis and treatment desirable. For patients not cured by surgery, lifelong medical therapy may be required to reduce hormonal hypersecretion or to replace hormonal deficiencies in other pituitary axes. Regular follow-up of pituitary tumor size using MRI is recommended for clinically-apparent pituitary adenomas if cure has not been achieved. Treatment-resistant pituitary tumors will require multimodal therapy that also includes radiotherapy. Taken together these facts indicate that a patient with a pituitary tumorsmay accrue significant costs over the course of treatment. Any increase in prevalence figures would have the effect of suggesting an increased economic allocation to endocrinology for the management of pituitary adenomas in order to match resources adequately with previously underestimated needs. A similar argument may be defensible in the setting of research funding, as improved understanding of pituitary pathology and tumorigenesis could lead to greater strides being made in earlier diagnosis and in the development of more effective therapies.

The Liège-based study, although novel, requires international validation to ensure that unrecognized local factors were not skewing the true estimates of pituitary adenoma prevalence. No racial or geographic propensity factors for pituitary adenomas have been identified from different autopsy studies worldwide and an international study would be valuable to determine if global rates are actually constant.

To this end, an international study on the prevalence of pituitary adenomas was designed and launched in late 2005. This study follows the same intensive, case-finding methodology used in Liège as described in Chapter 6. It has been performed in collaboration with local and community-based specialists in order to again maximize the thoroughness of patient identification within rigorously defined regions. Again, areas were chosen that avoided local clustering around referral centers. In addition to the Liège dataset which has been updated through 2007, three centers in France (Reims, Nantes and Bordeaux), three in Italy (Rome, Naples and Aosta), one in Austria (St Pölten) and one in Switzerland (Freibourg) were scrutinized. A large multi-site center on Réunion island in the Indian Ocean was also included as it is both a geographically distant and more ethnically diverse region that the continental European sites (Figure 1). Finally, a center in Brasilia, Brazil was included in mid-2007. Altogether, the scrutinized population comprises a total of approximately 900,000 people across 18 defined sampling sites on three continents, which comprises the largest international epide-

miology study performed to date in endocrinology.

Interim data from thirteen regional sample sites involving a total population of 723124 individuals have been reported [2]. The prevalence of clinically-relevant pituitary tumors was 0.75 per 1000 (range: 0.55-1.1/1000 population; Figure 2). The mean prevalences of individual adenoma types was: prolactinomas: 1 in 2703 individuals, non-functioning adenomas: 1 in 5263 individuals; acromegaly: 1 in 11,111 individuals, and Cushing's disease: 1 in 19,000 individuals. Thyrotropinomas remain very rare with some centers reporting no cases, making reliable prevalence data difficult to estimate.

In summary, this multicenter, international study confirmed and extended the findings from the Liège, showing clinically-apparent pituitary adenomas are much more prevalent than previously believed (0.75 cases per 1000). The mean prevalence of acromegaly at approximately 1 in 11,000 individuals is double the previous estimates and in itself is likely to alter perceptions of the disease as being very rare. As noted above, should these data be finally confirmed in all 900,000 of the study population, it may make an argument for significant increases to future healthcare and research allocations to pituitary disease.


Figure 1. International epidemiology study into the prevalence of clinically relevant pituitary adenomas. Completed study centers as of July 2007 denoted with a yellow arrow. Belgium: Liège (3 sampling sites); France: Reims (1 sampling site), Nantes (1 sampling site), Bordeaux (2 sampling sites), Reunion (3 sampling sites); Switzerland: Fribourg (1 sampling site), Austria: St Pölten (1 sampling site); Italy: Rome (3 sampling sites). Further completed study centers added in November 2007 in Italy: Naples (1 sampling site) and Aosta (1 sampling site). Data collection remains ongoing at sampling sites in Brasilia, Brazil. Images courtesy of Google Earth.

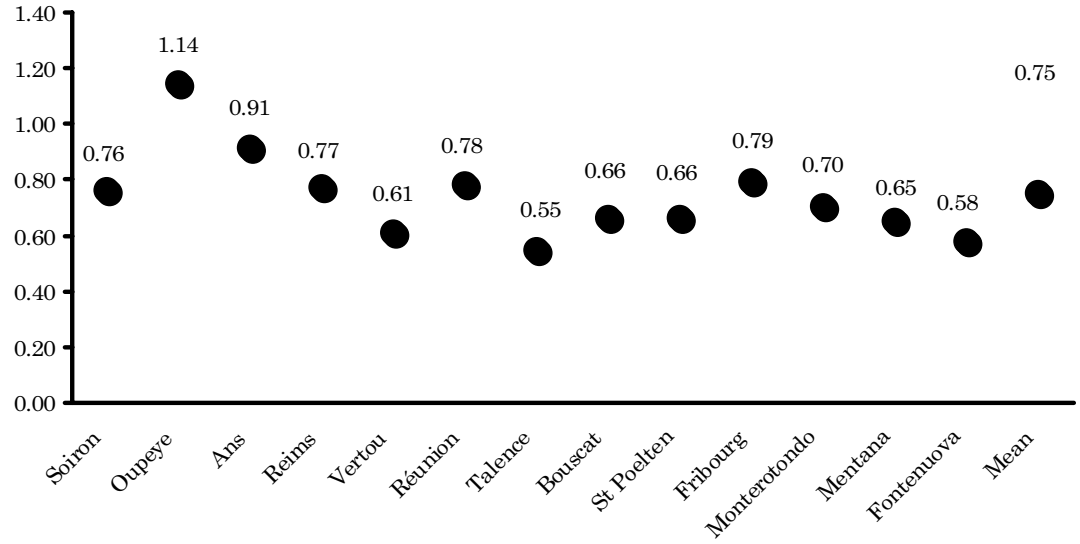


**Figure 2. Mean prevalences of clinically apparent pituitary adenomas in 13 sampling sites across continental Europe (Belgium, France, Italy, Austria and Switzerland) and Réunion Island, an overseas department of France in the Indian Ocean.**

## Familial Isolated Pituitary Adenomas (FIPA)

Other aims of the work described in previous Chapters were:
(a) to collect and characterize for the first time kindreds exhibiting pituitary adenomas of all phenotypes in multiple family members in the absence of diseases known to be associated with an increased risk of pituitary tumors.
(b) To compare the demographic, radiological and pathological characteristics of patients with the new clinical classification, familial isolated pituitary adenomas (FIPA), with those with non-inherited pituitary tumors.
(c) To determine the role played by genetic risk factors in the etiology and pathogenesis of FIPA kindreds and in patients with non-familial sporadic adenomas.

Overall, the work undertaken has permitted the emergence of FIPA as a novel entity with practical clinical utility. In addition the description of FIPA has been accompanied by improved understanding of the differences in disease characteristics between patients with sporadic and familial pituitary adenomas. Before the description of FIPA very few familial cases of pituitary adenomas had been described outside the setting of MEN1, CNC or familial acromegaly. Among those cases in the literature were individual case/-family reports of familial prolactinoma [3], [4], one Cushing's disease family [5], and two unrelated families each with two members with non-secreting adenomas [6]. Familial acromegaly had been described, but no study whatsoever had been made to assess whether all pituitary phenotypes could occur in a family setting. In 2000, FIPA was first defined in the literature in a small study from the Department of Endocrinology at the Centre Hospitalier Universitaire de Liège involving 27 patients [7]. Even at that stage it was clear that pituitary adenomas of different clinical and hormonal phenotypes could occur homogeneously (same tumor in all affected members of a family) or heterogeneously (different tumors among affected members). As described fully in Chapter 7, this feature had not been appreciated previously. The basis of the study described in Chapter 7 had its genesis in the efforts between 2000 and 2002 to seek FIPA families among other centers across Europe, particularly France and Italy [8]. With the comprehensive description of FIPA contained in Chapters 7 and 8, the condition has been studied internationally. As of late 2007, new FIPA families have been identified among clinical centers not affiliated with the initial studies and currently approximately 120 FIPA kindreds have been identified (Beckers, Daly; Unpublished observation).
As outlined in Chapters 7 and 8, FIPA families can vary in size from two to four affected members. The proportions of different types of tumors seen in FIPA differs from the proportions noted in the sporadic and MEN1 settings. As described in Figure 3, FIPA is characterized by a predominance of prolactinomas and somatotropinomas, which account nearly 75% of the total. In contrast, the sporadic adenoma population from Liège described in Chapter 6 is comprised of more frequent prolactinomas (66%), and less frequent somatotropinomas. In MEN1, prolactinomas are also more frequent (62%) than in FIPA.

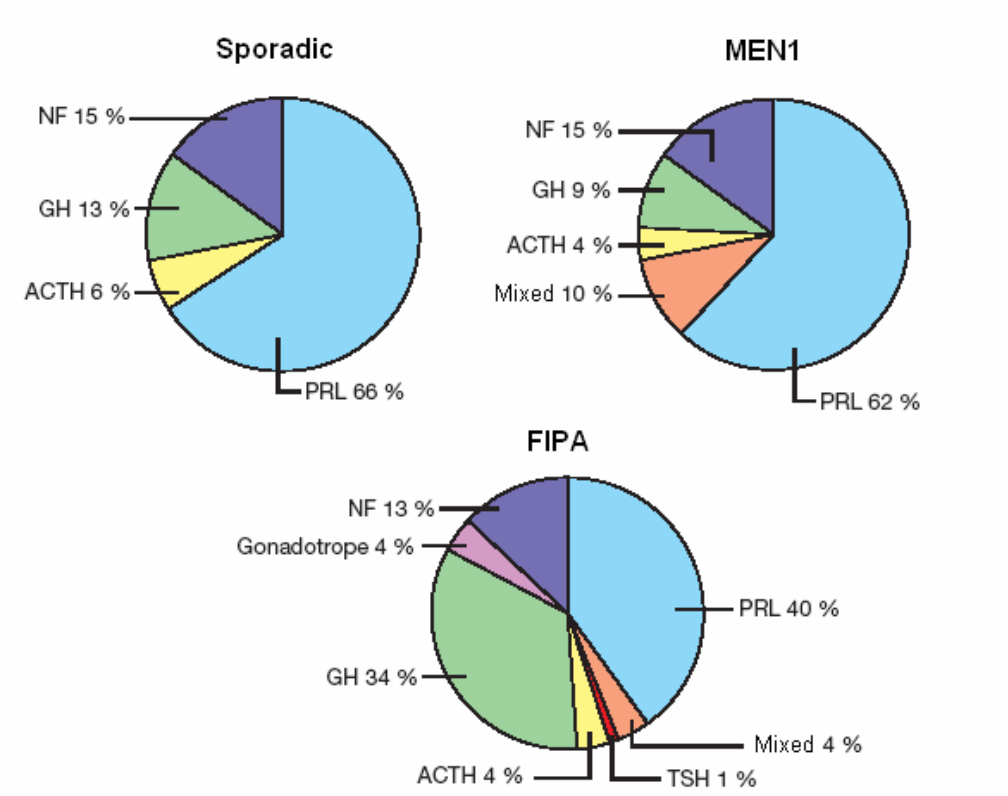


**Figure 3. Pituitary tumor phenotypes observed in the settings of FIPA, MEN1 and sporadic pituitary adenomas (redrawn from [9])**

The characteristics of the FIPA cohort reveal some other interesting features. The cohort is comprised of relatively more females (62%) than males. There is a frequent occurrence of prolactinomas in women within the FIPA cohort, which may shift the overall numbers in favor of a female predominance. There is a close relationship among affected members in the FIPA cohort with 75% being first-degree relatives.

Affected members of FIPA kindreds are statistically significantly younger at diagnosis than patients with sporadic pituitary adenomas. In particular, those with homogeneous familial acromegaly are more than 10 years younger at diagnosis than sporadic acromegaly cases; however all patients among homogeneous FIPA kindreds are diagnosed earlier than in heterogeneous kindreds irrespective of tumor type. One remarkable feature is that in multigenerational families, the children/grandchildren are, in general, more than 20 years younger at diagnosis than their parents/grandparents were. A number of factors may be involved. Firstly, diagnostic tests like MRI have become much more readily available over the last two decades and hormonal diagnostics are both accurate and cheap to perform. So in the case of suspicion of a clinically active pituitary adenoma, both general practitioners and endocrinologists can undertake much more comprehensive studies quite rapidly than were available in previous generations. Awareness of health concerns has also increased over the last two decades. While public health efforts have certainly played a part, there is also a much lower threshold among patients for

seeking medical advice for genitourinary or fertility problems, which may permit a more complete and earlier diagnosis of patients with prolactinomas. Awareness of pituitary disease within a family probably also plays a role in earlier diagnosis in younger affected individuals. The family may be "sensitized" to recognize symptoms or features of a pituitary adenoma due to their experience with other members. This is supported by the fact that 75% of affected members are first-degree relatives of one another. It cannot be excluded that the pituitary adenoma may occur earlier in the younger generation for a reason related to the molecular pathophysiology (or pathophysiologies) of FIPA.

Tumor size characteristics in FIPA also show some interesting features. Non-secreting adenomas are more frequently found in heterogeneous tumor families (85%) and more commonly large tumors (72% macroadenomas). In contrast, homogeneous prolactinoma families often had only females with microprolactinomas. These two features determined that heterogeneous FIPA families have significantly larger tumors overall than in homogeneous FIPA families.

The analysis of kindreds indicated that one or more underlying genetic factors played a role in the pathophysiology of FIPA. The original clinical characterization studies families that had on average 15 individuals between affected patients and unaffected relatives. Overall 14% of these kindreds were affected with pituitary tumors. As mentioned above, three quarters of affected individuals had affected parents, children or siblings. This translated into a high mean familiality or degree-of-relatedness score of 0.62, which implies dominant inheritance, with incomplete penetrance.

In Chapter 8 the role of mutations in the *aryl hydrocarbon receptor interacting protein* (*AIP*) gene (also known as Ara9 or XAP2) in FIPA were described in 156 individuals from 73 kindreds. This, the first and largest study of AIP in FIPA, demonstrated mutations in a minority of FIPA families (15%). Indeed, the initial concept of *AIP* as a cause of familial acromegaly was shown not to hold, as 50% of FIPA cohorts with homogeneous familial acromegaly were negative for *AIP* mutations. Tumors in patients with *AIP* mutations were larger and occurred at an earlier age than in subjects from FIPA families without *AIP* mutations. Furthermore, *AIP* mutations in FIPA are not limited to occurring only in somatotropinomas. FIPA families with *AIP* mutations can have somatotropinomas, prolactinomas, mixed GH/prolactin-secreting tumors or non-secreting pituitary adenomas. The presence of a somatotropinoma is also not invariable in the setting of *AIP*-mutated FIPA kindreds, as one family has been described with a non-secreting tumor in one member that was immunohistochemically negative for GH and prolactin, that occurred in association with a prolactinoma in a sibling. In FIPA patients with *AIP* mutations, those described as having acromegaly comprise a varied group with 62% having elevations in GH and IGF-I alone, while 38% also had elevated prolactin. On immunohistochemistry, tumors from patients with *AIP* mutations and acromegaly can show staining for GH alone (59%), GH and prolactin (33%) or GH and FSH (8%). Furthermore, the same *AIP* mutation can cause

different phenotypes in different FIPA kindreds.

Assessing the field of studies published on AIP in pituitary adenomas [10, 11, 12, 13, 14] it appears that the original mutation noted frequently in Finland (Q14X) [15] is limited to that country and is in essence due to a founder effect, a feature that occurs commonly in bottlenecked populations, such as that of Northern Finland.

In FIPA and sporadic adenomas, a large number of *AIP* mutations have been identified. Not enough study has been done in the setting of sporadic adenomas to assess whether with kindred screening if previously unrecognized adenomas can be found in *AIP* mutation carriers. As outlined in Chapter 9, full genealogic study and screening is a laborious process even in a FIPA family setting where subjects are motivated to participate. However, initial work in "sporadic" cases has shown that apparently asymptomatic carriers can be identified, thus proving that these are not necessarily *de novo* mutations (Beckers, Daly; Unpublished Observation). Indeed, most of the *AIP* mutations described to date appear in a familial setting (Table 1). Family screening studies that are underway will provide greater clarity regarding whether sporadic pituitary adenomas occurring in the setting of *AIP* mutations are truly sporadic or represent previously unrecognized FIPA kindreds.

The functional implications of *AIP* mutations are unknown, as is the mechanism of pituitary tumorigenesis. AIP itself is an immunophilin protein of 330 amino acids that contains three tetratricopeptide repeat (TPR) domains and a FK506 binding protein-type peptidyl-prolyl cis-trans isomerase (FKBP-PPI) domain [16]. The third TPR domain is required for interaction of AIP with a dimer of heat shock protein 90 (hsp90) and with the aryl hydrocarbon receptor (AhR) [17]. Animal studies have shown that mutations in the third TPR domain or the last 4 to 5 amino acids at the carboxy terminal prevent or decrease interactions with hsp90 and AhR [18, 19, 20]. Given the structure of AIP it is unsurprising that many *AIP* mutations described to date in FIPA and sporadic pituitary adenomas are predicted to lead to truncation of the protein that would affect the third TPR and the carboxy terminal. Other mutations involving amino acid substitutions can affect the carboxy terminal or third TPR also; some mutations have no clearly understood effect due to a lack of knowledge about the structure-function relationship of various parts of AIP. Very recently a knockout mouse model of *AIP* was reported [21]. In this model, homozygotic *Ara9* $^{-/-}$ animals died early in embryonic life, surprisingly, due to cardiovascular malformations that included a double outlet right ventricle and ventricular-septal defects. This is the first evidence of an important role for AIP in the development of the circulatory system.

Interestingly, no pituitary abnormalities developed in the homozygotic *Ara9* $^{-/-}$ embryos and no such abnormalities have been seen to date in heterozygotes. Longer-term studies of aging Ara9/AIP knockout heterozygotes will be required to assess whether haplo-insufficiency plays a role in pituitary tumorigenesis in these mice. A conditional Ara9 knockout model, which can be targeted to the liver or other organs has also been developed, which will provide more specific information on this topic (C. Bradfield; Personal Commun-

ication). There is an important caution to be raised when extrapolating murine data on AIP to humans, as interactions between AIP with AhR in the mouse differ from those in the human in terms of subcellular AIP location [22]. Divergent AIP/AhR function between species may alter disease risks and tumor expression in humans as compared with mice.

To date, FIPA (whether with associated *AIP* mutations or not) has not been linked to consistent abnormalities apart from pituitary adenomas. *AIP* mutations themselves appear to occur rarely outside the setting of pituitary adenomas, although some cases of colorectal tumors with non-truncating *AIP* mutations have been reported [23], [24]. In the study described in Chapter 9, it was shown that carriers of *AIP* mutations in a FIPA family had asymptomatic elevations in IGF-I in the absence of pituitary adenomas. Furthermore, one *AIP* mutation carrier in this family was a child with features suggestive of precocious puberty. Longer-term follow up of this family and a greatly expanded familial screening study will provide information of the potential association of non-adenoma pituitary and other diseases with *AIP* mutations in the setting of FIPA.

The lack of *AIP* mutations in FIPA kindreds with strong familiality for pituitary tumors (3-member kindreds and 4-member families), suggests that additional causes are involved in the genetic patho-physiology of FIPA. Screening of FIPA cohorts for mutations in the *CDKN1B* gene ($p27^{kip1}$) has not shown a major role in the genetic pathogenesis of FIPA (Beckers, Daly, Unpublished Observation). Further studies involving loci at 11q13 and elsewhere will be necessary to identify novel genetic causes in FIPA.

Important questions still remain regarding FIPA, which will require comprehensive long-term studies. The increasing number of FIPA kindreds being identified will help to extend the clinical characterization of FIPA. Other important topics such as responses to treatment in FIPA as compared with sporadic pituitary adenomas remain to be explored. In FIPA kindreds with *AIP* mutations, it will be important to address the penetrance of disease among mutation carriers, as the risk of developing a pituitary adenoma in asymptomatic mutation carriers is unknown; however, current data in FIPA suggests that penetrance is at least 33-50% [25] (Beckers, Daly; Unpublished Observation). The genetic causes in the 85% of FIPA families that are negative for *AIP* mutations will be an important focus for research in the years ahead, particularly given that *CDKN1B* mutations play little if any role in FIPA. As mentioned above, intensive familial screening studies like that described in a FIPA family in Chapter 9 will help to clarify the presence or absence of other associated pathologies other than pituitary disease.

The clinical utility of widespread screening for *AIP* mutations in patients with sporadic pituitary adenomas is probably low, as shown in Chapter 10. However, taking into account the features of patients with FIPA, *AIP* sequencing studies are most likely to prove useful in the setting of young patients with large/aggressive pituitary adenomas. In the case of patients with pituitary adenomas and a proven *AIP* mutation, screening (clinical, hormonal and genetic) of close

relatives is advisable after appropriate counseling.[26].

Based on the experience obtained in the studies described here, it would be preferable that international consortia of well-equipped, tertiary referral endocrinology centers perform future studies on FIPA, assessing large populations of patients with pituitary adenomas. This will permit not only further information to be accumulated regarding the genetics and pathology of FIPA, but also maintain a focus on the clinical applicability and practical utility of the results obtained.

| *AIP* mutation | Predicted effect on protein | Pituitary tumor types reported | Instances of mutation (families/individuals) | Country of origin |
|---|---|---|---|---|
| c.40C>T | Q14X | Acromegaly, prolactinoma, mixed GH/PRL secreting | 1 family (multiple other sporadic cases in Finland) | Finland |
| c.47G>A | R16H | Acromegaly | 1 family | France |
| c.64C>T | R22X | Acromegaly | 1 sporadic case | France |
| c.66-71delAGGAGA | Not reported | Acromegaly | 1 family | Germany |
| c.138_161del24 | G46_R54 deletion | Acromegaly | 1 family | Argentina |
| IVS2-1G>C | Exon 3 splice acceptor site affected | Acromegaly | 1 sporadic case | United States |
| c.286-287delGT | P96 frameshift | Acromegaly | 1 family | Japan |
| c.404delA | H135Leu followed by a frameshift and a premature stop codon | Acromegaly | 1 sporadic case | France |
| c.424C>T | Q142X | acromegaly, prolactinoma | 1 family | Italy |
| IVS3-1G>A | Exon 4 splice acceptor site affected | Acromegaly | 1 sporadic case | Finland |
| c.469-2A>G | Exon 4 splice acceptor site affected | Acromegaly | 1 sporadic case | France |
| c.517-521delGAAGA | E174 frameshift followed by premature stop codon | Acromegaly and prolactinoma/mixed GH-PRL secreting tumor | 1 family | Brazil |
| c.542delT | Not reported | Acromegaly | 1 family | Spain |
| c.601A>T | K201X | Acromegaly | 2 individuals | France |

| *AIP* mutation | Predicted effect on protein | Pituitary tumor types reported | Instances of mutation (families/individuals) | Country of origin |
|---|---|---|---|---|
| c.649C>T | Q217X | Acromegaly, mixed GH/PRL secreting tumor | 1 family | Belgium |
| c.715C>T | Q239X | Acromegaly | 1 family | France |
| c.721A>G | K241E | Prolactinoma, non-secreting tumor | 1 family | Belgium |
| c.804A>C | Y268X | Acromegaly | 1 family | Brazil |
| c.824-825insA | Not reported | Acromegaly | 1 individual | United States |
| c.854_857delAGGC | Q285 frameshift followed by a premature stop codon | Acromegaly | 1 family | Italy |
| c.880_891delCTGGACCCAGCC | Not reported | Acromegaly | 1 individual | Germany |
| c.910C>T | R304X | Acromegaly | 2 families, 1 individual | Italy (n=2 families) France (1 individual) |
| c.911G>A | R304Q | Cushing's disease | 1 individual | Poland |
| c.896C>T | A299V | Acromegaly | 1 individual | Holland |

**Table 2. *AIP* mutations in FIPA families and sporadic pituitary adenoma cases reported worldwide. Proven or reported familial cases consistent with the classification of FIPA are outlined in blue. GH = growth hormone; PRL = prolactin**

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# Appendix

# Supporting Studies

## Supporting Studies

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INVITED REVIEW

# The clinical, pathological, and genetic features of familial isolated pituitary adenomas

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## Abstract

Pituitary adenomas occur in a familial setting in multiple endocrine neoplasia type 1 (MEN1) and Carney's complex (CNC), which occur due to mutations in the genes *MEN1* and *PRKAR1A* respectively. Isolated familial somatotropinoma (IFS) is also a well-described clinical syndrome related only to patients with acrogigantism. Pituitary adenomas of all types – not limited to IFS – can occur in a familial setting in the absence of MEN1 and CNC; this phenotype is termed familial isolated pituitary adenomas (FIPA). Over the past 7 years, we have described over 90 FIPA kindreds. In FIPA, both homogeneous and heterogeneous pituitary adenoma phenotypes can occur within families; virtually all FIPA kindreds contain at least one prolactinoma or somatotropinoma. FIPA differs from MEN1 in terms of a lower proportion of prolactinomas and more frequent somatotropinomas in the FIPA cohort. Patients with FIPA are significantly younger at diagnosis and have significantly larger pituitary adenomas than matched sporadic pituitary adenoma counterparts. A minority of FIPA families overall (15%) exhibit mutations in the aryl hydrocarbon receptor-interacting protein (*AIP*) gene; *AIP* mutations are present in only half of IFS kindreds occurring as part of the FIPA cohort. In families with *AIP* mutations, pituitary adenomas have a penetrance of over 50%. *AIP* mutations are extremely rare in patients with sporadic pituitary adenomas. This review deals with pituitary adenomas that occur in a familial setting, describes in detail the clinical, pathological, and genetic features of FIPA, and addresses aspects of the clinical approach to FIPA families with and without *AIP* mutations.

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## Introduction

The etiology of pituitary tumors is an issue that provokes continued interest among endocrinologists. This interest stems from the variability in clinical presentation and symptom burden, the unpredictability of tumor growth, and the often complex management. Historically, there has been considerable uncertainty concerning the actual prevalence of pituitary tumors, with a lack of connection between data from autopsy and radiological series and clinical data. Assessments based on unselected populations undergoing autopsy or magnetic resonance imaging (MRI) suggest that pituitary tumors – almost invariably anterior pituitary adenomas – occur very frequently. A meta-analysis suggested a mean pituitary tumor prevalence of 14.4 and 22.5% in autopsy and radiological series respectively (1). In contrast, the few epidemiological studies performed in the past indicated that pituitary tumors occurred infrequently, with a rate of 190–280 cases/million (1:3571 to 1:5263 individuals) being reported overall (2). Between these two extremes lies the most practically relevant information, namely the prevalence of clinically apparent pituitary tumors or those tumors that utilize health care resources during their diagnosis, treatment, and follow-up. Recently, we reported some of the first evidence regarding clinically apparent pituitary adenoma prevalence in the modern era. This newer epidemiological evidence suggests that pituitary adenomas occur relatively frequently in the general population, with an overall rate of one case in 1064 of the population (3). These results indicate that clinically apparent pituitary adenomas are more than three times more common than previously thought, which in turn increases the need to understand the pathophysiological mechanisms that give rise to these tumors.

A wealth of studies have been conducted on the molecular genetics of pituitary adenomas in an effort to determine their pathophysiology. Mutations in a series of genes, some relatively frequent and some rare, have been described and characterized in the experimental setting. Chief among these is the *gsp* gene that encodes the α-subunit of the Gs, a heterotrimeric G-protein. Activating mutations in *gsp* lead to constitutive activation of Gsα, increased adenylyl cyclase activity and overproduction of cAMP. Up to 40% of somatotropinomas have mutations in *gsp* (4). Other genetic abnormalities associated with pituitary tumorigenesis or abnormal proliferative characteristics are listed in Table 1.



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While many genetic abnormalities have been described in the setting of pituitary adenomas, few are involved in familial or inherited conditions. Familial pituitary tumors account for ~3% of pituitary adenomas (5). Multiple endocrine neoplasia type 1 (MEN1) and Carney's complex (CNC) are well-characterized inheritable syndromes that are associated with, among other features, pituitary adenomas. Isolated familial somatotropinomas (IFS) have been recognized as occurring in a familial setting for some time and the genetic pathophysiology has been the subject of intense interest. Over the last 7 years, a newer clinical condition, termed familial isolated pituitary adenomas (FIPA), has emerged, which encompasses a wider spectrum of pituitary adenomas occurring in a familial setting than only somatotropinomas. This review addresses the features of pituitary disease occurring in the familial setting with particular attention on recent information concerning the clinical, pathological, and genetic features of FIPA.

## Familial causes of pituitary adenomas

### *MEN1*

MEN1 syndrome, an autosomal dominant disease caused by mutations in the *MEN1* gene on chromosome 11q13 that encodes the regulatory protein menin, is characterized by the presence of typical patterns of endocrine active and inactive tumors and non-endocrine tumors (6). In patients with mutations in the *MEN1* gene, pituitary adenomas occur in ~40% of cases (7). These data are supported by murine models of *men1* gene knockout, in which ~37% of heterozygotic animals had pituitary tumors in adulthood (8). While no genotype–phenotype relation has been shown among the hundreds of MEN1 mutations now described, in familial MEN1, pituitary disease is significantly more frequent than in sporadic MEN1 cases (9). Prolactinomas predominate in MEN1, are larger than their sporadic counterparts and have a poorer response to dopamine agonist therapy (7). In MEN1, pituitary tumors are twice likely to be macroadenomas than in cases of sporadic pituitary adenomas (85% vs 42% respectively). In keeping with this, tumor signs caused by local compression are more frequent in MEN1 than in sporadic pituitary tumors. Females with MEN1 have an increased risk of developing a pituitary tumor and acromegaly demonstrates a female preponderance in the setting of MEN1 (7). Despite the in-depth characterization of MEN1 clinically and genetically, more than 20% of cases with clinical features characteristic of MEN1 have no demonstrable genetic mutation, raising the possibility of the involvement of other genes in this syndrome. Recently, a mutation in the *CDKN1B* gene, which codes for the cyclin-dependent kinase inhibitor $p27^{Kip1}$, was shown to lead to a MEN1-like syndrome in a rat model and a human kindred (10). In the human setting, a germline nonsense mutation in the *CDKN1B* gene on chromosome 12 was associated with acromegaly, primary hyperparathyroidism, renal angiomyolipoma, and testicular cancer among various members of the kindred. A second patient with a MEN1-like phenotype and no *MEN1* mutation was recently identified as having a *CDKN1B* gene mutation (11). The female patient had a small-cell neuroendocrine cervical carcinoma (in which loss of heterozygosity (LOH) for *CDKN1B* and lack of p27 protein staining were found), Cushing's disease, and hyperparathyroidism. No relevant family history of MEN1-like features was seen and limited family screening (one brother) was negative. Despite the MEN1-like features of the patients described, studies from Ozawa and colleagues at the NIH and from

**Table 1** Genetic mutations or alterations occurring in the setting of pituitary adenomas.

| Gene | Defect |
|---|---|
| *Cyclin D1* | Overexpression in non-secreting adenomas and somatotropinomas |
| *Gsp* | Somatic activating mutations in up to 40% of somatotropinomas<br>Mosaicism in McCune–Albright syndrome (somatotropinoma, somatomammotropinoma, and Cushing's syndrome in association with precocious puberty, hyperthyroidism, and dermal and bony lesions) |
| *PRKAR1* | Truncation mutations in Carney's complex leading to somatolactotrope hyperplasia and adenomas |
| *Pdt-FGFR4* | Alternative transcription initiation in pituitary adenomas |
| *PTTG* | Increased expression in more aggressive pituitary tumors |
| *BMP-4* | Diminished expression in prolactinoma |
| *GADD45G* | Promoter methylation in non-secreting adenomas, prolactinomas, and somatotropinomas |
| *MEG3a* | Promoter methylation in non-secreting adenomas and gonadotropinomas |
| *MEN1* | Inactivating mutations in all pituitary adenoma types |
| *PKC* | Point mutations in invasive pituitary adenomas |
| *p16* | Promoter methylation in pituitary adenomas |
| *CDKN1B* ($p27^{Kip1}$) | Germline heterozygous nonsense mutation in MENX, a novel, rare MEN1-like syndrome |
| *Retinoblastoma* | Promoter methylation in pituitary adenomas |
| *ZAC* | Promoter methylation in non-functioning adenomas |
| *AIP* | Germline mutations and loss of heterozygosity in 15% of FIPA cases. Seen in familial/sporadic somatotropinomas, somatolactotrope adenomas, prolactinomas, non-secreting adenomas, and Cushing's disease (sporadic only) |

Georgitsi *et al.* indicate that CDKN1B mutations appear to account for only a minority of patients with a MEN1 phenotype in the absence of *MEN1* gene mutations (11, 12)

### *CNC*

CNC is a rare predominantly familial condition characterized by lentigines, myxomas, Schwann cell tumors, adrenal hyperplasia, and pituitary abnormalities (13, 14). CNC is associated with mutations in the protein kinase A Iα regulatory subunit gene (*PRKAR1A*) in 60% of cases (15). Pituitary disease in CNC is characterized by frequent (up to 75% of cases) hypersecretion of prolactin, growth hormone (GH), and insulin-like growth factor-I (IGF-I), which can lead to acromegaly in occasional cases. Acromegaly in CNC is not particularly aggressive, with a mean age at diagnosis of 35.8 years in the largest series from Stratakis's group at the National Institutes of Health in the United States (16). One particular feature of pituitary tumors in CNC is the presence of somatomammotropic cell multifocal hyperplasia that occurs against a background of normal pituitary and may give rise to adenomas (17). Interestingly, murine models of CNC with *prkar1a* knockout, while mirroring many of the tumor abnormalities seen in humans, do not develop marked pituitary disease.

## *Isolated familial somatotropinomas*

IFS, defined as $\geq 2$ cases of acromegaly or gigantism in a family in the absence of MEN1 or CNC, has long been recognized as a clinical entity. If members of the FIPA cohort are included, more than 50 IFS families including over 120 individuals have now been described in the literature (18–20). IFS is characterized by a slight male predominance and a much younger age at onset (25 years) when compared with sporadic acromegaly, with gigantism being a characteristic feature of IFS kindreds. Tumors in patients with IFS are almost invariably macroadenomas. Before the identification of aryl hydrocarbon receptor-interacting protein (*AIP*) gene mutations as a potential culprit in some cases of non-MEN1, non-CNC familial pituitary tumorigenesis (21), genetic linkage in IFS to a defined region of chromosome 11q13 was well demonstrated by the collaborative efforts of Frohman, Teh, Gadelha, and others (19, 22, 23). Indeed, by 2004, Luccio-Camelo *et al.* had narrowed the linkage to between microsatellite markers D11S956 and D11S527 on chromosome 11q13.1-q13.3 (24). Despite the advent of mutations in *AIP* as potential causative features in IFS, the genetic pathophysiology of IFS remains to be fully described as we have found that in 50% of IFS families, no *AIP* mutations exist (25).

## FIPA

### *Background*

In the late 1990s, we became interested in the issue of pituitary adenomas that occurred in a familial setting but were not related to MEN1 or CNC (19). As noted above, IFS had been clearly identified as a clinical entity (19). Little or no evidence of familial links in the setting of other pituitary tumor phenotypes had been published and apart from a handful of case reports on familial prolactinoma (26, 27), Cushing's disease (28), and non-secreting (NS) adenomas (29), there had been no organized effort to study the clinical and genetic characteristics of other pituitary tumors occurring in a familial setting. At our own center, we began to collect and classify kindreds with two or more pituitary adenomas of any type that were unrelated to MEN1 or CNC, a clinical condition that we termed FIPA. In our initial single-center study in 2000, we identified 27 patients who came from FIPA families, which constituted ~1% of our total pituitary adenoma patient population (30). At that early stage, we noted that patients within the same family could exhibit either the same pituitary tumor type or different tumor types; these were classified as homogeneous and heterogeneous FIPA kindreds respectively. In order to expand the cohort, we began a multicenter collaborative study among tertiary referral centers in France, Italy, and the United States. By 2002, this collaboration had led to the identification and the clinical and genetic characterization of 80 patients among FIPA cohorts (31). Further expansion to 22 centers in France, Italy, and The Netherlands permitted the identification of 64 FIPA kindreds in 2004 which included ~140 patients (32). At that time, the study was closed and a full series of clinical, biochemical, radiological, and pathological analyses were performed on the entire group. Since then, FIPA kindreds have continued to be reported and more than 90 families have been identified worldwide by our collaborative group.

As an initial step to delineate the profile of patients with FIPA, we undertook a detailed retrospective study of the most clinically relevant features of these FIPA kindreds, namely their hormonal, radiological, and pathological characteristics (18). At least one affected member of each FIPA kindred underwent *MEN1* genetic screening, while negative family history and a normal serum calcium and parathyroid hormone (and normal gastrin, vasoactive intestinal polypeptide, and pancreatic polypeptide levels wherever available) were used to rule out MEN1 clinically in all patients. CNC was ruled out by the sequencing of the *PRKAR1A* gene in one affected member of each family exhibiting homogeneous GH-secreting tumors; thorough clinical profiling and echocardiography were also performed to exclude other CNC features, such as cardiac myxomas in patients with acromegaly. In order to assess whether there were

differences between the FIPA cohort and non-familial pituitary tumor patients, a control group of 288 non-MEN1, non-CNC sporadic pituitary patients, was analyzed. This control series was matched with the FIPA cohort for the year of diagnosis.

We used a combination of biochemical and clinical data to classify pituitary tumors according to their secretory profiles as prolactinomas, somatotropinomas, somatolactotrope tumors, Cushing's disease (adrenocorticotropin-secreting tumors), and thyrotropin (TSH)-secreting tumors. Gonadotropinomas and NS tumors were grouped separately. Tumors were assessed using computed tomography (pre-1986) or MRI, and were classified as microadenomas ($\leq$10 mm), macroadenomas (>10 mm), or giant adenomas (>40 mm); invasive characteristics (cavernous or sphenoid sinus) were also assessed. Surgical findings were collected and analyzed to add direct visual information about tumor size and invasion; results of immunohistochemistry for pituitary hormones were collected wherever available.

## *Clinical characteristics*

Among the FIPA cohort, families with two, three, and four affected members are seen (18, 25). FIPA is characterized by a predominance of prolactinomas and GH-secreting tumors, which account for about 75% of the cohort (Fig. 1). There is a female preponderance (62%) which may be related to the frequent occurrence of prolactinomas in women within the FIPA cohort. Affected members are mainly close relatives, with 74.6% demonstrating a first-degree relationship (i.e. sibling or filial relationships). As noted above, it had been evident for some years that the tumor phenotype within individual FIPA kindreds could present homogeneously or heterogeneously. FIPA is divided evenly between homogeneous and heterogeneous kindreds. In heterogeneous FIPA kindreds, all tumor phenotypes can occur, but almost invariably at least one prolactinoma or GH-secreting adenoma is seen per family.

Patients from FIPA kindreds are younger at diagnosis – on average 4 years younger – than patients with sporadic pituitary adenomas. When multi-generational families are assessed separately, patients from the later generations (children and grandchildren) have a significantly younger mean age at diagnosis as compared with their forebears (29.0 vs 50.5 years, $P<0.0001$). It is not known whether this generational effect is related to some form of anticipation at the genetic level, or earlier disease recognition due to increased awareness on the part of parents. Patients from homogeneous FIPA kindreds are significantly younger at diagnosis than their heterogeneous kindred counterparts; this effect is significant following multivariate analysis correcting for tumor type.

Overall, tumors from FIPA patients have size characteristics similar to those of the general sporadic pituitary tumor population. Macroadenomas occur in 63% of FIPA cases and about two-thirds of sporadic cases, and the rates of suprasellar extension and invasion of surrounding tissues do not differ between FIPA and sporadic pituitary tumors. Tumors from heterogeneous FIPA kindreds are more frequently macroadenomas than in the homogeneous FIPA group (72% vs 53% respectively; $P<0.04$). This is due to a predominance of NS tumors in the former group (all macroadenomas) and a high frequency of microprolactinomas among homogeneous FIPA kindreds.

### *Characteristics by tumor type*

- Prolactinomas comprise about 40% of tumors that occur in the setting of FIPA and as a whole do not

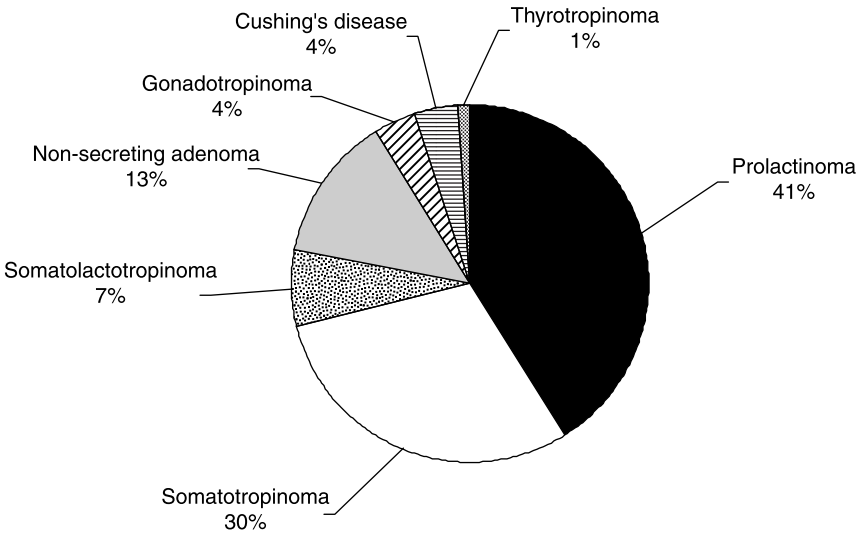


**Figure 1** Composition of the FIPA cohort by tumor phenotype.

differ from sporadic tumors (female predominance, presentation in second to fourth decades of life, microadenomas (33)). All males with prolactinomas in the FIPA cohort had macroadenomas, again in keeping with more aggressive disease in males from the sporadic population. Prolactinomas appear more aggressive when they occur in heterogeneous FIPA kindreds, with suprasellar extension and cavernous sinus invasion being significantly more frequent than in sporadic prolactinomas. Furthermore, the only malignant prolactinoma that occurred at our center was seen in a male from a heterogeneous FIPA family (34).

- Familial somatotropinomas account for 30% of tumors seen in FIPA. A further 7% of tumors could be classified as somatolactotropes, although their characteristics are not different from somatotropinomas in the FIPA cohort. Somatotropinomas are equally divided between homogeneous FIPA (essentially IFS) and heterogeneous tumor families. Notably, patients with IFS are diagnosed about 10 years before those with somatotropinomas in heterogeneous FIPA kindreds or in sporadic cases, a finding that echoes previous results on IFS from other groups. Those with IFS within the FIPA cohort are also significantly more likely to exhibit extra- or suprasellar extension of their tumors, with a trend toward more frequent invasion of surrounding structures.
- In FIPA, NS adenomas generally occur in heterogeneous kindreds ($>85\%$), are diagnosed significantly earlier ($\sim 8$ years), and are more frequently invasive than their sporadic counterparts.
- Gonadotropinomas, Cushing's disease, and TSH-secreting adenomas are all relatively infrequent in the setting of FIPA (each is $<5\%$ of the total population). They occur in association with other tumor types in heterogeneous kindreds, although two homogeneous Cushing's disease families and one family with homogeneous gonadotropinoma phenotype were described in the FIPA cohort.

Pituitary tumors in FIPA differ from those seen in the setting of MEN1. FIPA patients with homogeneous acromegaly (i.e. IFS) or Cushing's disease are younger at diagnosis than those with MEN1 (7). Prolactinomas are the most frequent tumors seen in FIPA and MEN1 (as they are in the sporadic setting); however, in MEN1, they account for a much greater proportion of the total (63%) when compared with FIPA (40%). In FIPA, somatotropinomas are about four times more frequent (34.1%) when compared with MEN1 (8.8%).

## *Genealogical information*

Among the full FIPA genealogies studied (mean family size 15.4 individuals), pituitary tumors occur in $\sim 14\%$ of family members. The familiality (degree of relatedness among affected individuals) of FIPA is high at 0.62, suggesting that genetic inheritance is at least partly dominant in character. Maternal transmission is more common among homogeneous than heterogeneous FIPA, potentially due to a high number of mother–daughter homogeneous prolactinoma kindreds (Fig. 2). A paternal transmission pattern is seen predominantly in patients with heterogeneous somatotropinomas, whereas homogeneous somatotropinomas (IFS) was characterized mainly (65%) by presentation in siblings.

## *The genetics of FIPA*

By definition, patients with FIPA have mutations in neither the *MEN1* nor the *PRKAR1A* gene. In the clinical studies outlined above, screening for *MEN1* mutations was performed in at least one affected member of each kindred, while *PRKAR1A* screening was performed in relevant kindreds with acromegaly. In IFS kindreds, a series of genetic studies have been undertaken to out-rule the involvement of mutations in candidate genes, some in the region of chromosome 11q13. No mutations in *gsp*, the GH releasing hormone (*GHRH*)-receptor gene, or the requiem gene were seen in IFS kindreds (35–37).

In an important advance, a study by Vierimaa *et al.* in May 2006 described a detailed genome-wide screening and DNA mapping study for genes involved in the pathogenesis of pituitary tumors that occurred in a familial setting (21). In these families, combinations of somatotropinomas, mixed GH–prolactin-secreting tumors and prolactinomas were seen. In affected members, the group discovered inactivating mutations in the gene that encodes AIP on chromosome 11q13.3. Analysis of tumor samples from affected individuals noted loss of heterozygosity at the AIP locus, suggesting that tumors were null for AIP. Two mutations were found among a Finnish cohort, Q14X in familial and sporadic pituitary adenoma cases and an IVS3-1G>A mutation in splice acceptor site of exon 4 in one patient with a sporadic pituitary adenoma. In addition, an R304X mutation was described in an Italian sibling pair with acromegaly, while familial pituitary tumor kindreds in Turkey and Germany demonstrated no *AIP* mutations. Given the phenotypic similarities between families within the FIPA cohort and those reported by Vierimaa *et al.* (21), a study was performed to assess whether *AIP* mutations contributed significantly to the pathogenesis of tumors in the setting of FIPA.

In an international cohort from nine countries, 156 patients comprising 73 FIPA families were included and were classified according to the disease definitions used in earlier studies of the clinical characteristics of FIPA (18, 25). A total of 11 out of 73 (15.1%) FIPA families harbored ten different germline *AIP* mutations, one FIPA family from Italy (unrelated to that reported by Vierimaa *et al.*) had an R304X mutation, and the

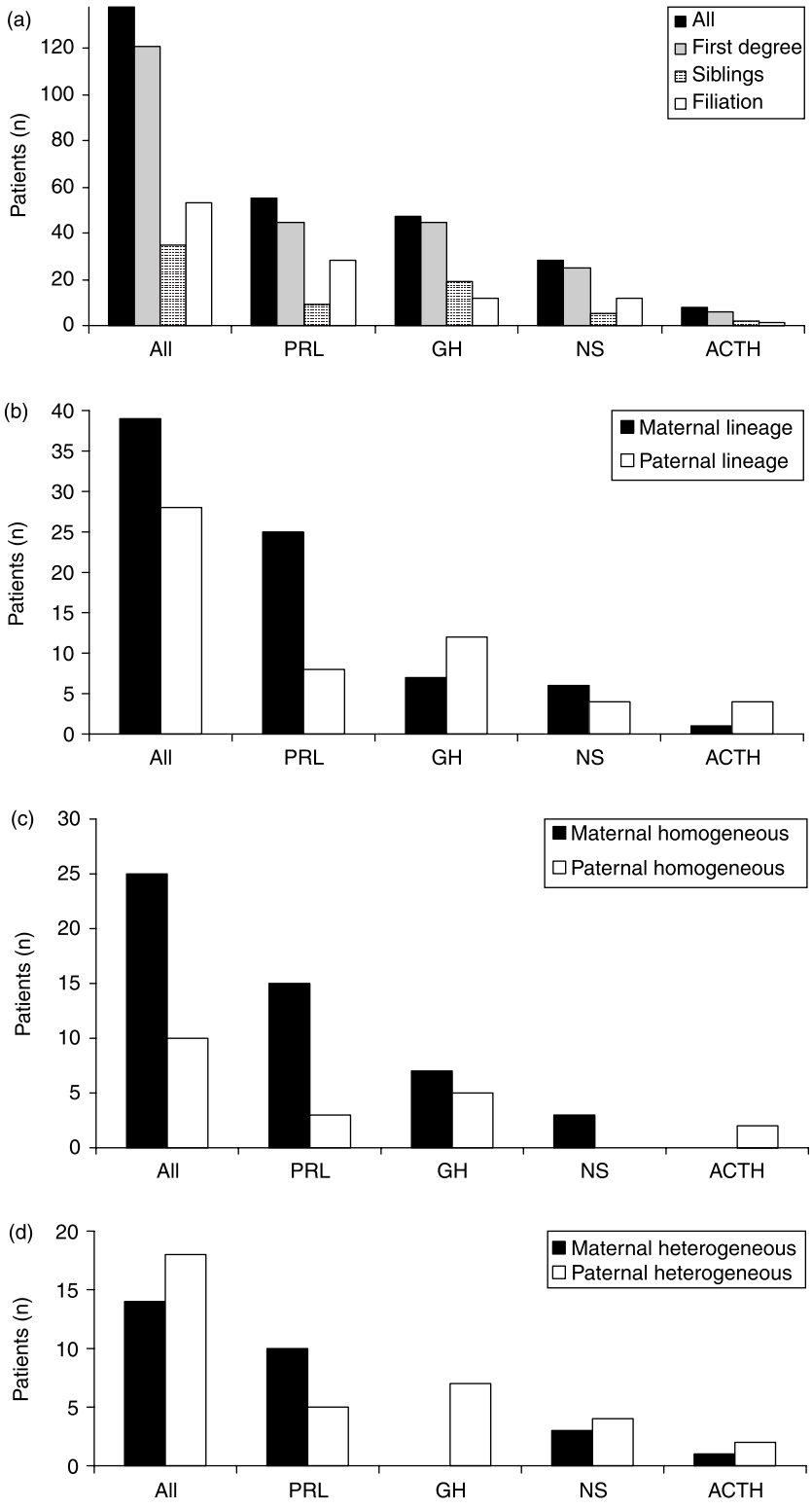


**Figure 2** Distribution of first-degree-related affected members from isolated pituitary adenoma families. Affected members are shown by (a) type of relation; (b) potential parental lineage; (c) potential parental lineage in families with homogeneous tumor phenotype expression; and (d) potential parental lineage in families with heterogeneous tumor phenotype expression.

rest were novel. Importantly, only 50% of the FIPA families with homogeneous acromegaly/IFS demonstrated *AIP* mutations. Indeed, the lack of *AIP* mutations in FIPA kindreds with apparently strong familiality for pituitary tumors (multiple three-member kindreds and a four-member family) indicates that additional causes are involved in the genetic pathophysiology of FIPA.

The characteristics of the AIP mutation-bearing FIPA families are outlined in Table 2. The range of patient and tumor characteristics seen in *AIP* mutation-positive FIPA kindreds is quite variable. FIPA patients with AIP mutations are significantly younger at diagnosis than those without *AIP* mutations (~12 years), while their maximum tumor diameter is significantly larger in the group with *AIP* mutations than those without. The majority of FIPA families with *AIP* mutations have somatotropinomas or mixed GH–prolactin-secreting tumors, but in one FIPA family, a patient with a NS tumor (negative GH and prolactin immunohistochemistry) occurred in association with a prolactinoma in the other affected family member. When immunohistochemical and hormonal secretion patterns in FIPA patients with *AIP* mutations are considered, a further degree of complexity is added. For instance, patients classified as having 'somatotropinomas' are in fact not a uniform group, with 62% having elevations in GH and IGF-I alone, while 38% also had elevated prolactin. In terms of immunohistochemistry, 'somatotropinoma' patients with *AIP* mutations can demonstrate staining for GH alone (59%), GH and prolactin (33%), or GH and FSH (8%). Indeed, the same *AIP* mutation can lead to various clinical phenotypes in different familial kindreds, with a somatotropinoma and a prolactinoma occurring in one family with an R271W mutation, and only acromegaly occurring in another unrelated family with the same mutation. The Q14X mutation seen relatively frequently among familial and sporadic pituitary tumor patients from Finland was not identified within FIPA families. The fact that this mutation was also not identified among larger sporadic and familial pituitary tumor populations in Europe, Japan, and the U.S. suggests that it is due to a founder mutation and is particularly characteristic of pituitary disease in Northern Finland (38–41).

Many AIP mutations have now been demonstrated in the setting of isolated pituitary adenomas, both familial and more rarely sporadic (Fig. 3). The functional implications of these mutations in *AIP* remain to be determined as relevant studies of protein expression and ligand–receptor interactions relating to these reported *AIP* mutations are wholly lacking. Experimental data on aspects of the structure of AIP do, however, provide some useful indicators. AIP, a member of the immunophilin family of proteins, is 330 amino acids in length and contains a number of conserved regions. Among these are three tetratricopeptide repeat (TPR) domains and a FK506 binding protein-type peptidyl-prolyl *cis–trans* isomerase (FKBP-PPI) domain; the latter is characteristic of immunophilin proteins. Most information available on the structure–function relationships of AIP relate to the third TPR domain and the carboxy-terminal amino acids (42). The third TPR domain is required for the interaction of AIP with a dimer of heat shock protein 90 (hsp90) and with the aryl hydrocarbon receptor (AhR) (43). Mutations of the AIP third TPR domain in the mouse prevent or decrease interactions with hsp90, AhR, or both (44, 45), while removal of the final carboxy-terminal amino acids prevents binding to AhR (46).

Many *AIP* mutations leading to protein truncations would either prevent AIP being encoded entirely or would remove the vital third TPR domain and the carboxy-terminal. Relatively little is known regarding the function of the amino terminus of AIP; however, its amino acid sequence is highly conserved across species. This suggests that amino acid substitutions, such as R16H for instance, could be expected to have functional significance. Indeed, since we reported germline R16H changes in a FIPA family, others have noted similar mutations in four sporadic pituitary adenoma patients and also in the germline and tumors of two patients with colorectal carcinoma (and family histories of colorectal, carcinoid, and other tumors) (47, 48). A full appreciation of the effect of R16H on AIP expression and/or function will be required to determine whether this is

**Table 2** Characteristics of patients and asymptomatic carriers with *AIP* mutations in the familial isolated pituitary adenomas cohort.

| ***AIP* mutation** | **Relation between members** | **Disease phenotype** | **Mutation-positive asymptomatic family members** |
|---|---|---|---|
| R16H | Two first cousins | Acromegaly | 0 |
| G47_R54del | Two siblings | Acromegaly | 2 (mother and sister of affected subjects); sister with hyperprolactinemia but no tumor |
| Q142X | Three siblings and one daughter of one affected sibling | Acromegaly/gigantism and prolactinoma | 0 |
| E174fs | Two siblings and a maternal aunt | Acromegaly/gigantism and prolactinoma | 7 (grandparent, parent, and siblings of affected subjects) |
| Q217X | Two siblings | Acromegaly | 1 (nephew of affected subjects) |
| Q239X | Parent and one offspring | Acromegaly/gigantism | 3 (grandparent, uncle, and sibling of affected offspring) |
| K241E | Two siblings | Prolactinoma and non-secreting adenoma | 2 (children of affecteds) |
| R271W | Parent and one offspring | Acromegaly | 0 |
| R271W | Parent and one offspring | Acromegaly and prolactinoma | 0 |
| Q285fs | Two siblings | Acromegaly/gigantism | 0 |
| R304X | Two siblings and one nephew | Acromegaly/gigantism | 4 (siblings of affected subjects) |

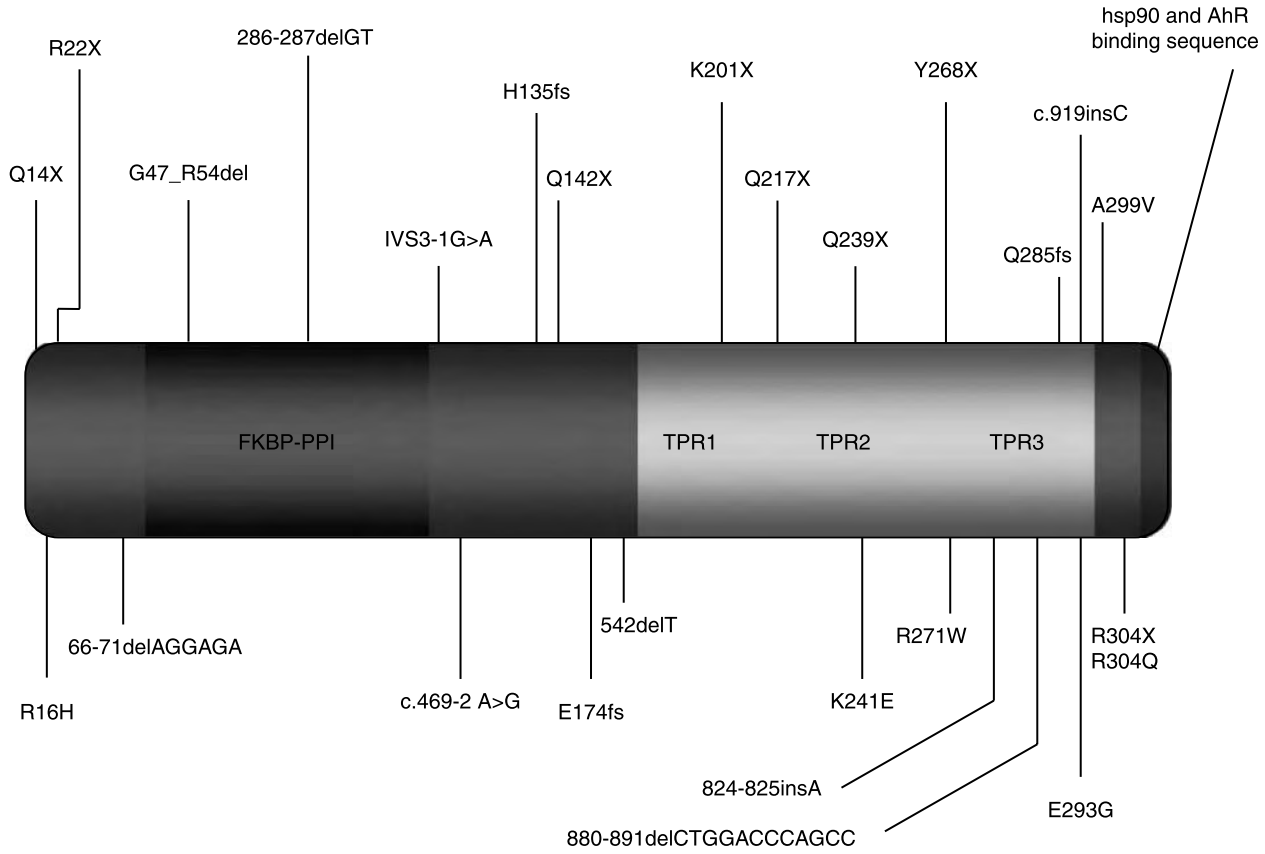


**Figure 3** The AIP protein with functional domains highlighted and reported genetic mutations noted. FKBP-PPI, FK506 binding protein-type peptidyl prolyl cis-trans isomerase; TPR, tetratricopeptide repeat domain; hsp90, heat-shock protein 90, AhR, aryl hydrocarbon receptor. Adapted with permission from Daly AF *et al.* Aryl hydrocarbon receptor-interacting protein gene mutations in familial isolated pituitary adenomas: analysis in 73 families. *Journal of Clinical Endocrinology and Metabolism* May 2007 **92**(5) 1891–1896. Copyright 2007, The Endocrine Society. Reference (25).

truly a pathogenic mutation. Other missense mutations involving conserved residues (R271W and K241E) have been identified in FIPA families; the former concerns a highly conserved arginine that forms part of the third TPR domain. Mutation studies of amino acids in this region of AIP in the mouse prevent hsp90/AhR binding. Similarly, K241E is a conserved amino acid, although its functional role is indeterminate at this time.

To date, most studies related to AIP have focused primarily on the modulation of dioxin-related cellular responses via the interaction of AIP with its receptor AhR and an hsp90 dimer. This is clearly an important function, with depletion and augmentation of intracellular AIP concentrations leading to enhanced and decreased ubiquitin-mediated degradation of AhR (49). However, AhR has been shown to have a wide range of effects on cellular signaling cascades, not only those limited to dioxin-mediated responses, e.g. induction of hepatic cytochrome P450 subtype 1A1 expression. AhR shares affinity with hypoxia inducible factor-1α (HIF-1α) for the aryl hydrocarbon nuclear translocator (ARNT), through which multiple cellular cascades can be modulated; newer evidence suggests that AhR, HIF-1α, and ARNT are involved in a complex crosstalk at a transcriptional level involving multiple response elements and DNA motifs (50). Crosstalk between AhR and other transcription factors has been shown to include nuclear factor-κB, retinoblastoma protein, and estrogen receptor-α (51). Interestingly, it has been suggested that AhR–ARNT can modulate estrogen receptor signaling, potentially explaining the role of dioxin-related toxins as environmental 'endocrine disruptors' (52). Furthermore, AIP appears to play a separate role in the selective modulation of the cAMP-specific phosphodiesterase PDE4A5, with AIP reversibly inhibiting PDE4A5, and can reduce the ability of protein kinase to phosphorylate PDE4A5 (53). Interestingly, a mutation at the arginine at position 271 (a mutation site in two FIPA families) reduced the interaction of AIP with PDE4A5. While the effects of AIP modulation of PDE4A5 on cell proliferation remain to be determined, a mutation-sensitive effect of AIP on cAMP levels would

appear to be a relevant line of investigation in determining the role of AIP mutations in pituitary tumorigenesis. Recently, a specific interaction of phosphodiesterase PDE2A with AIP has been reported that serves to inhibit dioxin- and cAMP-related AhR nuclear translocation and gene transcription (54). Given the important role of AIP in modulating AhR levels, inactivating mutations of AIP appear likely to interfere with multiple physiological signaling cascades and xenobiotic responses.

## Perspectives

The occurrence of tumors in a familial setting represents a useful starting point for the investigation of their genetic and molecular tumorigenesis, as evidenced by the study of MEN1 and CNC. Familial pituitary adenomas are a typical feature of these and other newer hereditary neuroendocrine tumor syndromes related to mutations in *CDKN1B* and *AIP*. However, despite molecular genetic advances, the clinical recognition and practical management remain the initial step and final goal in dealing with novel familial endocrine syndromes.

As described above, FIPA encompasses a wider phenotypic definition of familial pituitary tumors than permitted by the established condition of IFS, and virtually all combinations of pituitary tumors can occur in homogeneous and heterogeneous patterns within families. Thus, families exhibiting isolated somatotropinomas and prolactinomas can be readily described using the term FIPA. Furthermore, FIPA can be used to describe such kindreds in the presence or absence of *AIP* mutations, whereas the alternate description of pituitary adenoma predisposition is limited to the minority of families with a confirmed *AIP* mutation.

Some years ago, we chose the term FIPA for the description of our cohort for a number of reasons. First, it is broad yet clinically descriptive, as befits a label for an investigative condition of often uncertain molecular etiology. With the advent of *AIP* mutations as a causative agent for familial pituitary adenomas, we believe that FIPA remains valid as a terminology particularly given that only 15% of FIPA cases are linked to *AIP* mutations. Furthermore, as AIP is thought to be a culprit in only 50% of IFS cases, IFS also remains a disease classification with clear clinical utility. Secondly, FIPA readily follows a naming format similar to that used in other forms of hereditary hormone excess and endocrine cancers occurring in single organs, such as familial isolated hyperparathyroidism (FIHP) or familial isolated medullary thyroid carcinoma (FMTC) (55). Both of these conditions, like FIPA, are described in terms of their clinical and familial nature (56), but can be caused by various identified genetic mutations (e.g. MEN1 and HRPT2 for FIHP (57–60), while linkage to other genes and chromosomal regions has been suggested in cases without a defined genetic pathophysiology (61, 62). Similarly, FIPA permits the accurate clinical description of isolated pituitary tumors occurring in families while allowing for multiple genetic causes, such as *AIP*, and others yet to be described.

Currently, the FIPA cohort, including those with *AIP* mutations, remains free of other discrete endocrine and non-endocrine conditions that would suggest a characteristic clinical syndrome. However, it cannot be discounted that germline *AIP* mutations could be associated with a predisposition to tumors other than pituitary adenomas particularly as missense *AIP* mutations have been noted in the setting of colonic adenoma tissue. While other highly prevalent tumors, such as thyroid adenomas, are present in certain FIPA patients, demonstration of LOH for *AIP* or abnormal AIP protein expression would be required before expanding the clinical phenotype beyond pituitary adenomas. These questions will be answered in part by the clinical phenotype exhibited by *aip* knockout mouse models now at an advanced stage of development. However, there is an important caveat in extrapolating murine data on AIP function to humans because the interactions of AIP with AhR in the mouse differ from that in the human in terms of AIP-cytoplasmic localization and AIP-induced shuttling to the nucleus (63, 64). It may be that this inter-species divergent AIP/AhR function may lead to different disease risks and tumor expression in humans when compared with mice.

With the identification of *AIP* mutations as being involved in the etiology of familial pituitary tumors, the issue of screening has been raised (47). In particular, it has been suggested that immunohistochemistry for AIP in pituitary tumor tissue be used to screen operated patients for mutations. While it is indeed feasible to undertake AIP immunohistochemistry, further information will be required on a number of fronts. Initially, the presence or absence of AIP in pituitary tumor samples requires information regarding the patterns of expression of AIP in the nucleus and cytoplasm of normal pituitary cells. Also, it remains to be determined as to whether various normal pituitary cells (somatotropes, lactotropes, or corticotropes) themselves have distinctive levels of AIP expression. Pituitary adenomas associated with *AIP* mutations in FIPA and the sporadic setting are heterogeneous in terms of hormonal immunohistochemistry and clinical phenotype and include all types except for thyrotropinomas. Again, the range of AIP expression patterns in these adenomatous cells when compared with normal tissue remains to be determined before immunohistochemical screening can permit identification of *AIP*-mutated specimens. The effects of common first-line and adjunctive treatments (somatostatin analogs, dopamine agonists, and radiotherapy) on AIP expression patterns in somatotropinomas, prolactinomas, and NS tumors have not been studied to date and could theoretically alter immunohistochemical results. AIP immunohistochemistry has, to date, focused on samples derived from

patients with early truncating mutations of AIP (e.g. the Finnish founder mutation, Q14X). Such mutations would be expected to lead to an absence of AIP protein and a negative immunostain. Mutations that lead to the disruption of crucial amino acids in the third TPR (R271W) or the carboxy-terminal amino acids (R304X) may abrogate or decrease biological AIP function without decreasing protein expression; this could lead to tumor samples containing mutated AIP protein appearing normal on immunohistochemistry. Finally, AIP immunohistochemistry has been based on murine polyclonal antibodies that have unknown cross-reactivity patterns with human immunophilins. For fundamental immunohistochemistry studies on AIP expression in normal and adenomatous pituitary tissues, it would be preferable that monoclonal antibodies directed against known epitopes on the human AIP protein should be used.

Widespread genetic screening for *AIP* mutations in patients with sporadic pituitary adenomas and in relatives of those bearing *AIP* mutations requires careful consideration. First, there is considerable divergence in the reported penetrance of pituitary adenomas among kindreds with AIP mutations. In the original report regarding AIP mutations in familial pituitary adenomas, it was suggested that this was a low penetrance disease (21). However, that study was based on a relatively small number of families (three families with two distinct AIP mutations) and limited clinical screening of the kindred. In contrast, we suggest that the penetrance of pituitary disease in *AIP* mutation-bearing FIPA kindreds is high. Our preliminary data would suggest that well in excess of 50% of individuals from families with *AIP* mutations have pituitary adenomas, data that are supported by other groups (65) and are in keeping with the strong reported familiality of FIPA (18). Before widespread genetic screening for *AIP* mutations among pituitary adenoma patients can be contemplated, the issue of true penetrance will need to be addressed in order to provide informative counseling to patients and to address the vital ethical issues relevant to the study of familial neuroendocrine tumor syndromes (for review see Sukarai *et al.* (66)). We agree with Melmed that the widespread use of genetic and radiological screening in unselected patients with sporadic pituitary adenomas is not warranted at this time (67). However, given the characteristics of patients with *AIP* mutations (young age at diagnosis, large tumor size), screening of young patients with aggressive pituitary tumors for AIP mutations should be considered. In the case of relatives of patients with *AIP* mutation-related pituitary adenomas, *AIP* screening is recommended.

Patients with pituitary tumors and *AIP* mutations may have a poorer response to therapy, like many young patients with larger pituitary tumors (68), although this issue has not been studied *per se*. From a clinical perspective, it may be practical to bear in mind the main characteristics of patients from FIPA kindreds: (1) pituitary adenomas of all types can occur in a familial setting; (2) pituitary tumors in FIPA are larger and diagnosed earlier than sporadic adenomas; (3) 15% of FIPA and 50% of IFS families are linked to mutations in *AIP*; and (4) in the absence of a close family history of pituitary adenomas, *AIP* mutations are rare. Whatever the genetic pathophysiology is, good endocrine practice involving a detailed family history, standard comprehensive testing of pituitary axes, and pituitary MRI should be applied to all patients with pituitary adenomas. In the case of young patients with aggressive tumors, germline *AIP* genetic study may be useful (69) and is warranted in the presence of a family history of pituitary tumors without an identified genetic pathophysiology.

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# Clinical and Genetic Features of Familial Pituitary Adenomas



**Abstract**

Inherited or familial pituitary tumor syndromes such as multiple endocrine neoplasia type 1 and Carney complex provide an important insight into the genetics and molecular pathology of endocrine cancers. Our understanding of these conditions is expanding rapidly due to the identification of the genes and proteins affected and the availability of murine knockout models. The successes achieved to date in understanding multiple endocrine neoplasia type 1 and Carney complex have helped in the identification and study of new inherited pituitary tumor syndromes such as isolated familial somatotropinomas. This review assesses the current status of research into the clinical features, genetics and molecular pathologies of multiple endocrine neoplasia type 1, Carney complex, and isolated familial somatotropinomas, and details ongoing work to delineate familial isolated pituitary adenomas, a potentially new clinical entity.

## Introduction

Our understanding of familial cancer syndromes has assisted greatly in elucidating the genetic and molecular mechanisms that underlie tumorigenesis. This process has involved a movement from initial linkage analysis studies through to the mapping and identification of relevant gene(s), ending with an understanding of how abnormal protein expression dictates neoplastic changes at the molecular level. The occurrence of pituitary adenomas in a familial setting is a useful example that helps to illustrate how interesting clinical observations can stimulate basic research to understand mechanisms of tumorigenesis.

This review outlines the current state of knowledge regarding recognized syndromes that are associated with inherited pituitary adenomas such as multiple endocrine neoplasia type 1 (MEN1) and Carney complex (CNC). The characteristics of emerging heritable pituitary tumor syndromes, such as those of isolated familial somatotropinomas and familial isolated pituitary adenomas, are also discussed.

## Multiple Endocrine Neoplasia Type 1-associated Pituitary Adenomas

### Background

MEN1 syndrome is an autosomal dominant condition that is associated with the occurrence of endocrine-active parathyroid, enteropancreatic and anterior pituitary tumors amongst others [1]. Endocrine inactive tumors, such as lipomas, angiofibromas and collagenomas are a frequent finding in MEN1 [1]. Larsson et al. first linked the gene involved in MEN1 to a locus on chromosome 11q13 in 1988 [2], which was followed in the late 1990s by the cloning of the *MEN1* gene by Chandrasekharappa et al. [3]. The *MEN1* gene is organized into 10 exons, of which exons 2 to 10 encode the nuclear protein, menin [4]. The *MEN1* gene has a

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complex upstream promoter apparatus, elements of which are regulated by menin activity; this echoes the known interactions of menin with the transcription of endocrine gene promoters [5–8]. Differential regulation of menin expression in different tissues via upstream genetic elements may partly explain how mutations of *MEN1* preferentially involve cells of the endocrine system, although menin is also expressed in a variety of non-endocrine cells and tissues. More than 350 germline mutations of the MEN1 gene have been described to date [9]; these occur predominantly in coding exons, but also among intronic sequences [10]. These include point mutations and small deletional or insertional mutations, which are thought to significantly alter the structure or biological function of menin. In particular, a number of non-sense and frameshift mutations lead to the synthesis of a truncated protein. Approximately 10% of cases that are clinically suggestive of MEN1 do not exhibit any *MEN1* mutation, suggesting the existence of unrecognized abnormalities in the regulation of menin expression and/or molecular function [11]. For example, the phenotypic consequences of mutations in the *MEN1* promoter region have yet to be established. Also, the involvement of other unrecognized molecular pathways cannot be ruled out. Patients with clinical features of MEN1 lacking genetic evidence of *MEN1* mutation – referred to as MEN1 phenocopy – can present either sporadically [11] or as part of MEN1 kindreds [12]. These patients often show an incomplete MEN-1 phenotype with less frequent enteropancreatic tumors and a tendency to more frequent GH-secreting rather than prolactin-secreting pituitary tumors.

Menin is a nuclear protein with a wide variety of molecular interactions, most of which have recently been described, whose biological significance is gradually becoming clear [1,13]. For example, it has recently been demonstrated that interactions of menin with *JunD* (an AP-1 family member) lead to the formation of a growth-inhibiting complex, which can be disrupted by specific mutations in either component [14]. In the last four years, menin has been shown to interact with nuclear factor κB, the Smad family, DNA, cell cycle regulators and a variety of other transcription factors, and cell structural elements [15–18]. Recent work has demonstrated that menin may play a role in promoting apoptosis via Bax and Bak; menin loss reduces apoptosis and also decreases the mRNA expression of procaspase 8, which is a key regulator of death receptor-mediated apoptosis [19]. In contrast, upregulation of menin augments both apoptosis and procaspase 8 expression. Little is known about how menin interacts with these other molecules to regulate cell function-apart from nuclear localization sequences [20], no other functional domains have been characterized to date.

## Menin Function in Pituitary Tumorigenesis

Orthologs of menin have been found in many species, including mice [21]. This has permitted the development of murine menin knockout models to study the development of tumors in MEN1 [22,23]. Mice that are homozygous knockouts for *Men1* (*Men1-/-*) die as embryos and have multiple severe developmental defects [24]. Biondi et al. and others have used conditional homozygous inactivation methods using the Cre recombinase *lox*P system to circumvent this lethality and create adult mice with a constitutional *Men1-/-* genotype restricted to the pituitary gland [25]. The pituitaries of these mice developed normally in the absence of menin, but as in humans with MEN1, prolactinomas were common. Also – as in MEN1 – affected humans-pituitary tumorigenesis in *Men1-/-* mice lagged behind the development of adenomas in other tissues. Bertolino et al. followed *Men1+/-* mutant heterozygotic mice over a period of up to 26 months to assess the penetrance of various tumors over time [24]. Enlargement of the *pars distalis* of the mouse pituitary, which corresponds to the anterior pituitary in humans, was a common finding in mice aged over 13 months. Pituitary tumors were noted in 19% of mice at 13–18 months, increasing to 36.6% at 19–26 months; wild-type controls did not develop pituitary tumors. These tumors were more common in female mice, and over 50% of all pituitary tumors were carcinomas. Of 15 tumors that were characterized immunohistochemically, nine stained for prolactin only, while six were strongly growth hormone (GH)-immunoreactive, and no tumor stained positive for adrenocorticotropic hormone (ACTH). The complete or partial loss of the wild-type *Men1* allele was found to have occurred in all 34 islet, pituitary, adrenal, thyroid and gonadal tumors analyzed after microdissection in these heterozygotic mice [24]. These experimental results are in line with what has been demonstrated from microdissection studies performed in a variety of tissues from patients with MEN1 [26].

At a molecular level, menin appears to have a number of interactions of particular relevance to the pituitary. Menin overexpression has been shown to inhibit the activity of the prolactin gene promoter [8]. Prolactin expression among lactotropic pituitary cells is negatively regulated by activin, a member of the TGFβ family [27], an action that is regulated by menin and the Smad pathway [17]. Inhibition of TGFβ signaling appears to follow menin inactivation and is regulated in somatomammotropes via Smad3 [17]. In studies on pituitary lactotrope cells, Lacerte et al. have reported that menin plays a crucial role in the activin/TGFβ-induced regulation of prolactin expression and pituitary cell growth [28]. Some of these actions involving menin appear to be mediated via activin-induced downregulation of the pituitary transcription factor, Pit-1. Inactivation of menin leads to disruption of activin-induced repression of prolactin expression and pituitary cell growth [28].

As noted originally by Zhuang et al. [29], we [30] and others [31–36] have confirmed that somatic mutations of the *MEN1* gene are not invariably a causative factor in the tumorigenesis of non-MEN1-linked sporadic pituitary adenomas. In a series of 35 sporadic pituitary adenomas of various secretory phenotypes, we found only one tumor to exhibit homozygosity (or hemizygosity) for a mutation close to the MEN1 promoter region [30]. More recently, Theodoropoulou et al. used menin immunohistochemistry and immunofluoresence to study a series of 68 sporadic non-MEN1 pituitary tumor tissue samples, and found – with only one exception – that menin was detectable, albeit often at lower levels than in normal pituitary tissue [37]. Interestingly, one patient with a metastatic prolactin-secreting pituitary carcinoma exhibited weak menin staining in tumor tissue at initial resection, but lost menin expression completely in locally invasive tumor and in distant metastases that were studied *post mortem*.

### Clinical Characteristics of MEN1-related Pituitary Tumors

The prevalence of pituitary adenomas in patients with MEN1 is approximately 40%, although reported rates vary [38–40]. As part of the multicenter Franco-Belgian GENEM (*Groupe d'Etude des Neoplasies Endocriennes Multiples*) study involving 324 MEN1 patients, the characteristics of pituitary disease were compared with those of 110 non-MEN1 patients with pituitary adenomas who were matched for age, year of diagnosis and follow-up period [41] (Fig. **1**). Among the MEN1 patients, 136 (42%) had pituitary tumors, and this was the initial presenting tumor in 17% (56/324) of cases (Table **1**). On average, presentation with MEN1 occurred seven years earlier in patients presenting with pituitary tumors compared with those with enteropancreatic lesions. The mean delay to presentation with the next MEN1-related tumor was significantly longer in those with a pituitary tumor at initial diagnosis (9 ± 8.1 years) compared with those initially presenting with a parathyroid (5.2 ± 5.1 years) or enteropancreatic tumor (4.1 ± 4.0 years). Importantly, pituitary disease among the familial MEN1 cases was significantly more frequent compared to sporadic MEN1 cases (59% *vs.* 34% respectively, $p < 0.0001$). A multivariate analysis found that female sex was the only factor associated with increased risk of developing a pituitary tumor. Pituitary adenomas were significantly more aggressive in MEN1 cases compared with non-MEN1 cases, with macroadenomas present in 85% of the former, compared with only 42% of the controls ($p < 0.001$). Unsurprisingly, therefore, MEN1-associated tumors were significantly more likely to cause signs due to tumor size ($p < 0.001$) and had a significantly lower rate of hormonal normalization than non-MEN1 pituitary tumors ($p < 0.001$) (Table **2**). Prolactinomas predominated among both MEN1 associated and non-MEN1 pituitary adenomas, and the proportions of prolactinomas, GH-secreting, ACTH-secreting, non-secreting and co-secreting adenomas were similar between the MEN1 and non-MEN1 patients. The MEN1-related prolactinomas were predominantly macroadenomas (84%) and of these, 20 were invasive. The response of MEN1-related prolactinomas to dopamine agonists was relatively poor, with a normalization rate of only 44% of patients. Overall, these data suggest that pituitary adenomas in MEN1 are characterized mainly by prolactinomas; pituitary tumors in MEN1 appear to be larger and more aggressive than in patients without MEN1 [42].

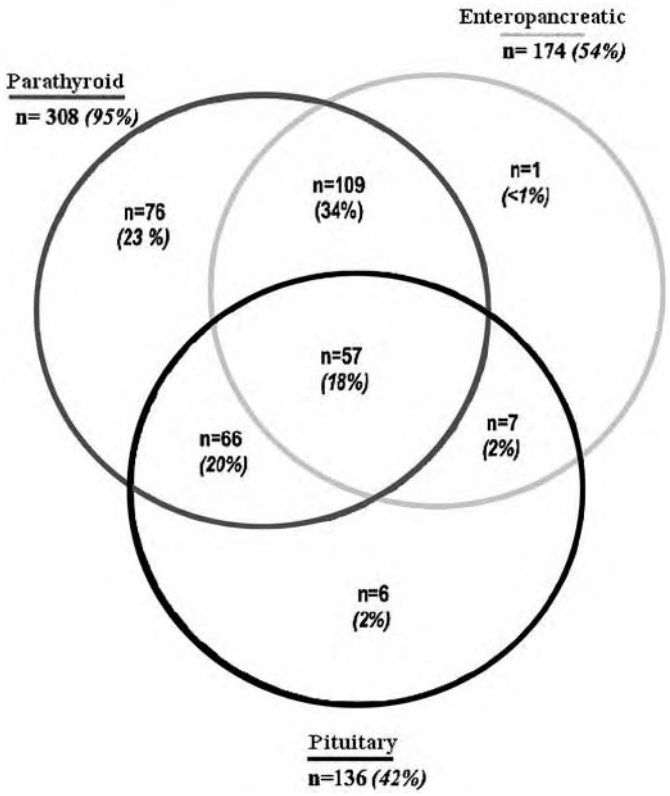


Fig. **1** Distribution of the main endocrine lesions among 324 MEN1 patients. The number of cases and the percentage of the total number of MEN1 patients are given for each endocrine lesion. Adrenal and neuroendocrine tumors are not shown. Among the 54 adrenal tumors, 8 are associated with parathyroid adenoma alone, 21 are associated with both parathyroid and enteropancreatic tumors, 2 with both parathyroid and pituitary tumors, 16 with parathyroid, enteropancreatic and pituitary tumors, and 2 with parathyroid, enteropancreatic, pituitary and neuroendocrine tumors. Among the 25 neuroendocrine tumors, 2 were isolated, 2 are associated with parathyroid tumors alone, 11 with both parathyroid and enteropancreatic tumors, 1 with both parathyroid and adrenal tumors, 3 with parathyroid, enteropancreatic and pituitary tumors and 2 with parathyroid, enteropancreatic, pituitary and adrenal tumors. Adapted with permission from Vergès B et al. J Clin Endocrinol Metab. 2002; 87: 457–465. Copyright 2002, The Endocrine Society.

There is no apparent relationship between the site or type of genetic mutation in the *MEN1* gene and the MEN1 disease phenotype expressed [43]. A variety of combinations/clusters of similar tumor types may occur in individual families with MEN1, and the severity of disease expression may vary [44]. While a variety of phenotypic subtypes of MEN1 have been reported, the most robustly demonstrated is the prolactinoma variant of MEN1, which include $MEN1_{BURIN}$ and $MEN1_{TASMAN}$. $MEN1_{BURIN}$ was initially described 25 years ago in a family from the Burin peninsula of Newfoundland, Canada, which exhibited a syndrome of prolactinomas and carcinoid tumors without pancreatic involvement [45, 46]. $MEN1_{TASMAN}$ was described initially by Burgess and colleagues and consisted of patients with prolactinomas and nonfunctioning adenomas [39]. Recently, Marx's group from the NIH reported two prolactinoma variant MEN1 kindreds that had undergone long-term scrutiny to avoid various forms of potential bias, such as non-standardized surveillance, young age and short follow-up times as well as small family size [47]. They concluded that the phenotype appears to be caused by different *MEN1* mutations although the prolactin MEN1 variant appears to be robustly expressed among the large kindreds reported.

In the Franco-Belgian multicenter MEN1 study, GH-secreting tumors were noted in twelve patients (9% of the total); all but one displayed the classical signs and symptoms of acromegaly, and five had tumor size-related signs [41]. The mean age at diagnosis was 43.6 ± 16.1 years, which is similar to that in sporadic acromegaly. However, in contrast to sporadic acromegaly, three times as many GH-secreting adenomas occurred in females as in males. Following treatment (surgery, radiotherapy and medical therapy, alone or in combination) 50% of patients had hormonal normalization, 33% had persistent hypersecretion, and the remainder had hypopituitarism. Although the patient number is relatively small, it appears that the response to treatment in MEN1-related acromegaly approximates that seen in sporadic GH-secreting macroadenomas. MEN1-related gonadotropin-secreting adenomas that

Table 1 Characteristics of a large series of MEN1 pituitary tumors (n = 136)

| Pituitary Adenoma (n) | Tumor size* Macro (n) | Micro (n) | Age (yrs) | Sex M | F | Diagnosis 1st tumor diagnosed | Simultaneous diagnosis | Diagnosed after other tumor | Familial | Sporadic |
|---|---|---|---|---|---|---|---|---|---|---|
| Prolactinoma (85) | 71 | 13 | 35.1 ± 14.8 | 25 | 60 | 36 | 25 | 24 | 51 | 34 |
| GH-secreting (12) | 12 | 0 | 43.6 ± 16.1 | 3 | 9 | 8 | 1 | 3 | 3 | 9 |
| ACTH-secreting (6) | 6 | 3 | 43.0 ± 12.1 | 4 | 2 | 2 | 2 | 2 | 3 | 3 |
| Co-secreting (13) | 10 | 10 | 38.3 ± 13.7 | 3 | 10 | 7 | 4 | 2 | 7 | 6 |
| Non-secreting (20) | 20 | 20 | 46.2 ± 12.2 | 7 | 13 | 3 | 5 | 12 | 11 | 9 |

*No tumor size data for 1 patient.
GH, growth hormone; ACTH, adrenocorticotropic hormone; micro, microadenoma; macro, macroadenoma.
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Table 2 Comparison of pituitary tumor characteristics between MEN1 and non-MEN1 patients

| | Pituitary adenoma MEN1 | Non-MEN1 | p |
|---|---|---|---|
| Age (yr) | 38.0 ± 15.3 | 36.2 ± 14.6 | ns |
| Mean follow-up (yr) | 11.1 ± 8.7 | 10.0 ± 6.3 | ns |
| Adenoma type | | | |
| Prolactinoma | 85 | 68 | ns |
| GH-secreting | 12 | 15 | ns |
| ACTH-secreting | 6 | 7 | ns |
| Co-secreting | 13 | 2 | ns |
| Non-secreting | 20 | 18 | ns |
| Tumor size | | | |
| Micro (n, %) | 19 (14%) | 64 (58%) | < 0.001 |
| Macro (n, %) | 116 (85%) | 46 (42%) | |
| Clinical signs due to tumor size (n, %) | 39 (29%) | 15 (14%) | < 0.01 |
| Normalization of pituitary hypersecretion (n, %) | 49 (42%) | 83 (90%) | < 0.001 |

Micro, microadenomas; macro, macroadenoma.
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occur in the settings of MEN1 are increasingly diagnosed using immunohistochemistry, and are clinically relevant mainly due to the physical effects of the tumor on local structures. In contrast, ACTH and TSH-secreting adenomas remain rare in MEN1 syndrome. Cushing's disease in MEN1 is mainly caused by ACTH-secreting pituitary microadenomas. TSH-secreting adenomas are usually macroadenomas causing clinical hyperthyroidism. We have described two sporadic cases with classical MEN1 features in a series of 42 TSH secreting adenomas, but no *MEN1* germline mutation could be found in either case [48].

## Carney Complex (CNC)-associated Pituitary Adenomas

### Background

A complex of spotty-skin pigmentation, myxomas, endocrine overactivity and schwannomas was described by Carney in the mid-1980s [49]. Carney complex (CNC), as it has since become known, is rare, having been described in about 400 people in largest database [50]. CNC is inherited in 70% of cases, occurs in all racial groups, and has a slight female preponderance [51]. Two gene loci have been identified-one on chromosome 17q22 – 24 [52] and the other on chromosome 2p16 [53]. The former is associated with the gene encoding the Iα regulatory subunit of protein kinase A type I (*PRKAR1A*), and mutations have been identified in up to 60% of CNC patients [50,54]. The 2p16 locus has not yet been localized to an individual gene, but has been narrowed down to a region of 100,000 base pairs [55]. A variety of *PRKAR1A* mutations have been described to date and almost all lead to mRNA instability and decreased/absent production of the mutated protein, leading to PRKAR1A haploinsufficiency in CNC cells [54,56]. In addition, LOH at the 17q22 – 24 locus and allelic loss have been shown in CNC tumors [55,56]. The loss of PRKAR1A function enhances intracellular response to cAMP in CNC tumors [55]. However, why mutations that affect *PRKAR1A* only produce pathologic changes in certain tissues but not in others is not fully understood. One explanation could be that since PRKAR1A only represents one of the major regulatory subunits of the PKA enzyme, the relative importance of its regulatory activity could vary depending on cell type [55,57].

Mouse models have been recently developed in order to overcome the embryonic lethality of homozygous *Prkar1a* knockout [54,58,59]. No typical CNC features are encountered in heterozygous *Prkar1a* +/- mice; in particular, altered pigmentation and cardiac myxomas are lacking, although sarcomas with myxomatous differentiation commonly develop [54]. Notably, no endocrine abnormalities have described yet in this model. In contrast, a transgenic mouse bearing an antisense construct of the *PRKAR1A* exon 2 develops multiple endocrine abnormalities paralleling features of CNC, reinforcing the concept that *PRKAR1A* may function as a tumor suppressor gene [58,59]. As pituitary tumors have not been reported in this model, this suggests that additional events may be necessary to promote pituitary tumorigenesis in CNC.

No somatic mutations on the *PRKAR1A* gene have been found in sporadic pituitary tumors, and LOH at the 17q22 – 24 locus occurs infrequently, suggesting that *PRKAR1A* is not commonly involved in sporadic pituitary tumorigenesis [60]. However, there is recent evidence that low levels of PRKAR1A protein in tumor cells may contribute to an imbalance in the ratio between the regulatory subunits of PKA R1/R2 and favor cAMP dependent proliferation in somatotrophs [61].

### Clinical Characteristics of CNC-related Pituitary Tumors

The main endocrine abnormalities seen in CNC are primary pigmented nodular adrenocortical disease (PPNAD), thyroid tumors and nodules, testicular tumors (large cell calcifying Sertoli cell tumor, Leydig cell tumors), and acromegaly due to a pituitary adenoma [62]. Acromegaly itself is relatively uncommon in CNC; however, three-quarters of the patients exhibit asymptomatic elevations in GH, IGF-1 and prolactin levels, or abnormal responses to dynamic pituitary testing (oral glucose tolerance test or thyrotropin-releasing hormone test) [62]. The restriction of pituitary abnormalities to those involving GH and prolactin points to the involvement of the somatomammotropic cells that co-secrete both hormones.

In a histological analysis of eight CNC patients undergoing surgery for acromegaly, the NIH group reported that all tumors were prolactin and GH-positive, while a minority also stained for thyroid-stimulating hormone, luteinizing hormone, or alpha-subunit [63]. A crucial distinguishing feature of CNC-related acromegaly was the discovery of multifocal hyperplasia of somatomammotropic cells that included non-adenomatous pituitary tissue within the pituitaries of CNC patients. Hyperplasia zonation was not well delineated, but characterized microscopically by increased cellularity and alterations in reticulin staining that merged with normal pituitary tissue. No consistent genetic abnormalities were seen on comparative genome hybridization. The same group also described the electron-microscopic structures of two GH-secreting adenomas in CNC patients, but the intracellular abnormalities were heterogeneous [64]. Thus, in patients with CNC, acromegaly seems to develop insidiously, beginning in ostensibly normal somatomammotropic tissue that undergoes multifocal hyperplasia to form GH/prolactin-secreting adenomas.

### Isolated Familial Somatotropinomas (IFS)

#### Background

From a genetic and molecular viewpoint, comparatively little is known about the IFS syndrome, particularly when compared with MEN1 or CNC. IFS is defined as the occurrence of two or more cases of acromegaly or gigantism in a family in the absence of MEN1 or CNC [65]. The most recent overview has calculated that just over 100 cases of isolated familial acrogigantism have been reported among 44 families [66]. Clinically, patients with IFS appear to have more aggressive disease than their sporadic counterparts, with a median age of diagnosis of 25 years; the disease was apparent by the age of thirty in nearly two-thirds of patients. Unlike the equal sex ratio in sporadic acromegaly, the male-to-female ratio was 1.5 to 1. Of the 83 patients analyzed by Frohman & Eguchi, all but two harbored macroadenomas.

Genetic linkage to chromosome 11q13 has been demonstrated in IFS, although MEN1 germline mutations have been shown to be absent in various studies [67–70]. Frohman & Eguchi recently reviewed work from their laboratory on the genetic study of IFS, and noted that loss of heterozygosity over an extensive area of chromosome 11q13 was a very frequent finding [66]. Significant linkage in some cases has been narrowed to a region of 3.9 Mb between the markers D11D4908 and INT-2 [65]. In a recent study on a two-generation family (mother, affected daughter, unaffected fraternal twin sister) with IFS, Luccio-Camelo et al. defined a linkage to between microsatellite markers, D11S956 and D11S527, on chromosome 11q13.1-q13.3 [65]. Studying all family members revealed that the haplotype co-segregated with the disease in the two affected members, while the unaffected twin had a recombination event within the linkage region. A somatotropinomas developing in this unaffected individual could narrow the genetic focus significantly.

### Familial Isolated Pituitary Adenomas (FIPA)

As described above, extensive genetic, molecular and clinical studies have delineated three syndromes in which pituitary tumors can occur in a hereditary or familial setting. Given the complexity of the genetics of endocrine cell regulation, it would appear likely that other as yet undefined syndromes of inherited pituitary adenomas remain to be described. For example, there are several interesting reports on families with isolated pituitary adenomas not genetically or clinically attributable to MEN1, CNC or IFS. Familial pituitary adenomas occurring independently of known genetic syndromes include familial prolactinomas, non-functioning adenomas, Cushing's disease and thyrotropinomas in a familial setting [43, 71–73]. A hereditary prolactinoma model has also been reported in the rat [74]. As a first step in assessing whether a rare syndrome of familial isolated pituitary adenomas (FIPA) exists, a collaborative study was designed and implemented to collect clinical, biochemical, histological and genetic data from multiple academic centers [75]. This retrospective, multicenter study has recently been completed. Data from non-consanguineous families with two or more affected siblings presenting with pituitary tumors and no evidence for MEN1 or CNC were collected. Pituitary tumors were classified as hormonal, biochemical or neuroradiological, and data were compared with control groups of patients with pituitary adenomas that did not occur in a familial setting. A total of 138 familial pituitary adenomas were collected in 64 families, and these kindreds exhibited various patterns of homogeneous and heterogeneous pituitary tumor expression. The proportion of secreting to non-secreting pituitary tumors was approximately 80% to 20%; of the secreting pituitary adenomas, about 50% were prolactinomas. When fully completed, this work will cast more light on the possible existence of FIPA as a distinct clinical entity.

### Conclusions

Our understanding of the complex mechanisms governing inherited pituitary tumor syndromes is growing at a rapid pace. This work provides a good example of how collaborative investigation of intriguing conditions can reveal important information regarding the functioning of cell in health and disease (Table 3). The coming years should see genetic linkage data being transformed into concrete molecular pathological disease models. Although a great deal is currently known about pituitary disease in MEN1, the future promises to reveal much more about the function of menin in pituitary tumors, which will be widely applicable within endocrinology and oncology. Similarly, the study of

Table 3 Familial pituitary tumor syndromes

| *Syndrome* | *Gene (genetic linkage)* | *Molecular Pathology* | *Pituitary Tumor* |
|---|---|---|---|
| MEN1 | MEN1 (Ch11q13) | Decreased menin expression/function | All pituitary tumor types (prolactinomas, non-secreting adenomas and GH-secreting adenomas most frequent) |
| CNC | PPKR1A (Ch17q22 – 24) ? (Ch2p16) | Decreased protein kinase A regulatory subunit Ia expression/function | GH and GH/prolactin secreting adenomas |
| IFS | ? (Ch11q13; between markers D11D4908 and INT-2) | ? | GH secreting adenomas |
| ?FIPA | ? (not linked to known MEN1, CNC, related loci) | ? | All pituitary adenoma subtypes |

MEN1, multiple endocrine neoplasia type 1; CNC, Carney complex; IFS, Isolated familial somatotropinoma; FIPA, Familial isolated pituitary adenoma.

pituitary tumors in CNC, and selectivity of the disease for somatomammotropes in particular, may help our understanding of the roles of protein kinases in normal cell function and tumorigenesis. Specific genetic culprits still remain to be found in some CNC patients and in IFS, although the search is focusing rapidly on a region close to the *MEN1* locus in the latter. Finally, collaborative investigations are currently being conducted to determine the clinical and biological characteristics of familial isolated pituitary adenomas (FIPA); this the first step on the road to a fuller genetic and molecular understanding of this potential new syndrome.

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# Aspects **génétiques** et diagnostic **étiologique**

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La prévalence réelle des adénomes hypophysaires a été longtemps sujet de controverse vu la discordance entre les données des séries autopsiques et radiologiques et les données cliniques. Les quelques études épidémiologiques réalisées dans le passé [1, 2] présentaient les adénomes hypophysaires comme une pathologie rare avec une prévalence de 190-280 cas/million d'habitants (1 : 3571 à 1 : 5263), soit 0,02-0,03 %. Cependant, plusieurs études portant sur des autopsies ou des examens IRM dans des populations non sélectionnées ont suggéré que les adénomes hypophysaires sont plus fréquents qu'on ne le pensait, affectant une personne sur six [3]. Ces discordances ont stimulé la réalisation d'une étude épidémiologique précise rapportant dans une région déterminée et une population exactement estimée le pourcentage de patients présentant des adénomes hypophysaires à la population normale [4]. Cette étude, la première du genre dans la pathologie hypophysaire, a permis de montrer un pourcentage bien plus élevé que dans les études antérieures. Avec une prévalence d'un cas pour 1064 habitants, soit plus de 4 à 5 fois ce qui était décrit précédemment, les adénomes hypophysaires sont actuellement reconnus comme une pathologie assez commune, susceptible de remettre en question les moyens nécessaires à son diagnostic, son traitement et son suivi.

Avant la réalisation de l'étude épidémiologique liégeoise [4], la prévalence de l'acromégalie était estimée à 36-69 cas pour un million des habitants [5]. Notre étude récente retrouve 13,2 % d'adénomes à GH parmi 68 adénomes dans une cohorte de 71 972 habitants, soit approximativement 100 cas pour un million d'habitants, le double de la prévalence « classique ».

Les mécanismes physiopathologiques impliqués dans l'apparition des tumeurs hypophysaires ont un support génétique qui commence à peine à être élucidé. Notre compréhension de la présentation clinique des adénomes hypophysaires a changé au rythme des découvertes génétiques. A présent, nous pouvons identifier des adénomes sporadiques, familiaux ou appartenant aux syndromes tumoraux, et les associer à des anomalies génétiques distinctes.

Dans cet article nous proposons de revoir les aspects génétiques et l'étiologie de l'acromégalie (Tableaux 1 et 2). Le plus souvent, la cause de l'acromégalie est un adénome hypophysaire à GH d'apparition sporadique. Un à trois pour cent des adénomes à GH appartiennent à la pathologie familiale héréditaire ou syndromique : la néoplasie endocrinienne multiple NEM1, la NEM X, le complexe de Carney, le syndrome de McCune-Albright et le FIPA (*familial isolated pituitary adenomas*). Rarement, une hypersécrétion tumorale hypothalamique ou périphérique de GHRH peut déterminer une hyperplasie voire une transformation adénomateuse des cellules somatotropes [6]. Les carcinomes à GH sont très rares et seuls quelques cas ont été décrits.

## L'acromégalie liée aux mutations du gène GNAS1

Les mutations activatrices du gène *GNAS1* (connu également comme l'oncogène *gsp*) situé sur le chromosome 20q13 ont été décrites dans des tumeurs endocriniennes diverses (y compris certains adénomes hypophysaires sporadiques) et dans le syndrome de McCune-Albright. *GNAS1* code la sous-unité Gsα d'une protéine G et les mutations activatrices faux-sens qui substituent les résidus Arg201 ou Gly 227 dans la protéine diminuent l'activité GTPasique de la protéine G et déterminent une surproduction d'AMP cyclique. Depuis 1989 [7], les mutations acti-

**Tableau 1. L'étiologie de l'acromégalie.**

| Acromégalie sporadique | Acromégalie familiale et syndromique |
|---|---|
| Source hypophysaire de GH<br>- Adénome hypophysaire à GH ou mixte GH-PRL/sous unité α/TSH<br>- Carcinome à GH (très rare)<br><br>Source non hypophysaire de GH<br>- adénomes ectopiques<br>- tumeurs des îlots pancréatiques, lymphome<br>- iatrogénique<br><br>Sécrétion excessive de GHRH<br>- centrale (tumeurs hypothalamiques)<br>- périphérique (carcinoïde bronchique, tumeurs des îlots pancréatiques, cancer pulmonaire à petites cellules, adénome surrénalien, cancer médullaire thyroïdien, phéochromocytome) | Le syndrome de McCune-Albright<br>La NEM1<br>La NEM X<br>Le complexe de Carney<br>Le FIPA |

**Tableau 2. Anomalies génétiques décrites dans l'acromégalie (adapté selon Beckers A [24] ).**

| | |
|---|---|
| **Acromégalie sporadique** | **Anomalies génétiques**<br>– mutations somatiques activatrices du gène GNAS1/Gsα (40 % des adénomes à GH)<br>– mutations du gène AIP/AIP (rare)<br>– surexpression de la PTTG<br>– surexpression de la cycline D1<br>– mutations de la GADD45G (promoteur de la methylation) |
| **Acromégalie familiale et syndromique** | |
| Le syndrome de McCune-Albright | – mosaïque du gène GNAS1/Gsα |
| Le NEM1 | – mutations germinales inactivatrices du gène MEN1/menine |
| Le NEM X | – mutations germinales du gène CDKN1B/p27Kip |
| Le complexe de Carney | – mutations inactivatrices du gène PRKAR1A/sous unité Iα de la protéine kinase A dans 60 % des cas |
| Le FIPA | – mutations germinales du gène AIP/AIP dans 15 % des cas |

vatrices de *GNAS1* ont été associées à l'apparition d'environ 40 % des somatotropinomes sporadiques [8], indépendamment de l'ethnie. Il est possible que seul l'allèle maternel de *GNAS1* soit exprimé au niveau de l'hypophyse normale. Dans les adénomes sporadiques à GH, les mutations de *GNAS1* atteignent quasiment toujours l'allèle maternel [9]. L'allèle paternel peut être exprimé dans les adénomes à GH positifs ou négatifs pour une mutation de *GNAS1*, mais la relation possible avec une progression tumorale n'est pas claire.

*Le syndrome de McCune-Albright* est déterminé par une mosaïque de *GNAS1*. Le syndrome rassemble plusieurs anomalies cutanées, osseuses et endocriniennes, dont l'acromégalie dans 20 % des cas. Chez un tiers des patients acromégales, un adénome hypophysaire a pu être identifié. L'empreinte maternelle semble intervenir seulement dans la tumorigenèse hypophysaire des patients avec le syndrome de McCune-Albright; dans les autres tumeurs présentes dans cette maladie, les deux allèles sont exprimés, ensemble ou séparément [10]. La transmission héréditaire du syndrome de McCune-Albright est théoriquement possible, mais la mutation germinale est probablement létale puisque aucun cas n'a été rapporté jusqu'à aujourd'hui.

## Le syndrome de néoplasie endocrinienne multiple de type 1 (NEM 1)

Le syndrome de néoplasie endocrinienne multiple de type 1 est une maladie autosomique dominante déterminée par des mutations germinales inactivatrices du gène *MEN1*. Des cas sporadiques sont décrits, mais le syndrome est le plus souvent héréditaire. *MEN1* est un gène suppresseur de tumeur qui code la ménine et qui est situé au niveau du chromosome 11q13. La ménine est une protéine régulatrice de la transcription génétique et elle interagit avec plusieurs facteurs de transcription dont JunD, Smad3 et NF-kB, avec des promoteurs de certains gènes (insuline, prolactine) et avec des protéines régulatrices du cycle cellulaire [11]. Plus de 500 mutations de la ménine ont été décrites jusqu'à présent, sans qu'une corrélation génotype-phénotype puisse être établie [12]. La pénétrance de la maladie parmi les porteurs de la mutation germinale est estimée à 82-99 % à l'age de 50 ans, d'où l'intérêt d'un dépistage précoce chez les membres asymptomatiques des familles NEM1 (par exemple le dépistage d'un dysfonctionnement hypophysaire est recommandé à partir de l'âge de 5 ans) [13]. Les patients appartenant au syndrome NEM1 présentent des tumeurs endocrines (adénomes parathyroïdiens, gastrinomes, insulinomes et d'autres tumeurs entéro-pancréatiques, tumeurs carcinoïdes, adénomes hypophysaires, adénomes nonfonctionels du cortex surrénalien et rarement phéochromocytomes), et non-endocrines (lipomes, collagenomes, angiofibromes faciaux). Le développement tumoral est lié a la perte du 2ème allèle de *MEN1* ; la mutation de l'autre allèle est héritée dans les cas familiaux. Les mutations somatiques du gène de la ménine sont très rares chez les patients non-NEM1 qui présentent des adénomes hypophy-

saires [14]. Les adénomes hypophysaires sont présents chez environ 40 % des patients NEM1 [15] et 10 % des patients atteints de NEM1 présentent une acromégalie (Figure 1, b). L'âge au diagnostic des patients acromégales NEM1 est similaire à l'âge des patients présentant des adénomes sporadiques à GH. Dans la population NEM1, les femmes acromégales sont trois fois plus nombreuses que les hommes [5]. Quand la tumeur hypophysaire est la première manifestation du NEM1, le diagnostic précède de 8 ans celui des patients NEM1 qui se présentent en première instance avec d'autres types de tumeurs.

## Autres types de NEM (NEM X)

Chez 20 % des patients qui présentent un tableau clinique de néoplasie endocrinienne multiple, aucune mutation de *MEN1* n'a pas pu être démontrée, suggérant l'intervention d'autres facteurs génétiques. Des travaux récents sur des modèles murins [16] et des rapports sur deux cas humains, dont un familial, ont décrit des mutations du gène *CDKN1B* qui code l'inhibiteur de kinase dépendant de cycline $p27^{Kip1}$, associées à un phénotype qui rassemble, entre autres, une acromégalie et une hyperparathyroïdie [17]. Cependant, les mutations de *CDKN1B* ne semblent responsables que d'une minorité des cas négatifs pour une mutation de *MEN1* [18].

## Le complexe de Carney (CNC)

Le syndrome de Carney ou le complexe de Carney est une pathologie rare, le plus souvent familiale, caractérisée par la présence de myxomes, lésions cutanées lentigineuses, schwannomes, hyperplasie surrénalienne et anomalies hypophysaires. 60 % des cas présentent des mutations inactivatrices du gène *PRKAR1A* qui code la sous unité régulatrice Iα de la protéine kinase A. Un peu plus de la moitié des patients sont des femmes [19]. Le bilan biologique trouve souvent une hyperprolactinémie (75 %), rarement symptomatique,

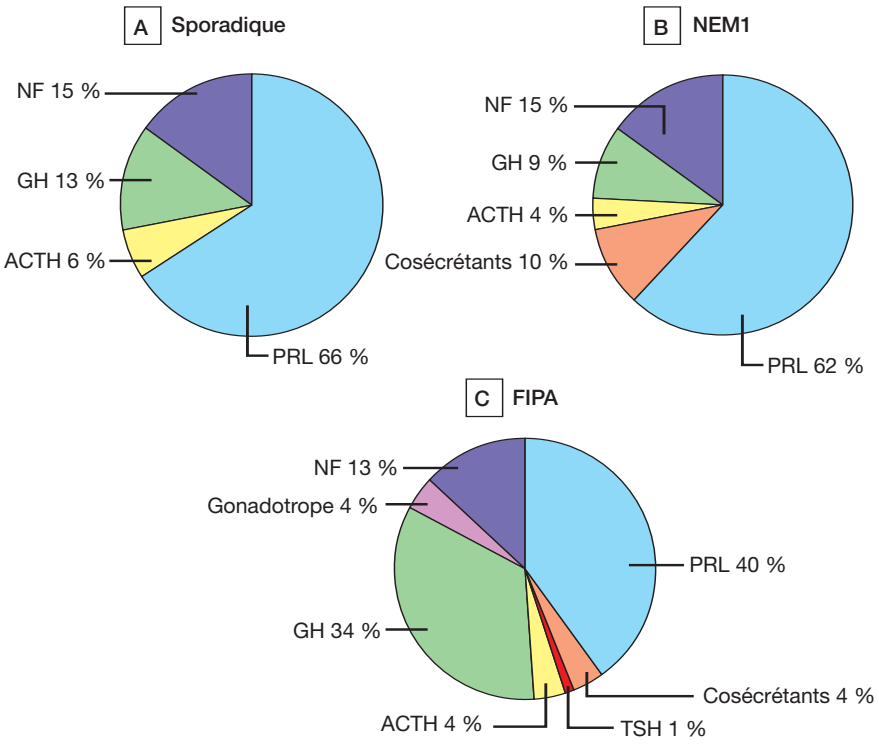


**Figure 1.** **Le prévalence des adénomes à GH parmi les adénomes hypophysaires sporadiques (A) [4], dans la NEM1 (B) [15] et dans le FIPA (C) [21].**

et une hypersécrétion de GH, responsable, dans 10 % des cas, d'un tableau clinique d'acromégalie [6]. L'hyperplasie multifocale des cellules somatomamotropes pouvant évoluer vers une transformation adénomateuse est une particularité du CNC.

## Les adénomes hypophysaires familiaux isolés (FIPA)

A la fin des années 90, la pathologie tumorale hypophysaire familiale autre que la NEM1 et le complexe de Carney comptait quelques rapports de prolactinomes, adénomes corticotropes et non sécrétants familiaux, par ailleurs non étudiés génétiquement. Seule l'acromégalie familiale isolée avait obtenu une identité clinique et des efforts pour élucider sa pathogénie étaient en cours. En 1999, 23 familles d'acromégales avaient été décrites mais leur étude génétique demeurait très incomplète [20]. A cette époque, nous nous sommes intéressés aux familles qui présentaient au moins 2 tumeurs hypophysaires de même type (groupe homogène) ou de lignée différente (groupe hétérogène). Nous avons appelé cette nouvelle entité clinique FIPA (*familial isolated pituitary adenoma*). Au départ, confinée à notre centre, l'étude de caractérisation de la population FIPA a pris, à partir de 2002, une dimension multicentrique, la participation de 22 centres européens permettant d'identifier jusqu'à 2004, 138 patients dans 64 familles FIPA [21]. Les critères d'inclusion dans la cohorte FIPA ont été des critères cliniques, biologiques et génétiques permettant d'exclure les syndromes NEM 1 et CNC. L'étude, rétrospective, a utilisé un groupe témoin de 288 adénomes sporadiques non syndromiques à titre de comparaison.

Dans la cohorte FIPA, 75 % des adénomes étaient des prolactinomes et des somatotropinomes (39,9 %, respectivement 34,1 %). Les femmes étaient plus souvent atteintes, surtout par des prolactinomes. 74,6 % des patients étaient des parents de premier degré.

Dans le groupe hétérogène FIPA, au moins un prolactinome ou un somatotropinome était présent par famille.

Les patients FIPA sont diagnostiqués plus tôt que ceux porteurs d'un adénome sporadique. Avec la succession des générations dans la même famille, l'âge au diagnostic diminue, peut-être par effet d'anticipation.

Les somatotropinomes étaient également distribués entre les deux groupes mais le groupe homogène, superposable à l'IFS (*isolated familial somaotropinomas*), avait bénéficié d'un diagnostic plus précoce, dû à une présentation plus agressive. En général, les adénomes sont diagnostiqués précocement dans le cadre d'un syndrome familial, qu'il s'agisse de la NEM1 ou du FIPA. Quant au type tumoral, FIPA compte presque 4 fois plus d'adénomes à GH que la population NEM1 (Figure 1, c).

L'étude de Vierimaa et al. [22] concernant le rôle dans la genèse des tumeurs hypophysaires familiales des mutations inactivatrices du gène *AIP* situé sur le chromosome 11q13.3 a permis d'identifier, en 2006, trois mutations, dont deux dans un contexte familial. Les familles étudiées présentaient des adénomes à GH, à PRL et des adénomes mixtes PRL-GH. La cohorte FIPA élargie (156 patients dans 73 familles) a permis d'identifier 9 mutations nouvelles dans 11 familles FIPA mutées, représentant 15,1 % de la cohorte [23]. Les patients porteurs d'une mutation d'*AIP* sont plus jeunes au diagnostic (25 *vs* 38 ans) et ont des tumeurs plus grosses et plus agressives (24 *vs* 14 mm de diamètre) en comparaison des patients FIPA non-mutés. Des mutations *AIP* sont décrites tant dans le groupe homogène que dans le groupe hétérogène ; la majorité des mutations ont été identifiées dans les adénomes à GH ou les adénomes mixtes GH-PRL. La corrélation avec les études immunohistochimiques montre que la même mutation peut donner des phénotypes tumoraux différents.

Le gène *AIP* code une protéine qui fait partie de la famille des immunophilines, avec lesquelles elle a en commun la région de liaison de la protéine FK506. La protéine AIP forme un complexe cytosolique avec un dimère de la protéine de choc thermique hsp90 et le récepteur AhR. Des mutations au niveau des domaines tétratricopeptides de l'AIP ou au niveau des derniers aminoacides de la catène carboxy-terminale empêchent la formation de ce complexe. Le rôle du complexe AhR-AIP a été étudié surtout dans la voie métabolique de la dioxine, mais des interactions avec certains facteurs de transcription, comme NF-kB, la protéine du retinoblastome ou le récepteur aux œstrogènes, ou avec la phosphodiesterase PDE4A5 de l'AMP cyclique suggèrent que l'AIP intervient dans des nombreuses voies de signalisation intracellulaires [24].

L'acronyme *l'IFS (les somatotropinomes familiaux isolés)* dénommait jusqu'à présent une entité clinique qui comprend plus de 2 cas d'acromégalie ou de gigantisme, négatifs pour les mutations NEM1 et CNC dans la même famille [20]. La présentation clinique est plus agressive que dans l'acromégalie sporadique, avec un plus jeune âge au diagnostic et des tumeurs quasiment toujours de type macroadénome. Depuis sa caractérisation clinique, un locus de susceptibilité situé au niveau du chromosome 11q13 a été incriminé dans la pathogénie de l'IFS [25], plus tard identifié comme étant le gène AIP. Une mutation AIP n'a néanmoins pu être mise en évidence que dans 50 % des cas IFS. Etant donné que dans les familles IFS on peut trouver des adénomes différents des adénomes à GH (souvent des prolactinomes), nous proposons d'inclure l'IFS dans l'appellation FIPA, sans éluder la possibilité d'un support génétique plus vaste.

## L'acromégalie et la GHRH

Des études sur des modèles animaux montrent que l'hyperstimulation chronique avec de la GHRH peut induire ou entretenir une hyperplasie, voir une transformation adénomateuse des cellules somatropes [26]. Dans la pathologie humaine, la sécrétion tumorale hypothalamique ou périphérique de GHRH peut déterminer une hyperstimulation des somatotropes suivie parfois d'une hyperplasie cellulaire, responsables d'un tableau clinique et biologique d'acromégalie. Une fois la tumeur à GHRH enlevée, l'hypersécrétion de GH est souvent normalisée.

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# *The Epidemiology of Prolactinomas*

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***Abstract.*** **Prolactin-secreting tumors (prolactinomas), the most frequently occurring pituitary tumor, have a frequency that varies with age and sex. They occur most frequently in females aged 20 to 50 years old, at which time the female-to-male ratio is approximately 10:1. In the pediatric-adolescent age group, prolactinomas have a prevalence of 100/million population, and account for less than 2% of all intracranial tumors. Prolactinomas occur in approximately 30% of patients with multiple endocrine neoplasia type 1 and in this setting, they may be more aggressive than their sporadic counterparts. Patients with Carney complex or McCune-Albright syndrome may exhibit hyperprolactinemia due to a pituitary tumor derived from somatomammotropic cells that secrete both growth hormone and prolactin. Few familial cases of prolactinoma unrelated to MEN-1 are reported in literature.**

***Key Words.*** **prolactin, prolactinoma, pituitary, tumor, inherited, neoplasm, epidemiology**

## *Introduction*

Pituitary tumors appear to occur commonly in the general population based on data derived from autopsy series and radiological imaging studies [1–3]. In autopsy series, the generally accepted mean prevalence approaches 10%, although both higher and lower rates have been reported [1,2]. Hall *et al.* noted a similarly high incidence of visible pituitary tumors in a magnetic resonance image (MRI) study of a cohort of healthy individuals without previously known pituitary disease [3]. The corresponding rate of clinically-active pituitary disease is unknown, and the impact on diagnosis rates of the widespread availability of accurate laboratory tests and MRI, is currently under investigation.

Hyperprolactinemia is one of the most frequently diagnosed clinical disorders in routine endocrine practice [4,5]. The most frequent symptoms are hypogonadism and/or galactorrhea in both sexes. Microprolactinomas (<10 mm) or macroprolactinomas (>10 mm) are the most common causes of hyperprolactinemia, although the pathogenesis of the disorder is diverse (Table 1). Any process that interferes with dopamine synthesis, its transport to the pituitary gland or its action at lactotroph dopamine receptors may produce hyperprolactinemia. Hyperprolactinemia is noted in 15–20% of women with secondary amenorrhea or oligomenorrhea, in approximately 30% of those with galactorrhea or infertility, and in 75% of those with both amenorrhea and galactorrhea [4,5]. In men, hyperprolactinemia is often present for many years without symptoms; the most important symptom in males is decreased libido and/or sexual potency. Consequently, the mean age at diagnosis is 10 years greater in men than in women [4,5].

## *Sporadic Prolactinoma*

### *General*

Prolactinomas are the most common pituitary adenoma and account for up to 45% of pituitary tumors in the clinical setting [6,7]. They occur with an incidence of 6–10 cases per million population per year, which translates into a prevalence of approximately 60–100 cases per million [8]. Recent research indicates, however, that the prevalence of all pituitary tumors, including prolactinomas, may be 3–5 times higher than once thought [9]. In young adults, prolactinomas occur much more frequently in women than in men, while this sex-imbalance is not apparent in the middle aged population [7]. Women present earlier than men and hence frequently exhibit microprolactinomas at diagnosis; this earlier presentation may be a function of the greater symptom burden caused by hyperprolactinemia in women. Men on the other hand may present later due to the nature of their symptomatology, in which decreased libido predominates. Thus, males with prolactinomas have a higher frequency of macroadenomas and attendant mass effects on the pituitary and visual system than women [4–6]. However, it remains uncertain if this difference between the sexes is entirely due to delayed diagnosis or whether gender-specific differences in tumor behavior exist. In support of the latter, some data appear to show that at least some men have rapidly growing prolactinomas with elevated markers of cellular proliferation [10,11].

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***Table 1.*** *Causes of hyperprolactinemia*

a. Hypothalamic Disorders
  *Tumors*: craniopharyngioma, germinoma, third ventricle tumor, cyst, glioma, hamartoma, metastasis
  *Infiltrative diseases*: sarcoidosis, tuberculosis, Langerhans cell Histiocytosis
  *Pseudotumor cerebri*
  *Cranial irradiation*
b. Pituitary Disorders
  *Micro- or Macroprolactinoma*
  *Acromegaly*
  *Cushing's disease*
  *Pituitary stalk section*
  *Empty sella syndrome*
  *Pseudoprolactinomas*: non functioning adenoma, meningioma, intrasellar germinoma, metastasis that may produce functional stalk section
  *Infiltrative diseases*: giant cell granuloma, sarcoidosis
c. Drugs
  *Neuroleptics*: perphenazine, fluphenazine, thorazine, promazine, trifluoperazine, haloperidol, chlorpromazine, dopamine
  *Receptor blockers*: metoclopramide, sulpiride, domperidone, cimetidine
  *Antidepressants*: amoxapine, imipramine, amitriptyline
  *Antihypertensives*: $\alpha$-methyldopa, reserpine
  *Estrogens*
  *Opiates*
  *Phenylalkylamine class N-type channel calcium blockers*: verapamil
d. Primary Hypothyroidism
e. Chronic renal failure
f. Cirrhosis
g. Neurogenic
  *chest wall or spinal cord lesions, breast stimulation*
h. Stress physical or psychological
i. Idiopathic

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### *Elderly*

The diagnosis and treatment of prolactinomas in the elderly has received less attention over the years than disease characteristics in other age groups [12–16]. As suggested by Turner *et al.* [16], outcomes research may be scarce due to a lower likelihood that microprolactinomas would be diagnosed in elderly patients. In support of this, Kovacs *et al.* found that autopsy revealed the presence of prolactin-staining microadenomas in 13% of patients aged over 80 [12]; data which have been replicated elsewhere [13]. Only three clinical series of elderly patients aged 65 years or more with pituitary tumors have been reported in the literature, [14–16]. In contrast to the autopsy series, these studies showed a clear prevalence of non-functioning adenomas, while prolactinomas represented only the 4–8% of the total [14–16]. All but one tumor was a macroprolactinoma, which is not entirely surprising given that indicators of hormonal disturbance, such as, menstrual disturbance, reduced sexual function, and infertility are not as informative in elderly subjects; indeed macroprolactinomas are often diagnosed in the elderly when they produce local mass effect symptoms.

### *Children*

In children, pituitary adenomas comprise 2.7% of supratentorial tumors and prolactinoma is the most common of these. The female preponderance seen in adults is maintained in children. A large retrospective surgical series of 136 young patients with pituitary tumors reported that prolactinoma was the most frequent tumor type encountered; these cases presented almost exclusively during teenage years [17]. Clinical presentation with a prolactinoma in childhood varies by age and sex [18]. As noted by Lafferty and Chrousos, prepubertal children usually present with headache, visual disturbances and growth failure [19]. During puberty, females can present with hypogonadism, pubertal arrest and galactorrhea due to hormonal suppression or destruction of normal pituitary tissue by the encroaching adenoma [19]. In pubertal males symptoms relating to mass effects can accompany arrested growth and puberty, perhaps due to a higher frequency of macroadenomas in males [19]

Extremely rarely, young subjects with hyperprolactinemia may present in the setting of McCune-Albright syndrome, which is caused by a post-zygotic activating mutation of the cAMP regulating protein GNAS 1 gene product Gs$\alpha$ [20]. This results in the constitutive activation of adenylate cyclase and subsequent cAMP formation as a second messenger [20]. McCune-Albright syndrome is characterized by a triad of poly- or monostotic fibrous dysplasia, cafè-au-lait macules and endocrine hyperfunction. Hyperprolactinemia in patients with McCune-Albright syndrome is usually associated with hypersecretion of growth hormone and to date only 15 cases have been reported in the literature [21].

### *Malignant prolactinoma*

Pituitary carcinomas are very rare with only about 100 cases reported in the literature; of these 29 malignant prolactinomas have been described [22]. The diagnosis of a pituitary carcinoma is based on the patient's medical history and the demonstration of metastases. Malignant prolactinomas do not present with distinct clinical signs that distinguish them from benign tumors and the initial radiological appearance may mimic that of an adenoma. Histological examination does not allow easy differentiation between adenomas and well differentiated carcinomas [22]. The diagnosis is usually raised because of multiple recurrences and progressive inefficacy of treatment, but in many cases the definitive diagnosis is made only after metastases have been discovered. Malignant prolactinomas usually metastasize to the central nervous system and arachnoidal tissues, while distant metastases are rare. The prognosis is poor with only 50% of patients described in the literature surviving more than one year.

## *Inherited Prolactinomas*

### *Multiple Endocrine Neoplasia-I (MEN-I)*

Multiple endocrine neoplasia type 1 (MEN-1) is an autosomal dominant disorder with endocrine and other tumors with an estimated prevalence of 0.02–0.2 per 1000 [23]. MEN-1 is related to mutations in *MEN1* gene on chromosome 11q13 that encodes the protein menin. Ninety percent of affected cases express parathyroid adenomas, 64% enteropancreatic endocrine tumors and 35–40% anterior pituitary tumors. Overall 22% of patients with MEN-1 develop a prolactinoma. Verges *et al.* showed specific characteristics of prolactinomas in patients with MEN-1 [24]. In fact, among the 136 patients with pituitary adenomas, 85 were prolactinomas (62% of the whole series) [24]. Macroprolactinomas were noted in 71 of 85 patients (84%), including 20 invasive tumors [24]. Macroprolactinomas were more frequent in MEN-1 patients than in sporadic cases (84% vs. 24%, respectively) and normalization of plasma prolactin levels was significantly less frequent in MEN-1 patients than in sporadic, non-MEN-1 subjects (44% vs. 90%, respectively) [24]. These data are supported by those of [25], although other groups have considered the clinical behavior and response to treatment of MEN-1 and non-MEN-1 pituitary adenomas to be similar [26,27]. Finally a MEN-1 variant with unusually high prevalence of prolactinoma was reported in four large and seemingly independent kindreds, originating around the Burin Peninsula of Newfoundland [28]. Affected members of all four Newfoundland families with MEN-1 were recently shown to share not only the same *MEN1* germline mutation but also the same 11q13 haplotype [29,30] suggesting a correlation between genotype and phenotype, although the validity of this correlation remains to be proven.

### *Carney complex*

Carney complex is an autosomal dominant multiple endocrine neoplasia characterized by the complex of "spotty skin pigmentation, myxomas, endocrine overactivity and shwannomas" [31]. Two gene loci have been identified, one on chromosome 17q22-24 and the other on chromosome 2p16. The former is associated with the gene encoding the I$\alpha$ regulatory subunit of protein kinase A type I (*PRKAR1A*) and mutations have been identified in up to 60% of CNC patients. To date approximately 400 cases have been described in the largest case collection. Hyperprolactinemia, usually mild, occurs almost exclusively in association with clinical or subclinical acromegaly in patients with Carney complex. The disorder of prolactin and growth hormone secretion is due to multifocal hyperplasia of somatomammotropic cells within the anterior pituitary. Hence, asymptomatic hyperprolactinemia in addition to elevations in growth hormone and insulin-like growth factor-I are present in up to 75% of patients with Carney complex [31].

### *Familial Isolated Pituitary Adenomas (FIPAs)*

Familial pituitary adenomas have been characterized in the settings of MEN-1 and Carney complex, as noted above, while isolated familial acromegaly has been reported in about 100 patients. Interestingly, Berezin *et al.* and Poncin *et al.* have reported familial prolactinomas unrelated to MEN-1 or Carney complex [32,33]. Recently we have observed other pituitary phenotypes not linked to these previous syndromes, which may represent a new entity: familial isolated pituitary adenomas (FIPAs). To obtain further clinical and genetic insight of FIPAs, a retrospective European multicenter study was undertaken [34]. A hundred and forty cases have been identified in 64 families, including prolactinomas, acromegaly, clinically non-secreting adenomas, Cushing's disease and gonadotrophinomas. There were 54 families with two patients, 8 with three and two with four affected members. Prolactinomas were the most frequent, with 56 affected members observed in 41 families and the female predominance seen in sporadic prolactinoma was maintained.

## *Conclusions*

Prolactinomas are the most commonly diagnosed pituitary tumors. They occurs more ferquently in women than in men particularly between the second and third decades of life. Special attention is required for the diagnosis of prolactinoma in males and in the elderly, as signs and symptoms may not be as suggestive of hyperprolactinemia as in females of child-bearing age. Prolactinomas can occur in a familial setting in MEN-1, while pituitary adenomas and hyperprolactinemia can complicate other inherited conditions such as Carney complex.

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# The Epidemiology and Management of Pituitary Incidentalomas

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**Key Words**: Pituitary incidentalomas, Prevalence, Epidemiology

## Abstract

***Prevalence:*** The prevalence of pituitary tumors has been a topic of controversy for many years. Autopsy and radiological series show that pituitary incidentalomas may be present in one of six people. Recent epidemiological data suggest that clinically apparent pituitary adenomas have a prevalence of approximately one in 1000 people in the general population. The disconnect between these two prevalence rates underlines the common clinical quandary of how to manage pituitary incidentalomas, particularly those lacking clinical signs/symptoms or hormonal abnormalities.

***Management:*** The natural history of incidentalomas suggests that periodic hormonal, clinical and radiological follow-up is the optimal approach. In the absence of tumor growth or relevant symptoms, screening can be continued intermittently or curtailed based on the clinical judgement of the physician. In the presence of hormonal hypersecretion, the management of pituitary incidentalomas, whether they are micro- or macroadenomas, should follow accepted clinical guidelines. For incidental pituitary macroadenomas without hormonal hypersecretion, clinical management should also include assessments for visual field impairment or hypopituitarism. In such cases, regular radiological and hormonal follow-up is required to identify tumor growth or the appearance of new symptoms. In the presence of tumor growth or new hormonal abnormalities, surgical options should be considered and discussed with the patient.

## The Epidemiology of Pituitary Adenomas

According to data from tumor and cancer registries, primary brain and central nervous system tumors have a prevalence of approximately 130-230 cases/100,000 of the population, of which 75% are benign [1, 2, 3]. However, reporting of pituitary adenomas to centralized cancer databases has not been mandatory in some jurisdictions until relatively recently, thereby confounding prevalence assessments from these databases. Historically, few epidemiologic data regarding the prevalence of clinically apparent pituitary adenomas have been available. According to studies from the United Kingdom (UK) and Italy, clinically diagnosed pituitary adenomas occurred with a prevalence of 19 to 28 cases/100,000 population [4, 5].

Since the 1930s, pituitary adenomas have been a frequent incidental finding ("incidentalomas") at autopsy in the general unselected population [6]. The advent of ready access to computerized tomography (CT) and magnetic resonance imaging (MRI) led to the recognition that pituitary tumors are frequently seen in asymptomatic patients. In a fundamental work, Ezzat et al. undertook a systematic review of all autopsy and radiological studies of the prevalence of pituitary adenomas [7]. They found that the

prevalence of pituitary adenomas was 14.4% in autopsy cases and 22.5% in individuals from CT/MRI studies; the mean prevalence was approximately 16.7% or one in six individuals. Of tumors noted at autopsy, immunohistochemistry showed that 25–41% of cells were prolactin positive, suggesting that some cases found at autopsy may represent undiagnosed clinically relevant pituitary tumors. These results underscore the clear disconnect between the relative rarity of clinically diagnosed pituitary adenomas and the relatively high prevalence of incidentalomas found in autopsy/radiology studies. To address this disconnect, an intensive cross-sectional study was conducted in Liège, Belgium, the results of which were reported recently [8]. This study of approximately 72,000 individuals in a tightly delineated geographical sampling zone found that clinically apparent pituitary adenomas occurred with a prevalence of 94 ± 19.3 cases/100,000 population (one clinically apparent case/1064 individuals). This is 3.5- to 5-fold higher than the prevalence reported in the UK and Italian studies, but is lower than the rate reported by Ezzat et al [7]. These findings raise important clinical questions regarding the natural history of incidentalomas and what proportion of incidentalomas progress to pituitary adenomas with attendant clinical signs and symptoms. Clinical decision-making regarding the management of the patient with an incidentally found pituitary adenoma can be challenging in terms of balancing appropriate investigation (and, if necessary, treatment) with the need to avoid unnecessary alarm or unwarranted interventions in the asymptomatic patient.

## Clinical Management of Pituitary Incidentalomas

In a study of a large series of incidentalomas, Sanno et al. reported that 2.4% (6 of 248) of patients had physiological hypertrophy [9]. Chanson et al. described the long-term follow-up of young women (aged 18–35 years) with pituitary enlargement (>9 mm maximal pituitary height) [10]. None of the women had evidence of hormonal abnormalities and none was pregnant. During a follow-up of 2-8 years, yearly review demonstrated no change in pituitary size and no hormonal abnormalities. Two of the subjects had undergone pituitary surgery at another center, and in both cases the histopathology and electron microscopy findings were entirely normal. Overall, Chanson et al. highlighted the importance of comprehensive clinical assessment of pituitary status, rather than limited focus on MRI findings, for optimal management of patients with asymptomatic pituitary hypertrophy. Clearly, it is important to differentiate between pituitary microadenomas and normal variations in pituitary size if unwarranted neurosurgical intervention is to be avoided. Indeed, once asymptomatic pituitary hypertrophy has been definitively diagnosed, intensive hormonal and radiological follow-up is probably not warranted.

Pituitary incidentalomas can occur as micro- or macroadenomas, with or without attendant hormonal abnormalities, local tumor effects or clinical signs and symptoms. Many incidentally discovered pituitary tumors are cystic in nature and may be Rathke's cleft cysts or, more rarely, arachnoid cysts or craniopharyngiomas. Sanno et al. reported that Rathke's cleft cysts accounted for 27.5% (39 of 506) of pituitary incidentalomas treated surgically or nonsurgically [9]. Clinically it is important to differentiate between Rathke's cleft cysts and craniopharyngiomas, as the former rarely

enlarge (only 5.6%), respond well to surgery if required and rarely recur, while craniopharyngiomas are associated with a poorer clinical response to surgery and often recur during long-term follow-up [11].

*Microadenomas*

There are some practical differences in the clinical management of incidentally discovered pituitary micro-adenomas and macroadenomas. With microadenomas, symptoms due to local tumor effects (visual field disturbance, hypopituitarism) are less likely, so management usually hinges on demonstration of hormonal excess and clinical examination for pathological correlates of hormonal hypersecretion. Ideally, all hormonal axes should be tested at the outset using basal and dynamic function tests. The most frequently observed hormonal excess is hyperprolactinemia and, in the absence of other physiological and pathological confounders, hyperprolactinemia will lead to the diagnosis of a prolactinoma and treatment with dopamine agonists in accordance with guidelines [12]. Confirmed hypersecretion of other hormones, such as growth hormone or adrenocorticotrophic hormone, similarly require management of the microadenoma according to accepted clinical guidelines for acromegaly and Cushing disease [13, 14]. Incidental microadenomas without hormonal hypersecretion should be managed conservatively, with planned MRI follow-up at least once a year accompanied by basal hormonal testing. If the tumor remains stable and devoid of hormonal abnormalities over a period of 2 or more years, the patient can be followed up less frequently (e.g., every 2 years). In an elderly patient with a long-term stable incidental pituitary microadenoma, the clinical judgment of the physician will be important in determining the practicality of long-term follow-up. Less than 10% of incidental pituitary microadenomas grow during long-term follow-up [9], but once tumor expansion has been definitively diagnosed, clinical management again will depend on the presence or type of hormonal hypersecretion. In expanding nonsecreting pituitary incidentalomas, surgery should be actively considered before tumor enlargement causes significant hypopituitarism or threatens local structures.

*Macroadenomas*

For subjects with incidentally discovered macroadenomas, visual field impairment, associated hypopituitarism and risk of pituitary apoplexy (in large tumors) are highly relevant issues. Fainstein Day et al. found macroadenomas in 63% (38 of 46) of patients with pituitary incidentalomas, although other groups have reported lower rates [15, 16]. Growth of incidentally discovered macroadenomas occurred in an average of 18.6% of cases, highlighting the importance of regular MRI follow-up [9, 15, 16, 17, 18]. There is a lower threshold for intervention in the clinical management of macroadenomas than is the case for microadenomas. However, the presence or absence of associated hormonal hypersecretion should guide management, with incidentally discovered large prolactinomas requiring treatment with dopamine agonists, for instance. Patients with macroadenomas secreting other intact pituitary hormones should also be considered for surgery or for primary medical therapy if appropriate, as in the case of acromegaly. In patients with nonfunctioning tumors, the presence of visual field impairment, hypopituitarism or the danger of apoplexy require early referral for surgery. As noted by Molitch, a trial of treatment with dopamine agonists may be of some value in 10% of these patients [19]. Given the inherent

propensity for incidentally discovered pituitary macroadenomas to grow, MRI follow-up of patients who elect to postpone surgery should be performed at least every 6 months for the first 12 to 24 months after discovery of the tumor [19].

## Conclusions

Since the autopsy studies conducted by Costello in 1936, reports on the prevalence of incidental versus clinically relevant pituitary tumors have been contradictory. The high number of pituitary tumors found at autopsy and in CT/MRI studies compared with the historical prevalence of clinically evident tumors suggests that clinically relevant pituitary adenomas are more prevalent then previously thought. Recent epidemiological data confirmed this hypothesis and indicate a prevalence of one in 1064 people. For subjects in whom a pituitary lesion is incidentally discovered, the initial evaluation should focus on comprehensive endocrine function testing and visual field assessment. The natural history of incidentalomas suggests that periodic radiological, hormonal and clinical follow-up is the optimal approach when signs and symptoms are initially absent or minimal. Once signs and symptoms become apparent or hormonal abnormalities occur, the management of an incidentally discovered pituitary adenoma should follow the accepted clinical guidelines in a similar fashion to pituitary tumors that present classically.

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