Novel Genetic Causes of Pituitary Adenomas
Francisca Caimari and MártA Korbonits

Abstract

Recently, a number of novel genetic alterations have been identified that predispose individuals to pituitary adenomas. Clinically relevant pituitary adenomas are relatively common, present in 0.1% of the general population. They are mostly benign monoclonal neoplasms that arise from any of the five hormone-secreting cell types of the anterior lobe of the pituitary gland, and cause disease due to hormonal alterations and local space-occupying effects. The pathomechanism of pituitary adenomas includes alterations in cell-cycle regulation and growth factor signaling, which are mostly due to epigenetic changes; somatic and especially germline mutations occur more rarely. A significant proportion of growth hormone- and adrenocorticotropic hormone-secreting adenomas have activating somatic mutations in the GNAS and USP8 genes, respectively. Rarely, germline mutations predispose to pituitary tumorigenesis, often in a familial setting. Classical tumor predisposition syndromes include multiple endocrine neoplasia type 1 (MEN1) and type 4 (MEN4) syndromes, Carney complex, and McCune-Albright syndrome. Pituitary tumors have also been described in association with neurofibromatosis type 1, DICER1 syndrome, and SDHx mutations. Pituitary adenomas with no other associated tumors have been described as familial isolated pituitary adenomas. Patients with AIP or GPR101 mutations often present with pituitary gigantism either in a familial or simplex setting. GNAS and GPR101 mutations that arise in early embryonic age can lead to somatic mosaicism involving the pituitary gland and resulting in growth hormone excess. Senescence has been suggested as the key mechanism protecting pituitary adenomas turning malignant in the overwhelming majority of cases. Here we briefly summarize the genetic background of pituitary adenomas, with an emphasis on the recent developments in this field. Clin Cancer Res; 22(20); 5030–42. ©2016 AACR.

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Introduction

The pituitary gland consists of an anterior lobe of epithelial origin and a posterior lobe of neuronal origin. The main cell types of the anterior lobe are the hormone-secreting cells (growth hormone (GH), prolactin, adrenocorticotrophin (ACTH), thyrotropin (TSH), or gonadotrophin (LH and FSH)) and the folliculostellate cells. The term “pituitary adenoma” is attributed to the usually benign tumors arising from the hormone-secreting cells of the anterior pituitary. Typically, pituitary adenomas are classified as either functioning pituitary adenomas with characteristic clinical symptoms, such as acromegaly or Cushing disease, or clinically nonfunctioning pituitary adenomas (NFPA), usually arising from cells secreting LH and FSH. These adenomas generally present as slowly growing lesions with low mitotic rate and Ki-67 labeling index (1). Symptoms are present due to hormonal disturbances, hypersecretion or lack of pituitary hormones, and compression symptoms that are secondary to local invasion and lead to hypopituitarism and visual field defects.

Data derived from autopsies and radiologic imaging studies have shown that pituitary adenomas are relatively common, estimated to be present overall in 17% of the general population (2, 3). Although most of these small lesions are incidental findings, with no obvious clinical impact (3), clinically relevant pituitary adenomas are present in 0.1% of the general population, and they represent the third most-frequent intracranial tumor type after meningiomas and gliomas (4).

Pituitary adenomas are monoclonal neoplasms in origin (5). A number of different molecular mechanisms that lead to pituitary adenomas have been identified, although in the majority of the sporadic cases, the exact molecular pathogenesis remains unknown. Factors hypothesized to contribute to pituitary neoplasia initiation and proliferation include altered growth factors and cell-cycle regulators that are the result of epigenetic changes (6), abnormal hormonal milieu, abnormal intrapituitary microenvironment (7), and inherited or somatic mutations (Fig. 1). The role of environmental factors remains questionable (8–10). In the following brief overview of the underlying pathomechanisms, we will concentrate on germline and somatic mutations that lead to pituitary adenomas.

Germline Mutations

Familial pituitary adenomas can be divided into (i) an isolated group, in which no other organs are involved in addition to the pituitary gland, and (ii) a syndromic group, which includes multiple endocrine neoplasia type 1 (MEN1), MEN4, Carney complex, DICER1 syndrome, SDHx gene-associated syndromes, and neurofibromatosis type 1 syndrome (Fig. 1; Table 1). Familial...
Isolated pituitary adenoma (FIPA) is the most common type followed by MEN1, which together represent 5% to 7% of patients with pituitary adenomas (11).

Isolated pituitary adenomas

FIPA is defined by the presence of pituitary adenomas in two or more family members with no other syndromic features present (12). FIPA is a heterogeneous condition that includes patients with mutations in the aryl hydrocarbon receptor–interacting protein (AIP) gene (13), patients with X-linked acrogigantism (XLAG) due to duplication of GPR101 (14), and patients with a family history of pituitary adenomas with no known genetic cause. Patients with AIP or GPR101 mutations or with AIP and GPR101-negative FIPA do not present with other types of tumors, hence the name "isolated" pituitary adenomas. Not all cases grouped under this category have a known family history, either due to low penetrance (such as in AIP mutation–positive simplex cases) or due to de novo mutations (most cases of XLAG).

AIP mutations. The prevalence of AIP mutations in FIPA families is 17% to 20% (15, 16), whereas in sporadic cases, it ranges between 3.6% (unselected pituitary adenoma patients) and 10% to 20% (pediatric pituitary adenoma cases; refs. 17, 18). About 50% of AIP mutation–positive probands have a positive family history (16), whereas mutations in the other half are found as a germline mutation in sporadically diagnosed pituitary adenoma patients, so-called "simplex cases." The lack of apparent family history in the latter group is due to low penetrance, as de novo mutations have only been found in two cases (19, 20). Penetrance in AIP-mutated families is incomplete (Fig. 2A): only every fifth mutation carrier manifests the disease. The age of onset is also characteristic; the disease usually manifests in the second decade of life and almost all cases are diagnosed before the age of 30 years (15, 16, 21, 22).

AIP encodes a 330 amino acid protein acting as a tumor suppressor. It has a wide tissue distribution; in the normal human pituitary, it is expressed in GH cells (somatotroph cells) and prolactin-secreting cells (23). Lack of interaction with cell type-
specific molecular partners might explain the specific clinical phenotype of AIP deficiency. AIP has numerous interacting partners (12). It is co-chaperone to several heat shock proteins and nuclear receptors including the aryl hydrocarbon receptor, and it interacts with phosphodiesterase 4A/5, which regulates the cAMP pathway (24–26); however, lack of AIP leads to reduced inhibitory G protein Goi-2 expression. The latter two aspects could be important to the tumorigenic role of AIP (27), as activation of the cAMP pathway is important in somatotroph tumorigenesis (Fig. 3): (i) activating mutations of the stimulatory G protein GNAS are present in approximately 30% of sporadic somatotroph (GH-secreting) adenomas and in McCune-Albright syndrome (28, 29), (ii) mutations in PRKAR1A and PRKACB lead to increased protein kinase A activity in Carney complex (30, 31), and (iii) the duplication of the cAMP-coupled orphan receptor GPR101 causes XLAG (14).

AIP mutations predispose individuals to childhood or young adult-onset disease with large, aggressive, poorly responsive, mostly GH or prolactin-secreting tumors, leading to gigantism in 40% of the cases (Fig. 2B and C; refs. 15, 16, 23). There is a phenotype–genotype correlation, as patients with truncating mutations are significantly younger at disease onset in comparison with patients with nontruncating AIP mutations (Fig. 2C; ref. 16). Patients with AIP mutations have an increased risk for pituitary apoplexy (Fig. 2B) compared with AIP mutation–negative young GH excess patients (16, 23, 32). Classical

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Genetic alteration (inheritance pattern)</th>
<th>Function</th>
<th>Location</th>
<th>Penetrance</th>
<th>Prevalence</th>
<th>Main clinical characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carney complex</td>
<td>PRKAR1A (AD)</td>
<td>TSG</td>
<td>17q24.2</td>
<td>&gt;95% overall, 80% for GH excess</td>
<td>Unknown</td>
<td>Skin pigmentation; myxomas; thyroid, testis and adrenal tumors, as well as somatotroph hyperplasia or adenomas</td>
</tr>
<tr>
<td>2p16 locus (unknown gene)</td>
<td>PRKACB</td>
<td>Oncogene</td>
<td>1p31.1</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Less severe Carney complex phenotype, mostly in sporadic cases</td>
</tr>
<tr>
<td>DICER1</td>
<td>DICER1 (AD)</td>
<td>TSG</td>
<td>14q32.13</td>
<td>Unknown, &lt;1% for pituitary</td>
<td>Unknown</td>
<td>Described in one case with Carney complex (31)</td>
</tr>
<tr>
<td>FIPA</td>
<td>AIP (AD)</td>
<td>TSG</td>
<td>11q13.2</td>
<td>30%</td>
<td>2.5%</td>
<td>Pituitary ACTH-secreting blastomas</td>
</tr>
<tr>
<td>McCune-Albrighta</td>
<td>GPR101 (X-linked)a</td>
<td>Oncogene</td>
<td>Xq26.3</td>
<td>100%</td>
<td>Unknown (very low, 10% of pituitary gigantism cases)</td>
<td>Gigantism due to pituitary hyperplasia or adenoma</td>
</tr>
<tr>
<td>GNAS (mosaic postzygotic mutation)</td>
<td>Oncogene</td>
<td>20q13.32</td>
<td>10–20% for pituitary</td>
<td>1:100,000–1:1,000,000</td>
<td>Polyostotic fibrous dysplasia, café-au-lait spots, and precocious puberty with GH/prolactin excess in 10%–20%</td>
<td></td>
</tr>
<tr>
<td>MEN1</td>
<td>MEN1 (AD)</td>
<td>TSG</td>
<td>11q13.1</td>
<td>&gt;95% overall, 30–40% for pituitary</td>
<td>1:30,000</td>
<td>Pancreatic, pituitary (typically prolactinomas), and parathyroid gland tumors with other tumors as well</td>
</tr>
<tr>
<td>MEN4</td>
<td>CDKNIB (AD)</td>
<td>TSG</td>
<td>12p13.1</td>
<td>Unknown overall, high for pituitary</td>
<td>Unknown (very low)</td>
<td>MEN1 like, typically somatotroph adenomas</td>
</tr>
<tr>
<td>Neurofibromatosis type 1</td>
<td>NFI1 (AD)</td>
<td>TSG</td>
<td>17q11.2</td>
<td>&gt;95% overall, very low for pituitary</td>
<td>1:4,000</td>
<td>Café-au-lait spots, Lisch nodules, neurofibromas, optic pathway gliomas, and pheochromocytoma. Unclear whether pituitary adenomas caused by NFI mutations</td>
</tr>
<tr>
<td>Paraganglioma/ pheochromocytoma and pituitary adenomas—3P association</td>
<td>SDHA (AD)</td>
<td>TSG</td>
<td>5p15.33</td>
<td>Low penetration</td>
<td>Unknown</td>
<td>Familial paraganglioma type 5 (PGL and PHEO)</td>
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<td></td>
<td>SDNB (AD)</td>
<td>TSG</td>
<td>1p36.13</td>
<td>~50% overall, very low for pituitary</td>
<td>Unknown</td>
<td>Familial paraganglioma type 4 (PGL with increased malignant potential)</td>
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<tr>
<td></td>
<td>SDHC (AD)</td>
<td>TSG</td>
<td>1q23.3</td>
<td>Low penetration overall, very low for pituitary</td>
<td>Unknown</td>
<td>Familial paraganglioma type 3 (head and neck PGL predominance, no pheochromocytoma, associated with GIST)</td>
</tr>
<tr>
<td></td>
<td>SDHD (AD)</td>
<td>TSG</td>
<td>11q23.1</td>
<td>Up to 80% overall, very low for pituitary</td>
<td>Unknown</td>
<td>Familial paraganglioma type 1 (head and neck PGL but also pheochromocytoma)</td>
</tr>
<tr>
<td></td>
<td>SDHA (AD)</td>
<td>TSG</td>
<td>11q22.2</td>
<td>Low penetrance, very low for pituitary</td>
<td>Unknown</td>
<td>Familial paraganglioma type 2 (head and neck PGL)</td>
</tr>
</tbody>
</table>

Abbreviations: AD, autosomal dominant; GIST, gastrointestinal stromal tumor; PGL, paraganglioma; PHEO, pheochromocytoma; TSG, tumor suppressor gene. 
*aGermline or somatic mosaicism. 
*bSomatic mosaicism. 
*cIn patients with pituitary adenomas.
pituitary apoplexy refers to a clinical syndrome that is characterized by sudden onset of headache, vomiting, visual impairment, and decreased consciousness that evolves over hours or days and is caused by hemorrhage and/or infarction of a pituitary adenoma. Patients with AIP mutations often need repeated surgery and external pituitary irradiation, and they are relatively resistant to treatment with somatostatin analogues (SSA) i.e., poor reduction in hormone levels and tumor size (15). The mechanism of somatostatin resistance could involve the somatostatin receptor type 2 (SSTR2)–ZAC1 pathway (33). AIP is upregulated by SSA (34, 35) and, in turn, AIP can upregulate ZAC1 mRNA expression (35, 36). Another possible mechanism explaining the somatostatin resistance of these patients is the reduced expression of the inhibitory G protein subtype Gαi-2. SSTR2 is known to regulate cAMP levels via inhibitory G proteins (37), and deficiency of Gαi-2 could play a role in the SSA resistance of AIP mutation–positive samples (ref. 35; Fig. 3).

In sporadic adenomas that do not harbor AIP mutations, low AIP protein expression is associated with reduced SSTR2 expression, which predicts a reduced responsiveness to SSA therapy (38). Tumors pretreated with SSA before surgery show an increase in AIP expression (34, 35). It has been suggested that AIP might be a better marker of invasiveness in somatotrophinomas (GH-secreting adenomas) than Ki-67 and p53, even in AIP mutation-negative adenomas (39).

Genetic screening and clinical follow-up of family members of AIP mutation–positive probands revealed numerous cases of prospectively identified patients, where the early diagnosis led to earlier treatment and potential avoidance of significant complications (16). This suggests that patients with gigantism or young-onset acromegaly and their families could benefit from genetic screening and clinical follow-up.

**GPR101–X-linked acrogigantism.** XLAG is a novel genetic cause of GH excess. It usually presents at a very early age as a sporadic disease due to a de novo microduplication on the X chromosome involving the GPR101 gene in patients with gigantism (40–42). The majority of the cases are females with germline microduplication (14, 40, 42). Two familial cases have been described with transmission from affected mother to an affected son and show full penetrance (14). Somatic mosaic mutation cases have also been described in males where the mutation was identified in the pituitary tissue and/or at low level in germline (18, 41, 42). Although the originally identified Xq26.3 duplicated area involves four genes (14), only one of these, the GPR101 gene,
has been found upregulated at the mRNA level in pituitary tissue. We have recently identified a patient with XLAG whose duplicated area includes only the GPR101 gene, but not the other three genes, indicating the pathogenic role of GPR101 (14, 42). Activation of GPR101, an orphan Gs protein–coupled receptor, leads to an increase in cAMP levels (refs. 43, 44; Fig. 3). GPR101 is expressed in the caudate putamen, nucleus accumbens, and hypothalamus (45). The endogenous ligand and the exact function of GPR101 remain unknown. It has been suggested, in a uterine cancer cell line, that a fragment of the gonadotrophin-releasing hormone [GnRH-(1–5)] indirectly stimulated GPR101 to induce the release of EGF and subsequent phosphorylation of the EGFR (46), leading to enhanced cellular migration. However, (i) GnRH-(1–5) does not change cAMP levels, which are important for somatotroph tumorigenesis, and (ii) an upregulated EGFR pathway seems to lead to corticotroph (i.e., ACTH-secreting) adenomas (see section on USP8), but not somatotroph adenomas. Therefore, the mechanism by which mutant GPR101 contributes to increased GH secretion is still unclear.

Patients with XLAG have significant GH excess leading invariably to gigantism due to their particularly early age of onset, even in comparison with other pituitary gigantism cases, occurring before the age of 5 years, with growth curves significantly exceeding the 97th percentile (14, 40–42, 47). Most of these patients secrete increased amounts of both GH and prolactin due to a somatomammotroph adenoma and mixed cell hyperplasia. In the adenomas, the Ki-67 index is low or moderate (except one case; ref. 48). SSTR expression is preserved, and AIP expression is moderate or high; GnRH is not expressed, whereas there is increased expression of GPR101 and the GH receptor (40, 42). Patients with XLAG provide a particular challenge in management due to the very young age of onset, the fact that some have hyperplasia rather than an adenoma, and due to partial or complete resistance to SSA and dopamine agonist therapy. Radical pituitary surgery or radiotherapy could be effective but often leads to hypopituitarism (40). The GH receptor antagonist pegvisomant in combination with somatostatin and dopamine agonists has been shown to control IGF-1 and excessive growth (40, 41).

Familial isolated pituitary adenoma with no known gene mutation. Approximately 80% of FIPA cases do not have an identified gene mutation. While AIP- and GPR101-related disease starts in young or very young patients, in this group, the age of diagnosis is often (>60%) after the third decade of life and only 2% of the cases have gigantism (16). In our cohort, the most frequent diagnosis was acromegaly followed by prolactinoma, while in another large cohort, macroadenomas were the most common (49). Fifty-eight percent of our families presented with the same pituitary adenoma type (homogeneous FIPA families), whereas different adenoma types were present in the same family in 42% of the cases. The penetrance seems to be lower than in AIP mutation–positive families, as approximately 80% of the families have only two affected family members. We cannot exclude the possibility that, given the prevalence of pituitary adenomas in the general population (1:1,000), some of these adenomas coexist in a family as a coincidence rather than due to an existing underlying germline mutation.

 Syndromic pituitary adenomas

**MEN1 syndrome.** MEN1 is characterized by the presence of the classical triad of hyperparathyroidism (in almost all patients by the age of 50 years), pituitary adenomas (in about 30%–40% of cases), and neuroendocrine tumors [in about 60% of cases; further data on neuroendocrine tumors can be found in the *CCR Focus* article by Maxwell and colleagues (50)]. In addition, other tumor types have also been associated with this syndrome, such as facial angiofibromas (85%), collagenomas (72%), adrenal cortical adenomas (40%), lipomata (30%), meningiomas (8%), pheochromocytomas (rarely). Breast cancer (6%) has also been suggested more recently.
The MEN1 gene encodes a ubiquitously expressed transcriptional cofactor that regulates cell-cycle proteins such as p27 (51) and cyclin-dependent kinase subunit 4 (CDK4; ref. 52), factors also found to be altered in sporadic pituitary adenomas (see the following section). The interaction with p27 is especially interesting, as mutations in the gene encoding p27 (CDKN1B) are also associated with a MEN1 syndrome–like phenotype (see the following section). MENIN also has a role in G1–S checkpoint regulation, response to DNA damage and apoptosis, regulation of histone deacetylation and methyl transferase complexes, interaction with transcription factors and nuclear receptors, as well as transport of β-catenin (53).

MEN1-related pituitary disease is dominated by prolactinoma cases; however, systematic screening reveals similar numbers of small nonfunctioning pituitary adenomas (42%), followed by somatotrophinomas (7%) and a smaller percentage of the other pituitary adenoma types (54). Nonfunctioning adenomas were more often microadenomas detected during screening. Pituitary adenomas could be the first manifestation of MEN1 syndrome in 15% to 20% of the cases, and the current guidelines suggest that pituitary screening should start at the age of 5 years in mutation carriers (55).

MEN4 syndrome. Not all patients with MEN1 syndrome harbor a mutation in the MEN1 gene. Loss-of-function mutations have been identified in CDKN1B, coding for p27, in a subset of these patients, and the phenotype was named MEN4 syndrome (56). The most common pituitary tumor type is somatotrophinoma. and a childhood-onset case has also been described (57). Other tumors include parathyroid adenomas, adrenal tumors, renal angiomylipomas, uterine fibroids, gastrinomas, neuroendocrine cervical carcinomas, bronchial carcinoids, papillary thyroid carcinomas, and gastric carcinomas. Interestingly, in addition to the usual mutation types (nonsense, frameshift, and missense), alterations in the 5′ open reading frame were also shown to disrupt the function of the p27 protein (57, 58). In a few MEN1-like syndrome cases, mutations in other cell-cycle inhibitors, such as p15 (CDKN2A), p18 (CDKN2C), and p21 (CDKN1A), have also been described (59). These cases, together with MEN1-related pituitary adenomas and with the gene expression data of sporadic pituitary adenomas (see below), support the hypothesis that cell-cycle dysregulation is an important factor in pituitary adenoma development.

Carney complex. Carney complex is characterized by the presence of endocrine and nonendocrine tumors with spotty skin pigmentation, as well as cardiac and cutaneous myxomas (60). More than two-thirds of patients present asymptomatic elevation of IGF-1, GH, and prolactin due to pituitary hyperplasia, and 10% of the patients present with adenomas and symptomatic acromegaly (61). The age of onset is usually after the third decade: however, a few cases of gigantism have also been described. Carney complex is caused in 70% of the cases by inactivating mutations in the regulatory subunit of protein kinase A (PRKAR1A), which leads to excessive cAMP signaling (ref. 62; Fig. 3). Large deletions within the gene lead to a more severe phenotype. Another genetic locus at 2p16 has also been shown to be associated with Carney complex (63). More recently, a single case was described of an activating mutation (gene duplication) of the catalytic subunit of protein kinase A (PRKACB; ref. 31). It has been shown that PRKAR1A haploinsufficiency leads to a dysregulated WNT signaling pathway, in addition to cell-cycle abnormalities (64). Interestingly, embryonic mutations in β-catenin (CTNNB1), a crucial element of the classical WNT pathway, leads to craniopharyngiomas, a nonhormone-secreting pituitary tumor (65).

DICER1 syndrome. Pituitary blastoma is a novel aspect of the DICER1 syndrome. Germline mutations in DICER1 lead to infant pleuropulmonary blastoma, differentiated thyroid carcinoma, multinodular goiter, nasal chondromesenchymal hamartoma, ovarian sex cord stromal tumor, Sertoli-Leydig cell tumor, pheochromocytoma, and pituitary blastoma. Pituitary blastomas develop before the age of 2 years and secrete ACTH, causing severe Cushing disease (66–68). The penetrance of pituitary blastomas is low (~1%; ref. 66). Approximately half of the children with confirmed pituitary blastoma die of the disease within months of diagnosis (66–68).

DICER1 is a cytoplasmic endoribonuclease that processes hairpin precursor miRNAs into short, functional miRNAs that downregulate targeted mRNAs, thereby modulating cellular protein production (69). In the more than 50 reported DICER1 mutation kindreds, germline mutations usually led to truncated proteins (67), whereas the “second hit” somatic mutations were typically in the metal-binding sites of the catalytic RNase IIIa and b domains. The exact mechanism explaining this special scenario or why DICER1 mutations in general lead to tumorigenesis remains unclear.

Succinate dehydrogenase mutations (SDHx): paraganglioma, pheochromocytoma, and pituitary adenoma association. Pheochromocytomas and/or paragangliomas (PGL) have rarely been associated with pituitary adenomas (70–74). Patient with these tumors harbor mutations in the SDHA-D or SDHAF2 genes. Loss of heterozygosity at the SDH locus in the pituitary adenomas, as well as data from animal studies, supports the causality between these genes and pituitary adenoma tumorigenesis (71, 73, 75).

SDH, a multimeric enzyme bound to the inner membrane of mitochondria, has two essential roles: (i) it is an important member of the Krebs cycle, and (ii) it plays a role in oxidative phosphorylation and controls activation of hypoxia-inducible factor 1a (HIF1α), leading to VEGF upregulation (75). SDH protein complex is formed by the subunits A, B, C, and D with its associated assembly factor (SDHAF2). Several mechanisms have been described to explain the tumorigenic effect of SDH mutations: (i) succinate accumulation that inhibits HIFα-prolyl hydroxylases, leading to activation of HIF1α and resulting in a state of tissue pseudohypoxia (76) and reactive oxygen species accumulation, and (ii) inhibition of histone demethylases that leads to epigenetic changes [ref. 77; see further details in the article by Jochmannova and Pacak in this CCR Focus (78)]. Interestingly, the HIF1α-VEGF pathway is upregulated by RSIUME, a gene with increased expression in sporadic pituitary adenomas (79). The upregulated VEGF pathway in SDH-related pituitary adenomas may play a role in their invasive and aggressive behavior (80).
The majority of the described SDH-related pituitary adenoma cases are prolactinomas, followed by NFPA- and GH-secreting adenomas. The age of onset of pituitary adenomas is comparable with that of sporadic tumors without germline mutations (~40–50 years), and the vast majority of them present as invasive macroadenomas with a unique vacuolated histologic phenotype (71). The penetrance of pituitary adenomas in patients with SDH mutations is low; whether imaging of the pituitary fossa should be added to at least the first MRI screening of the neck and skull base area, especially in SDHB mutation-positive cases, remains to be determined.

**Somatic Mutations**

Sporadic pituitary adenomas harbor a lower somatic mutation rate in comparison with malignant tumors (81, 82), which is consistent with their typically low proliferation rate and benign phenotype.

**GNAS**

The most frequently observed (up to 40%) genetic change in somatotroph adenomas is the somatic heterozygous gain-of-function mutation of the GNAS gene coding for the Gsα subunit (11, 83, 84). The mutations, known as gsp mutations, affecting codon 201 or 227 destroy the GTPase activity of the protein (Fig. 3). Prolonged adenyl cyclase stimulation and increased cAMP synthesis result in increased cell proliferation and GH secretion. GNAS is an imprinted gene in the pituitary; mutations are always located on the maternal allele (85). Some, but not all, studies found gsp-positive somatotropinomas to be more responsive to SSA treatment and mostly to be densely granulated somatotroph adenomas (6, 36). No other specific recurrent somatic mutations have been identified in somatotroph adenomas (82).

**USP8**

In ACTH-secreting adenomas, novel somatic gain-of-function mutations have recently been identified in the USP8 gene (86, 87). Although USP8 is expressed in all anterior pituitary cell types (88), USP8 mutations have only been identified in corticotroph adenomas (87). The pathomechanism leading to corticotroph adenomas has been linked to EGFR. USP8 encodes a deubiquitinase enzyme that can protect EGFR from degradation by removing lysosome targeting ubiquitin tags, allowing recycling of the receptor to the cell surface (ref. 89; Fig. 4). EGFR is known to be expressed in corticotrophinomas, where it leads to increased POMC levels, ACTH synthesis, and corticotroph cell proliferation, and its presence correlates with invasiveness (86, 87, 90–92). All the identified USP8 mutations are located in the 14-3-3 protein-binding motif of the protein, a domain highly conserved across species, suggesting that lack of 14-3-3 binding leads to increased cleavage and, therefore, increased catalytic activity of the shortened USP8 protein. Gain-of-function mutations in USP8 increase deubiquitination of EGFR, which inhibits its degradations and leads to activation of EGFR signaling. Corticotroph adenomas with mutated USP8 are more frequently found in females (67% vs. 38%; ref. 87), are smaller tumors, have higher ACTH production, and show better prognosis (87, 90). Not all studies found a correlation between EGFR expression and USP8 mutation status (93). USP8-mutated tumors express significantly higher levels of proopiomelanocortin (the gene encoding ACTH) and SSTR5. An important clinical question is whether these adenomas, treated preoperatively or after unsuccessful surgery, respond better to

![Figure 4. Mechanism of USP8 induced tumorigenesis. In the normal pituitary, there is a balance between EGFR ubiquitination and degradation and deubiquitination by USP8 leading to EGFR recycling. Mutation in the 14-3-3 protein-binding site of USP8 (yellow star) results in excessive cleavage and higher deubiquitination activity, leading to increased recycling of EGFR.](https://example.com/figure4.png)
multiligand somatostatin analogue pasireotide than USP8 mutation–negative adenomas. The USP8-related tumorigenesis also opens up the possibility of EGFR-directed therapy for corticotrophinomas; indeed, this concept has already been tested in various animal models using EGFR inhibitor gefitinib (94).

Prolactinomas and NFPAs
Some prolactinomas show loss of chromosome 11 (95) and trisomy of chromosomes 5, 8, and 12, whereas no specific recurrent single-nucleotide mutations have been identified to date.

No recurrent specific somatic mutations have been identified in NFPAs using exome sequencing (81); however, intronic mutations, copy-number variations, or other genetic mechanism, not detected with this method, could play a role.

All pituitary adenoma types
Point mutations and increased copy number of the gene coding for the catalytic subunit of phosphoinositide 3-kinase catalytic subunit PIK (phosphoinositide 3-kinase catalytic subunit PIK3CA) have been identified in 20% to 40% of various types of pituitary adenomas (96–98), but patients with Cowden syndrome who harbor germline mutations in the PI3K–PTEN–AKT pathway genes do not present with pituitary adenomas (99), suggesting that PIK3CA amplification could be a permissive phenomenon.

Sixty percent of oncocytic pituitary adenomas show mutations in mitochondrial DNA genes coding for various respiratory complex I components (MTND1, 2, 4, 5, MTTM, MTCYB, and MTRNR2) leading to the disruption of respiratory complex I (100). It has been suggested that this leads to lack of HIF1α stabilization and, therefore, the relatively benign nature of these adenomas (101).

Mosaic Mutations

Patients with somatic mosaicism with GNAS mutations have McCune-Albright syndrome (Fig. 3). One of the characteristic manifestations of this disease is pituitary hyperplasia or tumor resulting in increased GH and prolactin levels in addition to the classical triad of polyostotic fibrous dysplasia, café-au-lait spots, and precocious puberty (102). Other endocrine dysfunctions include testicular lesions, hyperthyroidism, phosphate wasting, and hypercortisolism. GH-secreting tumors (20%–30% of patients) usually present...
before 20 years of age, although the diagnosis can be delayed due to craniofacial fibrous dysplasia resembling features of acromegaly (103–106). This is one of very few diseases that can result in both abnormally short stature, due to precocious puberty, or gigantism, due to young-onset GH excess. Patients with GH excess and craniofacial fibrous dysplasia present a surgical challenge due to skull-base thickening and obliteration of the sphenoid sinus, and treatment often requires total hypophysectomy due to diffuse involvement of the pituitary (104). Somatostatin analogues, especially in combination with GH receptor antagonist treatment, can effectively reduce IGF-1 levels (103, 105).

**GPR101**

In addition to germ line mutations, GPR101 duplication can occur as somatic mosaicism (41, 107). The clinical phenotype of patients with mosaic GPR101 duplication, only described in male patients until now, does not differ from the germline cases. GPR101 sequence variants, apart from gene duplication, do not play a role in pituitary tumorigenesis (42, 108).

**Gene Expression in Pituitary Adenomas**

The pathogenesis of sporadic pituitary adenomas is influenced by multiple factors (Fig. 5), and we refer to other reviews for detailed discussion of these elements (84, 109–111). The number of somatic mutations identified are small compared with other neoplastic conditions, and no data are available for simultaneous multiple mutated gene sets that are so characteristic of many other tumor types (112, 113). Abnormalities in cell-cycle regulation are considered a crucial event in the formation of pituitary adenomas. Loss of CDK inhibitors, such as p16 or p27 (114, 115), or overexpression of CDKs, such as cyclin D (116), are involved in tumor development (109). It has been estimated that 80% of human pituitary adenomas display alterations in at least one cell-cycle regulator (110). A systematic review of epigenetic regulation of pituitary adenomas identified altered expression of tumor suppressor genes including genes coding for p16, p21, p27, p14, death associated protein kinase (DAPK), growth arrest, and DNA damage-inducible protein (GADD45γ), p73, retinoblastoma protein, BMP-4, oncogenes (P TTG and MAGA2), imprinted genes (GNAS, NNAT, MEG3), epigenome modifiers (DNMT3b), and transcription regulators (HMGA2). More recent examples of proteins involved in pituitary tumorigenesis are the mammalian sterile-20-like kinase (MST4) and CABLES1. MST4, which was found to be upregulated in NFPAs, stimulates p38, AKT, and HIF1—known factors in pituitary tumorigenesis—whereas an MST4 inhibitor showed promising results in *in vitro* studies (117, 118). Another recently identified protein is CABLES1, which was lost in 55% of human corticotrop adenomas, and its levels correlated with loss of p27. The feedback effect of glucocorticoids following upregulation of CABLES1 might link the tumorigenic process with glucocorticoid-regulated cell-cycle progression (119). Hormonal factors could also have a role as prolactinomas with lower expression of estrogen receptor α have a higher tumor grade, greater resistance to treatment, and worse prognosis (120).

**Pituitary Carcinomas**

Pituitary carcinomas are extremely rare. They are diagnosed when distant metastases are detected, and survival is usually less than 2 years after diagnosis, which is similar to some other malignancies such as adrenal carcinoma [see article by Payabyab and colleagues in this *CCR Focus* (121)]. The majority of the cases arise from prolactin- and ACTH-secreting cells, and more rarely GH- or LH/FSH-secreting cells. It is currently unclear whether they develop as *de novo* carcinomas or pituitary adenomas that gradually gain malignant features. Somatic changes in tumor suppressor genes (e.g., *TP53* and *RB1*) and oncogenes (e.g., *HRA s* and *MYC*) commonly present in other neoplasia, have been identified in only a few cases of pituitary carcinoma (122). A few MEN1-related pituitary carcinomas (123, 124) and one *SDH* -related (80) pituitary carcinoma have been described in patients with pituitary tumor-predisposing syndromes.

The precise mechanism of why pituitary adenomas do not turn cancerous, even after many years of disease, remains unknown; however, it has been suggested that oncogene-induced senescence plays an important role (125, 126). Senescence is characterized by a signal transduction program leading to irreversible cell-cycle arrest and represents an important protective mechanism against malignancy. Senescence restrains proliferation, but allows the cell to remain viable and perform its physiologic function. This process can be activated by DNA damage, telomere shortening, lysosomal or oxidative stress, chromosomal instability, aneuploidy, loss of tumor-suppressive signaling, or oncogenic activity (110, 127). These events trigger the activation of different cell-cycle regulators, such as p53 and pRb, to upregulate the senescence pathways. PTTG, an oncogene often overexpressed in pituitary adenomas, promotes chromosomal instability and aneuploidy, and lack of PTTG results in pituitary-specific senescent features (127). IL6 is a cytokine involved in pituitary tumor progression, but it is also required for induction and maintenance of oncogene-induced senescence via an autocrine mechanism (128). Senescence markers have been shown to be present in human adenomas where subtype-specific senescence induction pathways could play an important role (125, 129).

In summary, several new pituitary adenoma–predisposing genes have been identified in the last few years: germline mutations, causing isolated pituitary adenomas or syndromic disease, as well as somatic or mosaic mutations. The clinical characterization of patients with sporadic disease or genetic predisposition for pituitary adenomas, and the elucidation of the exact molecular mechanisms will contribute to the identification of novel physiologic pathways, leading to better understanding of tumorigenesis as well as novel therapies.

**Disclosure of Potential Conflicts of Interest**

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