Pituitary Tumors: Genetics and Heritable Predisposition

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Ricky R. Kalra, Philipp Taussky, Toba Niazi, and William Couldwell

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R.R. Kalra • P. Taussky • T. Niazi • W. Couldwell (✉)
Department of Neurosurgery, University of Utah, Clinical Neurosciences Center, 175 North Medical Drive East, Salt Lake City, UT 84132, USA
e-mail: neuropub@hsc.utah.edu

Abstract

Pituitary tumors are the most common intracranial tumors. Most pituitary tumors are thought to be sporadic, with estimates showing that genetic heritability in the form of traditional syndromes, including multiple endocrine neoplasia type 1 (MEN1) and Carney complex (CNC), accounts for only 5% of all cases. The monoclonality of pituitary tumors is a widely established model in which a genetic mutation in one cell leads to the formation of an adenoma. On a larger scale, however, the pituitary gland may contain multiple hyperplastic cells, each with its own origin. The predominant cell type within the adenoma is dependent on a variety of oncogenes and tumor suppressor genes including *GSP*, *RAS*, Cyclin D1, *PTTG,* and *p53.* Multiple other studies show germline mutations in a variety of additional genes, including *AIP, BMP-4, CDKN1B, CDKN2A, CDKN2C, GADD45G, PDt-FGFR4, PKC, PRKAR1A, RB, WIF1,* and *ZAC*. More recently, studies of genetic mutations leading to pituitary adenomas and population studies of patients and families with pituitary adenomas have revealed a significant heritable predisposition for pituitary tumors outside of traditional syndromes. These studies, while confirming the heritability of pituitary tumors, unfortunately only provide a glimpse into the multifactorial cause of pituitary adenomas.

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Introduction: Background and Epidemiology

The most common pathological condition of the pituitary gland is intrinsic tumor growth. The most common tumors, benign adenomas of the pituitary, are a diverse group of tumors that have historically been classified according to size as micro- (less than 1 cm) or macroadenomas (greater than or equal to 1 cm). They are also classified as either functional or nonfunctional, depending on their hormonal activity in vivo.

Pituitary tumors are the most common intracranial tumors, estimated to be present in 16.7% of the population (Ezzat et al. 2004). They comprise up to 10–15% of intracranial tumors found at surgery, 6–25% of intracranial tumors observed at autopsy, and 20% of all primary brain and central nervous systems tumors. They are the second most common type overall by histology in patients between the ages of 20 and 35 years, according to the Central Brain Tumor Registry of the United States (www.cbtrus.org). The majority of these tumors are thought to be sporadic, with estimates showing that genetic heritability in the form of traditional syndromes, including multiple endocrine neoplasia type 1 (MEN1) and Carney complex (CNC), accounts for only 5% of all cases (Tichomirowa et al. 2009). More recently, studies of genetic mutations leading to pituitary adenomas and population studies of patients and families with pituitary adenomas have revealed a significant heritable predisposition for pituitary tumors outside of traditional syndromes. These heritable tumors have been characterized as familial isolated pituitary adenomas (FIPAs) (Beckers and Daly 2007).

Pituitary Tumors

Nonfunctioning Adenomas

Nonfunctioning adenomas account for approximately 30% of pituitary tumors. The term nonfunctioning reflects the fact that these adenomas do not cause clinical hormone hypersecretion. These adenomas are generally heterogeneous and large and come to medical attention because of their compression effects on the chiasm and on the functioning pituitary, which can lead to hypopituitarism. Despite the lack of clinical hormone secretion, immunocytochemical staining reveals evidence of hormone expression in 80% of cases (Rengachary and Ellenbogen 2005). These are endocrinologically inactive peptides, with the dominant product being the α-subunits, which have no known systemic effects.

The enlargement of nonfunctioning adenomas into the suprasellar region results in optic chiasmal compression and visual field deficits. Additionally, the growing tumor causes progressive loss of native pituitary function over months to years. Gonadotropin function is generally lost first, followed by loss of growth hormone (GH) function, thyroid function, and, finally, adrenocorticotropic hormone (ACTH) function. Loss of vasopressin function is almost never a presenting or eventual symptom. Although progressive visual loss and clinical manifestations of hypopituitarism are generally the presenting symptoms, some patients present secondary to hemorrhage or infarction (apoplexy). With large hemorrhages into the gland, patients may present with sudden headache, a decreased level of consciousness, visual loss, and acute hormonal insufficiency.

Functioning Adenomas

Functioning pituitary adenomas are benign monoclonal tumors that arise from the cells comprising the anterior pituitary gland, which is responsible for secretion and regulation of peptide hormones and stimulating factors. Under physiologic conditions, the anterior pituitary gland depends on the hypothalamus, the portal circulation, and the pituitary stalk for normal hormone secretion. The anterior pituitary hormones ACTH, GH, prolactin, thyroid-stimulating hormone (TSH), luteinizing hormone (LH), and follicle-stimulating hormone (FSH) are controlled by the hypothalamic hormones corticotropin-releasing hormone (CRH), growth-hormone-releasing factor (GRF), dopamine, thyrotopin-releasing hormone (TRH), and

gonadotropin-releasing hormone (GnRH), respectively. This regulation is accomplished via the portal vascular system linking the hypothalamus and the pituitary gland.

Prolactin-Secreting Adenomas

Prolactin-secreting adenomas are the most common form of pituitary tumor, representing 40% of all pituitary adenomas. The normal regulation of prolactin is achieved by secretion of dopamine by the hypothalamus, which inhibits the anterior pituitary from producing prolactin. Aberrant growth of prolactin-secreting cells results in hyperprolactinemia and leads to amenorrhea, galactorrhea, and osteoporosis in women and diminished sexual drive and impotence in men.

GH-Secreting Adenomas

Acromegaly results from the hypersecretion of GH by the pituitary. Growth hormone is normally released in response to GRF from the hypothalamus, leading to increased levels and activity of insulin-like growth factor-1 (IGF-1), which, in turn, negatively feeds back to limit the production of GH. In addition, GH secretion is tightly regulated by somatostatin. The effects of uncontrolled hypersecretion of GH are gradual and result in gigantism in a child whose epiphyseal plates have not yet closed or in classic acromegalic features in an adult. There is typically an insidious coarsening of the facial features and an increase in the soft tissues. A significant number of patients present because of the local compressive effects of an expending pituitary mass, rather than somatic disturbances.

Glycoprotein-Secreting (TSH, FSH, LH) Adenomas

Glycoprotein-secreting tumors were traditionally thought to represent less than 1% of all pituitary tumors, but improved immunohistochemical techniques have shown that many "nonfunctioning" adenomas have evidence of glycoprotein production. Whereas TSH-secreting tumors cause hyperthyroidism, FSH- and LH-secreting tumors do not produce any specific clinical syndromes. Consequently, these tumors are usually only discovered when they cause symptoms relating to mass effect, and therefore they require surgical resection. Unfortunately, surgical cure is rarely achieved because of the suprasellar extension and involvement of the cavernous sinuses by the time they present to medical attention.

ACTH-Secreting Adenomas (Cushing Disease)

Hypercortisolemia causes multiple clinical problems; patients feel poorly and have diffuse muscle pain and weakness, emotional lability, and profound fatigue. They present with accelerated atherosclerosis, hypertension, diabetes mellitus, osteoporosis, obesity, susceptibility to infections, peptic ulcer disease, and thrombosis. Cushing disease is a serious medical condition that will shorten the life of afflicted individuals because of associated comorbidities. Most cases of Cushing disease in the adult population are caused by microadenomas of the anterior pituitary gland.

Genetic Syndromes Associated with Pituitary Tumors

Two autosomal dominant syndromes have been associated with a familial inheritance of pituitary adenomas: MEN1 and CNC. The familial syndrome MEN-1 affects the parathyroid gland, the endocrine pancreas, and, with less frequency, the pituitary gland. The syndrome is inherited in an autosomal-dominant fashion with reduced penetrance. Hyperparathyroidism develops in 90% of patients with MEN-1, and islet cell tumors of the pancreas develop in 60–70% of patients. Pituitary tumors occur in 24–45% of the patients, with symptoms consistent with hyperprolactinemia occurring in the majority of these patients (Lemos and Thakker 2008). The syndrome is caused by an inactivating mutation in the *MEN1* gene on chromosome 11q13, which encodes the nuclear protein menin. The clinical presentation of *MEN1* has been extensively characterized, and pituitary adenomas occur in 40% of patients with the syndrome (Lemos and Thakker 2008). All tumor phenotypes can occur, but prolactinomas predominate in this patient population. Over 350

mutations of the *MEN1* gene have been found, but more than 10% of patients with the clinical disease have no known gene mutations (Lemos and Thakker 2008). This suggests that epigenetic factors may also be involved in the development of the tumors (Lemos and Thakker 2008).

Carney complex is an autosomal-dominant familial neoplasia syndrome characterized by spotty skin pigmentation, cardiac myxomas, primary pigmented nodular adrenocortical disease, pituitary tumors, and schwannomas. Among the endocrine tumors, GH-producing pituitary adenomas are seen in approximately 10% of patients (Yin et al. 2008). Carney complex is a rare condition that is linked in more than 50% of cases to an inactivating mutation in the gene encoding protein kinase A at 17q24. The primary abnormality in CNC pituitary disease is multifocal cell hyperplasia. Thus, about 75% of patients with CNC exhibit subclinical increases in growth hormone, IGF-1, and prolactin levels, but only 10% of patients actually exhibit symptoms of acromegaly (Kirschner et al. 1998).

The recently defined syndrome FIPA has been identified with more frequency in the literature. Isolated familial somatotropinoma (IFS) was the first form of this syndrome described. It was defined as the occurrence of at least two cases of acromegaly in a single family in the absence of MEN1 and CNC. IFS was subcategorized within the broader FIPA syndrome category once data appeared to support the theory that pituitary adenomas of all types—not limited to IFS—can occur in a familial setting in the absence of MEN1 and CNC (Beckers and Daly 2007). The identification of FIPA as a "wastebasket" of heritable pituitary tumors indicates the vast amount of genetic mutations associated with pituitary tumors and the genetic predisposition linked to these mutations. Furthermore, heritable genetic mutations in families with incomplete penetrance indicate significant epigenetic factors associated with the development of pituitary adenomas.

Genetic studies have pinpointed one of the hereditary abnormalities associated with FIPA within a region on chromosome 11q13.3 in 15% of patients; however, multiple other chromosomes have been implicated in other forms of FIPA. The location of the 11q13.3 mutation is associated with the *aryl hydrocarbon receptor interacting protein (AIP)* gene (Vierimaa et al. 2006).

Pituitary Tumorigenesis: Genetic Basis

The monoclonality of pituitary tumors is a widely established phenomenon in which a genetic mutation in one cell leads to the formation of an adenoma. On a larger scale, however, the pituitary gland may contain multiple hyperplastic cells, each with its own origin. The predominant cell type within the adenoma is dependent on a variety of oncogenes and tumor suppressor genes. The most important oncogene implicated in sporadic tumors is *GSP*, which encodes a protein that regulates growth-hormone-releasing-hormone (GHRH) effects. The oncogene *RAS* has also been identified in tumorigenesis and is associated with aggressive pituitary tumors, including pituitary carcinomas. Cyclin D1, a cell cycle protein, is disrupted in a portion of pituitary adenomas and is overexpressed in approximately 70% of nonfunctioning tumors and 40% of somatotropinomas (GH-secreting pituitary adenomas) (Hibberts et al. 1999). The *pituitary tumor transforming gene (PTTG)* is usually down-regulated in healthy glands but shows up-regulation in functional adenomas (Zhang et al. 1999). Cell cycle regulator *p53* has also been shown to be mutated in a percentage of tumors (Tanizaki et al. 2007). Multiple other studies show germline mutations in a variety of genes, including *AIP, BMP-4, CDKN1B, CDKN2A, CDKN2C, GADD45G, PDt-FGFR4, PKC, PRKAR1A, RB, WIF1,* and *ZAC* (Tichomirowa et al. 2009). We will discuss some of the well-studied mutations further in this chapter.

Molecular Genetics and Epigenetic Alterations of Pituitary Tumors

Origin of Pituitary Adenomas

The clinical effects of pituitary tumors have been extensively studied, but until the 1990s researchers

lacked a good understanding of the initiating events of tumorigenesis. It was not known whether most pituitary adenomas arose from a single mutation or if they formed from multiple cells simultaneously stimulated by factors released from the hypothalamus. Using DNA restriction fragment length polymorphisms, Jacoby et al. (1990) studied X chromosome inactivation in DNA isolated from pituitary adenomas in women with three different hormonal subtypes. Analysis of the DNA fragments showed in each of the three samples that only one X chromosome was active in all cells within the adenoma, thus elucidating the monoclonal origin of pituitary adenomas. Multiple additional studies confirmed that pituitary adenoma tumorigenesis occurred in a single cell, leading to a clonal population of tumor cells (Jacoby et al. 1990).

Genetic Abnormalities of Carney Complex and Multiple Endocrine Neoplasia

The majority of genetic abnormalities in pituitary tumors result in dysregulation of hormone signaling, dysregulation of growth-factor signaling, dysregulation of signaling proteins, or cell cycle regulation (Spada et al. 2007).

CNC—PRKAR1A Gene

At the genetic level, CNC can be caused by null mutations in the *PRKAR1A* gene, encoding the type 1a regulatory subunit of protein kinase A. To further elucidate the role of this gene on pituitary tumorigenesis, researchers produced a tissue-specific knockout of this gene in a mouse model (Yin et al. 2008). The frequency of pituitary tumors was significantly increased in the knockout mice. At a hormonal level, GH levels in serum of knockout mice were markedly elevated compared with those of controls, regardless of whether a visible GH adenoma developed or not. Mice with heterozygous knockout did not have increased frequency of pituitary tumors. Thus, a null mutation at the *PRKAR1A* gene is necessary for development of pituitary tumors in the mouse CNC model.

Protein kinase A pathway abnormalities are well known to cause human pituitary adenomas. This is well illustrated by the activating mutations in G protein subunit Gs-α (encoded by the *gsp* oncogene). Mutations in the protein kinase A pathway lead to constitutive activation of the G protein, with subsequent stimulation of cAMP and protein kinase activity, leading to changes in GHRH effects and thus GH-secreting adenomas. *PRKAR1A* also causes changes the protein kinase A pathway, producing GH adenomas in patients with CNC.

Familial MEN-1 Syndrome

At the genetic level, the *MEN1* gene, which is responsible for the syndrome, encodes a 610-amino-acid protein, menin, on chromosome 11. The chromosome 11q13 germline mutation in *MEN-1* is revealed by a "second hit" on the remaining normal allele and is visualized as a loss of heterozygosity (LOH) using PCR technology to evaluate the gene. The "two-hit" requirement for phenotypic expression of the syndrome is further validated by the presence of truncated *MEN1* gene regions in up to 85% of families with the MEN-1 syndrome. LOH of the wild-type chromosome results in the development of tumors in patients with heterozygotic inheritance of the allele, including pancreatic (40% by 9 months after LOH), parathyroid (24% by 9 months after LOH), and pituitary (26% by 16 months after LOH) tumors (Williamson et al. 1995). The role of *MEN1* mutations in pituitary tumorigenesis in humans is not readily apparent yet. The predominance of prolactinomas in familial MEN-1 suggests that pituitary tumors might be caused by similar mutations. Since mutation of the wild-type allele in a heterozygous patient at the *MEN1* gene is observed in up to 30% of sporadic pituitary adenomas, the *MEN1* mutation may play a role in the progression but not the initiation of sporadic pituitary tumors. However, DNA sequence analysis shows that *MEN1* mutations occur in less than 2% of sporadic pituitary adenomas (Spada et al. 2007).

Genes Associated with Adenomas and Epigenetic Factors

Pituitary tumorigenesis, whether in the sporadic form or as part of a heritable syndrome, frequently involves mutations of oncogenes, tumor

Table 8.1 Germline and somatic gene abnormalities associated with pituitary adenomas

Gene	Product/Defect
Oncogenes	
Cyclin D1	Overexpression in nonsecreting adenomas and somatotropinomas
Gsp	Somatic activating mutations in up to 40% of somatotropinomas
PTTG	Increased expression in more aggressive pituitary tumors
RAS	Somatic activating mutations in pituitary carcinomas
c-myc	Transcription factor associated with PTTG gene mutations
Tumor suppressor genes	
MEN1	Inactivating mutations in all pituitary adenoma types
p53	Somatic inactivating mutations and overexpression in pituitary carcinomas
Retinoblastoma	Somatic mutations and promoter methylation in pituitary adenomas
Cell cycle regulators	
AIP	Germline mutations and loss of heterozygosity in 15% of FIPA cases
CDKN1B (p27)	Germline heterozygous nonsense mutations
PKC	Point mutation in invasive pituitary adenomas
PRKAR1A	Truncating mutations in Carney complex leading to hyperplasia and adenomas
ZAC	Promoter methylation in nonfunctioning adenomas
Growth factors and cytokines	
Pdt-FGFR4	Alternative transcription initiation in pituitary adenomas
BMP-4	Increased expression in prolactinoma

suppressor genes, cell cycle regulator genes, and epigenetic factors (Table 8.1). Recently published data indicate that pituitary tumors may possess a greater heritability than previously thought (Couldwell and Cannon-Albright 2010). Thus, an understanding of the genetic basis of these tumors is paramount in understanding the possible risk these tumors present to offspring of affected individuals (Tichomirowa et al. 2009).

Oncogenes

An oncogene is a gene that, when mutated or expressed at high levels, helps turn a normal cell into a tumor cell. Many abnormal cells normally undergo a programmed form of death, apoptosis. Activated oncogenes can cause those cells to survive and proliferate instead.

Several oncogenes have been implicated in the development of pituitary adenomas.

Cyclin D1

Cyclin D1, located on chromosome 11q13, regulates the cell cycle, specifically progression through the G_1 phase. Mutations in *cyclin D1* result in unregulated growth of pituitary cells. *Cyclin D1* amplification is more frequent in nonfunctioning adenomas and invasive tumors. The gene mutation is overexpressed in 70% of nonfunctioning tumors and 40% in somatotropinomas (Hibberts et al. 1999).

GSP

The most important oncogene implicated in sporadic tumors is *gsp*, which encodes a protein that regulates GHRH effects. Located on chromosome 20, mutation of the α subunit of the stimulatory guanine nucleotide-binding protein produces an unregulated active adenyl cyclase signaling system, which causes an inhibition of GTP hydrolysis and maintains Gsα in constitutively active state, thus increasing cAMP, leading to GH hypersecretion via upregulation of GHRH effects. Mutations of the *gsp* gene have been noted in approximately 40% of GH adenomas, 10% of nonfunctioning adenomas, and 6% of ACTH adenomas (Clayton 1999).

PTTG and C-myc

The PTT gene, also known as *securin*, is generally poorly expressed in normal pituitary glands but is up-regulated in most pituitary adenomas. *Securin*

appears to induce expression and secretion of basic fibroblast growth factor, a strong mitogenic and angiogenic factor (Zhang et al. 1999). Additionally, *securin* is involved in chromosome separation during mitosis, and mutations in the gene lead to improper cell cycle function, causing chromosomal instability and aneuploidy, and thus giving *PTTG* its oncogenetic potential (Zou et al. 1999). Basic fibroblast growth factor activation has also been shown to activate the *c-myc* oncogene. *C-myc* codes for proteins that bind to DNA and serves as a transcription factor. Overexpression of the *c-myc* oncogene, located on chromosome 8q24, has been reported in nearly one third of all pituitary adenomas (Pei 2001).

RAS

Expression of the *ras* oncogene, located on chromosome 5p13, has been reported in pituitary carcinomas and aggressive prolactinomas. The gene expression is absent in benign adenomas, which indicates that its activation may be a secondary event causing further de-differentiation to a more aggressive tumor type. By itself, *ras* does not likely play a significant role in initiating tumor development.

Tumor Suppressor Genes

A tumor suppressor gene, or anti-oncogene, is a gene that protects a cell from becoming a tumor cell. When this gene is mutated to cause a loss or reduction in its function, the cell can progress to cancer, usually in combination with other genetic changes. Unlike oncogenes, tumor suppressor genes generally follow the 'two-hit hypothesis,' which implies that both alleles that code for a particular gene must be affected before an effect is manifested. This is because if only one allele for the gene is damaged, the second can still produce the correct protein. Thus, mutant tumor suppressor alleles are usually recessive whereas mutant oncogene alleles are typically dominant. Several tumor suppressor genes have been implicated in the development of pituitary adenomas.

MEN1

Among the earliest recognized tumor suppressor genes associated with pituitary adenomas was the *MEN1* gene located on chromosome 11q13. Twenty-five to forty-five percent of patients with the autosomal-dominant inherited germ-line mutation in *MEN1* gene, who thus have *MEN1* syndrome, have pituitary tumors. *MEN1* mutations have been reported in sporadic tumors of all major subtypes, including ACTH and GH adenomas, prolactinomas, and nonfunctioning adenomas. The biological role of *MEN1* appears to be in part as a tumor suppressor gene with an immense series of interactions within the cell. Menin regulates or interacts with promoter regions of hundreds of genes and has a wide regulatory role in transcription (Agarwal et al. 2007).

p53

Cell cycle regulator and tumor suppressor gene *p53* has also been implicated in pituitary tumorigenesis. *p53* encodes a nuclear protein that regulates cyclin-dependent kinase (CDK) inhibitor p21. p21 induction has been shown to restrain cell cycle progression and thus pituitary tumor growth. Mutations in *p53* result in decreased p21 activation and uncontrolled replication (Chesnokova et al. 2008). The mutated *p53* gene, located on chromosome 17p13, has been reported in high percentage of patients with Cushing disease and in invasive nonfunctioning adenomas. Additionally, a recent study demonstrated *p53* mutations resulting in high expression of *p53* protein by pituitary tumor cells, both noninvasive and invasive ACTH adenomas (Tanizaki et al. 2007).

Retinoblastoma Gene (Rb)

The retinoblastoma protein Rb is a tumor suppressor protein that is dysfunctional in many cancers. A primary function of the versatile protein is to prevent excessive cell growth by inhibiting cell cycle progression until a cell is ready to divide. *Rb* also serves as recruiter of several chromatin remodeling enzymes such as methylases and acetylases. Differing results in regard to the role of the retinoblastoma gene (*Rb*) in tumorigenesis of pituitary adenomas have been reported. Somatic *Rb* gene loss via deletion in a locus on the long arm of chromosome 13 has been reported in invasive adenomas (Pei et al. 1995). Hypermethylation of the promoter region of the *Rb* gene has also

been implicated in tumor development. Overall, there are no definitive studies showing the specific mechanism by which *Rb* initiates or enhances pituitary tumor growth (Tichomirowa et al. 2009).

Cell Cycle Regulators

Cell cycle regulators function to initiate, control, and stop cell growth and development. Dysregulation of the cell cycle components may lead to aberrant cell cycle initiation and thus lead to hyperplasia and tumor development. The following cell cycle regulators have been implicated in the development of pituitary adenomas.

Aryl Hydrocarbon Receptor Interacting Protein (AIP)

Aryl hydrocarbon receptor interacting protein was discovered to cause adenomas in a comprehensive genetic study in families with multiple members diagnosed with pituitary tumors. Studies of patients in the FIPA cohort showed that *AIP* was associated with familial presentation of somatotropinomas and prolactinomas in 15% of cases (Vierimaa et al. 2006). Immunohistochemical methods showed that in normal cases, *AIP* was localized within GH- and prolactin-secreting cells; however, in sporadic and familial tumors, it was expressed in all tumor types (Tichomirowa et al. 2009).

The mechanism by which *AIP* mutations lead to adenomas in sporadic as well as FIPA cases is not well understood. To date, all germline *AIP* mutations reported have been heterozygous in nature. Some researchers have speculated that homozygous germline *AIP* mutations are not compatible with life. Thus, *AIP* mutations resulting in abnormal protein production are due to a 'second-hit' phenomenon. *AIP* mutations described to date result in either truncation or misfolding of the protein. The *AIP* gene product interacts with multiple cell cycle proteins including phosphodiesterases, heat shock proteins, cAMP, and aryl hydrocarbon receptors. Thus, abnormal cell cycle regulation is hypothesized to lead to aberrant growth (Daly et al. 2007).

CDKN1B (p27)

In pituitary adenomas, CDK inhibitor *p27*, located on chromosome 12, appears to have an important function, as evidenced by animal studies showing development of pituitary tumors in homozygous *p27* deletions. p27 protein quantitative reduction has also been shown to occur in adenomas but not in normal pituitary glands. The reduction in protein was most predominant in ACTH tumors (Lidhar et al. 1999).

Protein Kinase C

Protein kinase C (PKC) is an enzyme involved in the regulation of cellular growth, proliferation, and differentiation of the anterior pituitary gland. Increased PKC activity and expression have been reported in pituitary adenomas, especially in the invasive forms. In particular, the PKC α-isoform (α PKC) is overexpressed in these tumors. Furthermore, the α-isoform can undergo a point mutation, structurally altering the protein and resulting in a more aggressive tumor form (Couldwell et al. 1996).

PRKAR1A

Carney complex is associated with mutations in the *PRKAR1A* gene. This gene has been identified in 60% of patients with CNC (Veugelers et al. 2004). The *PRKAR1A* gene encodes the protein kinase A regulatory subunit Iα, and mutation of the gene results in mRNA instability leading to absent or decreased gene product. Decreased protein function within the cell increases cAMP effects and leads to tumor growth. *PRKAR1A* homozygous gene deletion is not compatible with life; *PRKAR1A* mutations resulting in deficient protein production are due to a 'second-hit' phenomenon (Kirschner et al. 2000).

ZAC

The *ZAC* gene encodes a zinc finger protein that also induces cell apoptosis and cell cycle arrest. The protein, located on chromosome 6, is normally expressed in high quantities in healthy pituitary glands. In nonfunctional adenomas, however, *ZAC* expression is virtually absent. Interestingly, loss of expression of the ZAC protein was not associated with a mutation of the *ZAC* gene; thus, an alternative epigenetic mechanism of gene inactivation must exist. One such mechanism implicated in the *ZAC* gene defect is promoter methylation leading to decreased protein product (Pagotto et al. 2000).

Table 8.2 Epigenetic factors associated with pituitary adenomas

Gene	Product/Defect
CDKN2A (p16)	Promoter methylation in pituitary adenomas
DAP	Promoter methylation in invasive/metastatic adenomas
GADD45G	Promoter methylation in nonsecreting adenomas, prolactinomas, and somatotropinomas
Gsp	Somatic activating mutations and relaxation of imprinting
MEG3a	Promoter methylation in nonsecreting adenomas and gonadotropinomas
Pdt-FGFR	Alternative transcription initiation in pituitary adenomas
Rb	Promoter methylation in pituitary adenomas
WIF 1	Promoter methylation in pituitary adenomas
ZAC	Promoter methylation in nonfunctioning adenomas

Growth Factors and Cytokines

Although pituitary adenomas arise from a clonal expansion of a single cell, the monoclonal growth may occur within a hyperplastic environment secondary to disturbances in paracrine regulation, including cytokines and growth factors. Abnormal responses to target organ feedback can also lead to paracrine abnormalities and play a role in creating this environment (Vallar et al. 1987).

Examples of such responses include:

1. Overexpression of FR-α. Pathological staining and microarray analysis has revealed that the folate receptor FR-α is significantly overexpressed in clinically nonfunctioning adenomas. It is a high-affinity folate transporter and thus may provide a growth advantage to dysplastic cells (Evans et al. 2008).
2. Potentiation of prolactin-producing cells by estrogen during pregnancy and lactation.
3. Loss of negative feedback resulting in tumor growth in the setting of adrenalectomy (Nelson syndrome) (Ando et al. 2001).
4. Mutations in fibroblast growth factor receptors (FGFRs). FGFRs assist in the growth and development of tissues. Mutations in such factors have been shown to lead to hyperplasia; specifically, a truncated pituitary tumor–associated form of FGFR4 has resulted in invasive pituitary tumorigenesis in animal models via alternative transcription initiation (Ezzat and Asa 2006).
5. Effects on bone morphogenic proteins (BMPs). These members of the tumor growth factor (TGF) β family interact with downstream regulators to control the cell cycle and cell proliferation. Specifically, BMP-4 has a stimulatory role on prolactin-secreting cells and supports the development of prolactinomas, but it has an inhibitory action on the corticotropic cells. Thus, cytokines in general, and the TGFβ family specifically, play a complicated role in tumorigenesis and tumor inhibition (Giacomini et al. 2007).

Epigenetic Factors

Epigenetics is the study of inherited changes in phenotype and gene expression caused by mechanisms other than changes in the underlying DNA sequence. Three main types of epigenetic inheritance have been studied: DNA methylation, genomic imprinting, and histone modification (Table 8.2). In many cancers, epigenetic changes have been implicated in development and growth of tumors, including pituitary adenomas (Tichomirowa et al. 2009).

DNA Methylation

Cell cycle regulators *p16* (CDKN2A) and *Rb* are absent in virtually all tumor types. The mechanisms implicated in their loss include gene deletion, point mutations, and promoter methylation. In pituitary tumors, DNA methylation of *p16* with gene silencing is present in approximately 70% of sporadic tumors and represents an early change in pituitary tumorigenesis. In regard to *Rb* absence, only a small portion is attributable to gene-silencing via methylation compared with deletion (Farrell 2005). The death-associated protein kinase (DAP kinase) gene product is an apoptotic mediator via the activation of *p19*/*p53*.

Several studies have implicated the methylation and deletion of DAP kinase gene with highly invasive and/or metastatic pituitary tumors (Simpson et al. 2002). Methylation of the growth arrest and DNA damage-inducible gene (GADD45G) also leads to gene silencing and is associated with the development of somatotropinomas, prolactinomas, and nonfunctioning adenomas (Zhang et al. 2002).

Imprinting

Genomic imprinting of the maternal and paternal DNA during gametogenesis establishes conditions whereby a specific allele is more abundantly or exclusively expressed in the offspring. Relaxation of imprinting by activation of the nonimprinted gene can lead to tumor development. Recent studies indicate that the oncogene *gsp* normally undergoes imprinting with the maternal allele exclusively being expressed (Weinstein et al. 2002). Tumor development due to gsp abnormalities is generally associated with mutation of the gene, but data suggest that some tumors express biallelic gene products, indicating a loss of imprinting. Relaxation of imprinting of gsp may represent a secondary feature in the progression of these tumors (Hayward et al. 2001).

Histone Modification

Histones are subject to a wide variety of post-translational modifications. These modifications occur primarily within the histone amino-terminal tails protruding from the surface of the nucleosome as well as on the globular core region (Cosgrove et al. 2004). Histone modifications are proposed to affect chromosome function through at least two distinct mechanisms. The first mechanism suggests modifications may alter the electrostatic charge of the histone resulting in a structural change in histones or their binding to DNA. The second mechanism proposes that these modifications are binding sites for protein recognition modules. Thus, post-translational modifications of histones create an epigenetic mechanism for the regulation of a variety of normal and disease-related processes, including pituitary tumor growth (Tateno et al. 2010). Recently published data suggest that MAGE-A3, a member of the MAGE-I family of cancer-related antigens, is abundantly transcribed in pituitary tumors and has been implicated in transcriptional silencing of *p53* through histone modification (Monte et al. 2006). As mentioned earlier, *p53* serves as a cell cycle regulator and tumor suppressor gene. *p53* encodes a nuclear protein that regulates CDK inhibitor p21. p21 induction has been shown to restrain cell cycle progression and restrain pituitary tumor growth. The silencing of the *p53* gene results in decreased *p21* activation and uncontrolled replication (Chesnokova et al. 2008).

Overview of Current Understanding of Heritability

Utah Population Data Base

Numerous researchers have undertaken the effort to understand genetic alterations that contribute to the development of different disease states associated with pituitary tumors. The ultimate goal of these studies is to develop processes that will enable us to screen and treat patients afflicted with these specific diseases and someday prevent their development altogether. In addition to the laboratory studies evaluating the genetic contribution to pituitary tumors, another resource has now been applied to the study of these tumors. The Utah Population Database (UPDB) was developed with the goal of investigating the genetic contribution to cancer. During the last 30 years, use of the UPDB has expanded to include many tumor types, and the genetic contribution to disease phenotypes other than cancer have also been evaluated. This resource links genealogical information representing Utah's pioneers and their descendants with the individual cancer records represented in the Utah Cancer Registry (UCR), which collects data on all patients with cancer diagnosed in the state, and with annual updates from the Utah Department of Health for births, deaths, marriages, divorces, as well as records from the Utah Driver's License Division. The UPDB now encompasses over seven million individuals and has a subset of over 2.5 million individuals with at

least three generations of genealogical data; and some pedigrees now extend to 11 generations linking back to the initial settlers in Utah.

Evaluation of Heritable Contribution

The genetic contribution to specific phenotypes can be evaluated using one of three different methods: the Genealogical Index of Familiality (GIF), which was developed specifically for use with the UPDB; the estimation of the relative risks (RR); and the identification of high-risk pedigrees with specific phenotypes observed in significant excess (Couldwell and Cannon-Albright 2010). The GIF examines the estimation of the average relatedness among affected individuals who share a specific phenotype (Couldwell and Cannon-Albright 2010). If individuals in the database with a selected phenotype have a significantly greater average relatedness than matched controls, there is evidence of excess familiality. Similarly, phenotypes with a genetic contribution should occur more often in relatives of those affected with that phenotype than in the control population (i.e., higher RR) (Couldwell and Cannon-Albright 2010). Finally, high-risk pedigrees found in the database may suggest the predisposition genes responsible for the observed phenotypes.

Use of the UPDB for Evaluation of Heritability of Pituitary Tumors

Using the data available in the UPDB and the UCR, Couldwell and Cannon-Albright (2010) analyzed the genetic relationships among individuals diagnosed with benign or malignant pituitary tumors to investigate whether there was evidence for a heritable contribution to the disease in non-syndromic cases. Twenty-one patients with a malignant pituitary tumor and 720 individuals with a benign pituitary tumor recorded in the UCR since 1966 also had Utah genealogical data. The analysis of the GIF testing the hypothesis of no excess relatedness for all pituitary tumor cases demonstrated that the pituitary cases had a higher degree of relatedness than expected ($p < 0.001$). The average relatedness of all pituitary tumor cases was also significantly higher than expected when all relationships closer than third-degree relatives were ignored. The RR assessment demonstrated a significantly elevated risk to first and third-degree relatives of affected individuals. Using these two methods, the authors found strong evidence for a genetic contribution to predisposition to symptomatic pituitary tumors.

Use of Heritability Information

This information is particularly valuable for counseling family members of patients treated for pituitary adenomas. Relative risks for first- and third-degree relatives were significantly elevated ($RR = 2.83$ and 1.63, respectively) (Couldwell and Cannon-Albright 2010). It also may raise a higher index of suspicion for the detection of symptomatic pituitary tumors in such individuals. At the present time, the cost effectiveness of screening close relatives for pituitary tumors using magnetic resonance imaging and endocrine studies has yet to be determined, but there is the potential in the future that this information may be useful for early detection. This information could be a stepping stone for gene mapping in these high-risk groups and for creation of a low-cost screening tool using the gene mapping information to help to lessen the burden of the disease.

In conclusion, extensive molecular research into pituitary tumors has revealed an exhaustive list of genes responsible for the growth of pituitary cells. Furthermore, epidemiological data has elucidated the genetic predisposition to clinically significant pituitary tumors in family members of patients. The data linking specific genes to relative risk to family members is still sparse. The discovery of familial pituitary tumor syndrome (FIPA) and the AIP gene has shown that no single gene is solely responsible for heritable pituitary adenomas. As such information linking specific genes with pituitary tumors becomes complete and available, it will be extremely valuable in counseling family members of patients

treated for pituitary adenomas and for raising a higher index of suspicion for the detection of a symptomatic pituitary tumor in such individuals.

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