REVIEW

Germline and mosaic mutations causing pituitary tumours: genetic and molecular aspects

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Abstract

While 95% of pituitary adenomas arise sporadically without a known inheritable predisposing mutation, in about 5% of the cases they can arise in a familial setting, either isolated (familial isolated pituitary adenoma or FIPA) or as part of a syndrome. FIPA is caused, in 15–30% of all kindreds, by inactivating mutations in the AIP gene, encoding a co-chaperone with a vast array of interacting partners and causing most commonly growth hormone excess. While the mechanisms linking AIP with pituitary tumorigenesis have not been fully understood, they are likely to involve several pathways, including the cAMP-dependent protein kinase A pathway via defective G inhibitory protein signalling or altered interaction with phosphodiesterases. The cAMP pathway is also affected by other conditions predisposing to pituitary tumours, including X-linked acrogigantism caused by duplications of the GPR101 gene, encoding an orphan G stimulatory protein-coupled receptor. Activating mosaic mutations in the GNAS gene, coding for the Ga stimulatory protein, cause McCune–Albright syndrome, while inactivating mutations in the regulatory type 1α subunit of protein kinase A represent the most frequent genetic cause of Carney complex, a syndromic condition with multi-organ manifestations also involving the pituitary gland. In this review, we discuss the genetic and molecular aspects of isolated and syndromic familial pituitary adenomas due to germline or mosaic mutations, including those secondary to AIP and GPR101 mutations, multiple endocrine neoplasia type 1 and 4, Carney complex, McCune–Albright syndrome, DICER1 syndrome and mutations in the SDHx genes underlying the association of familial paragangliomas and phaeochromocytomas with pituitary adenomas.

Key Words
- genetics
- pituitary
- pituitary adenoma
- mutation

Introduction

The human pituitary gland consists of an anterior lobe, which derives from the oral ectoderm, and a posterior lobe, which originates from the neuroectoderm. The anterior pituitary contains five types of endocrine cells, including the somatotroph (producing growth hormone (GH)), lactotroph (producing prolactin (PRL)), gonadotroph (producing the gonadotropins, LH and FSH), corticotroph (producing adrenocorticotropic hormone (ACTH)) and the thyrotroph (producing thyrotropin (TSH)) cells. The anterior pituitary also contains a non-endocrine cell...
population represented by the folliculostellate cells, which have a sustentacular function to the hormone-producing cells (Devnath & Inoue 2008).

Pituitary adenomas (PAs) are usually benign tumours arising from the endocrine cells of the anterior pituitary. These tumours are quite common and they are found in approximately 15–20% of the general population in radiological or autopsy studies (Ezzat et al. 2004, Daly et al. 2009), and they represent the third most common intracranial neoplasm after meningiomas and gliomas (Aflorei & Korbonits 2014). However, most of these tumours have no clinical relevance and often represent incidental findings (Freda et al. 2011). Clinically relevant pituitary tumours are rarer, occurring in about 0.1% of the general population (Daly et al. 2006a, Fontana & Gaillard 2009, Cannavo et al. 2010, Fernandez et al. 2010, Raappana et al. 2010, Gruppetta et al. 2013, Agustsson & Gaillard 2009). Although histologically benign, PAs can cause significant morbidity due to hormone excess, hypopituitarism and tumour mass effects on the surrounding structures, such as the optic pathway, the cavernous sinuses and the brain. The most common PAs are represented by prolactinomas (45–65%), followed by non-functioning PAs (NFPAs) (15–37%), somatotroph (9–15%), corticotroph (2–6%) and thyrotroph PAs (0–1%).

Pituitary tumours are believed to be monoclonal in origin (Herman et al. 1990). The exact molecular pathogenesis is still not clear; however, several mechanisms have been described, including, among others, dysregulation of cell cycle regulators (Jacks et al. 1992, Kiyokawa et al. 1996) or alterations of growth factors (Zhou et al. 2014). Somatic mutations can also occur, including activating GNAS mutations (found in 10–50% of somatotroph PAs (Peverelli et al. 2014)) or somatic mutations in the USP8 gene causing activation of the EGF signalling pathway (found in 20–60% of corticotroph PAs) (Ma et al. 2015, Reincke et al. 2015, Ballmann et al. 2018).

While most PAs arise sporadically, about 5% occur in a familial setting (Daly et al. 2006b). Familial PAs are often distinct from their sporadic counterpart, as they can present an aggressive behaviour, are frequently resistant to treatment and they often arise at an earlier age. Familial PAs can develop as part of a syndromic condition, such as multiple endocrine neoplasia type 1 (MEN1), multiple endocrine neoplasia type 4 (MEN4), Carney complex (CNC), McCune–Albright syndrome (MAS), phaeochromocytoma/paraganglioma with PA (3PAs) and DICER1 syndrome. However, as seen in the case of familial isolated PA (FIPA), PAs can develop in the absence of other clinical manifestations, as is the case for patients harbouring mutations in the aryl hydrocarbon receptor-interacting protein (AIP) gene and in patients with X-linked acrogigantism (XLAG), a rare condition of early-onset pituitary gigantism due to duplications involving the GPR101 gene. Nevertheless, in the majority of cases of FIPA, the causative genetic mutations still remain to be identified.

Owing to either incomplete penetrance or to de novo mutations, variants in genes associated with FIPA or syndromic conditions can also be identified in patients with sporadic PAs, especially in those with early-onset disease. The recognition of these patients is particularly important, as it can allow to identify unaffected carriers who will benefit from regular clinical screening which could result in early diagnosis and possibly improved treatment outcomes (Hernandez-Ramirez et al. 2015).

In this review, we aim to discuss the genetic causes of familial and sporadic pituitary tumours, focusing on germline and somatic mosaic mutations causing FIPA and syndromic conditions predisposing to pituitary tumours, including MEN1, MEN4, CNC, MAS, 3PAs and DICER1 syndrome (summarised in Table 1). As the clinical features of these conditions have been extensively reviewed elsewhere (Vasilev et al. 2011, Beckers et al. 2013, Caimari & Korbonits 2016, Marques & Korbonits 2017), here we will aim to focus on the genetic aspects and the mechanisms linking monogenic mutations with PA pathogenesis.

Familial isolated pituitary adenoma

FIPA is an inherited condition characterised by the occurrence of PAs in two or more members of the same family with no other associated manifestations (Beckers et al. 2013). It is estimated to account for about 2% of all PAs (Daly et al. 2006b). In a recent study looking systematically at the prevalence of familial PAs among patients with functioning pituitary tumours, FIPA was identified in 10/262 patients (3.8%) (Marques et al. 2017). FIPA is a highly clinically heterogeneous condition and can include families where affected family members have the same PA subtype (homogeneous FIPA) or families with different PAs (heterogeneous FIPA). Most homogeneous FIPA kindreds present with prolactinomas or somatotroph PAs, followed by NFPAs and, rarely, corticotroph PAs, while in heterogeneous FIPA families, all possible combinations of different PA subtypes can be observed, with the association of somatotroph PAs and prolactinomas being the most common.
## Table 1 Germline and mosaic mutations causing pituitary tumours.

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Gene (inheritance pattern)</th>
<th>Germline or mosaic Location</th>
<th>Penetrance for pituitary disease</th>
<th>Main clinical characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIPA</td>
<td>AIP (AD)</td>
<td>Germline 11q13.2</td>
<td>15–30%</td>
<td>Young-onset (typically in the second decade) somatotroph or mixed somatotroph–lactotroph PAs and prolactinomas. Responsible for 15–30% of FIPA kindreds and up to 20% of young-onset PAs (typically causing gigantism or early-onset acromegaly)</td>
</tr>
<tr>
<td></td>
<td>GPR101 (X-linked)</td>
<td>Germline or somatic mosaic in males with sporadic disease Xq26.3</td>
<td>100%</td>
<td>Early-onset (&lt;4 years) gigantism</td>
</tr>
<tr>
<td>MEN1</td>
<td>MEN1 (AD)</td>
<td>Germline 11q13.1</td>
<td>30–40%</td>
<td>Hyperparathyroidism, PAs (mostly prolactinomas and NFPA), GEP NETs, other neoplasms</td>
</tr>
<tr>
<td>MEN4</td>
<td>CDKN1B (AD)</td>
<td>Germline 12p13.1</td>
<td>Unknown</td>
<td>MEN1-like phenotype</td>
</tr>
<tr>
<td>Carney complex</td>
<td>PRKAR1A (AD)</td>
<td>Germline 17q24.2</td>
<td>10% (symptomatic acromegaly)</td>
<td>Skin pigmented lesions, cardiac and cutaneous myxomas, multiple non-endocrine and endocrine neoplasms including pituitary hyperplasia and PAs (mostly somatotroph and lactotroph or mixed, very rarely corticotroph PAs)</td>
</tr>
<tr>
<td></td>
<td>Unknown gene PRKACB</td>
<td>Germline 2p16</td>
<td>Unknown</td>
<td>Same as for PRKAR1A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Germline 1p31.1</td>
<td>Unknown</td>
<td>Described in one case with CNC phenotype (Forlino et al. 2014)</td>
</tr>
<tr>
<td>McCune–Albright syndrome</td>
<td>GNAS (not inheritable)</td>
<td>Somatic mosaic 20q13.32</td>
<td>20%</td>
<td>Café-au-lait spots, polyostotic fibrous dysplasia, precocious puberty, GH excess in about 20% of patients</td>
</tr>
<tr>
<td>Phaeochromocytoma/paraganglioma with pituitary adenoma</td>
<td>SDHA (AD)</td>
<td>Germline 5p15.33</td>
<td>&lt;1%</td>
<td>Familial PPGL</td>
</tr>
<tr>
<td></td>
<td>SDHB (AD)</td>
<td>Germline 1p36.13</td>
<td>&lt;1%</td>
<td>Familial PPGL</td>
</tr>
<tr>
<td></td>
<td>SDHC (AD)</td>
<td>Germline 1q23.3</td>
<td>&lt;1%</td>
<td>Familial PPGL</td>
</tr>
<tr>
<td></td>
<td>SDHD (AD)</td>
<td>Germline 11q23.1</td>
<td>&lt;1%</td>
<td>Familial PPGL</td>
</tr>
<tr>
<td></td>
<td>MAX (AD)</td>
<td>Germline 14q23.3</td>
<td>Unknown</td>
<td>Familial PPGL</td>
</tr>
<tr>
<td></td>
<td>DICER1 (AD)</td>
<td>Germline or somatic mosaic 14q32.13</td>
<td>&lt;1%</td>
<td>Early-onset pituitary blastomas (ACTH-secreting)</td>
</tr>
</tbody>
</table>

AD, autosomal dominant; CNC, Carney complex; FIPA, familial isolated pituitary adenoma; GEP NET, gastroenteropancreatic neuroendocrine tumour; GPCR, G protein-coupled receptor; MEN1, multiple endocrine neoplasia type 1; MEN4, multiple endocrine neoplasia type 4; NFPA, non-functioning pituitary adenoma; PA, pituitary adenoma; PPG, phaeochromocytoma and paraganglioma.
Most FIPA cases have no known genetic cause, while AIP mutations can be identified in 15–30% of FIPA families (Vierimaa et al. 2006, Daly et al. 2007, Leontiou et al. 2008, Hernandez-Ramirez et al. 2015). Owing to the low penetrance of the disease, AIP mutations can also be identified in subjects with early-onset PAs, and typically among those with gigantism and early-onset acromegaly (Tichomirowa et al. 2011, Cuny et al. 2013, Hernandez-Ramirez et al. 2015). Very rarely, duplications of Xq26.3 involving the GPR101 gene have been identified in families with XLAG (Trivellin et al. 2014, Gordon et al. 2016). To this date, most of the reported XLAG patients presented as isolated cases due to de novo germline or somatic mosaic mutations, and only three cases of familial XLAG have been so far described (Trivellin et al. 2014, Gordon et al. 2016).

**Aryl hydrocarbon receptor-interacting protein**

The AIP gene maps to chromosome 11q13.2, consists of six exons and encodes a 330 amino acid protein. The AIP protein is characterised by an N-terminal immunophilin-like domain and a C-terminal tetratricopeptide (TPR) domain containing three TPR motifs a C-terminal alpha helix. The TPR domain of AIP is considered important to mediate the binding between AIP and its numerous interacting partners, in line with the role of AIP as a co-chaperone (Morgan et al. 2012). The best characterised function of AIP is to form, together with the heat shock protein 90 (HSP90), a protein complex which regulates the nuclear translocation of the aryl hydrocarbon receptor (Ma & Whitlock 1997). However, AIP has several other interacting partners, including other members of the heat shock protein family, growth factor receptors, nuclear receptors and viral proteins (Trivellin & Korbonits 2011).

Despite AIP being ubiquitously expressed, no other manifestations other than PAs have been consistently associated with mutations in the AIP gene. Interestingly, in the normal human pituitary gland, AIP is exclusively found in somatotroph and lactotroph cells, while its expression has been described in all PA subtypes and is particularly abundant in NFPAs (Leontiou et al. 2008). The mechanisms underlying the pituitary-specific prostate tumorigenic effects of AIP mutations remain to be elucidated, but are likely to involve different pathways. The pro-tumorigenic effects of AIP mutations depend on the loss of its tumour suppressor function. AIP mutations in fact result, in most cases, in the premature truncation of the coding sequence (nonsense and frameshift mutation) or in highly unstable proteins with reduced half-life (missense mutations or segmental duplications) (Hernandez-Ramirez et al. 2016, Salvatori et al. 2017). Moreover, loss of heterozygosity (LOH) at the AIP locus is often found in AIP-related PAs (Gadelha et al. 1999, Vierimaa et al. 2006), confirming that the tumorigenic process depends on the loss of AIP. Several lines of evidence suggest a link between AIP and the cyclic AMP (cAMP)-dependent protein kinase A (PKA) pathway, which plays a central role in regulating GH expression and proliferation of somatotroph cells (Formosa & Vassallo 2014). The binding of GHRH to its receptor on the somatotroph cell determines the activation of a G stimulatory protein with consequent increase in cAMP levels and activation of PKA. The phosphorylation of the cAMP response element-binding protein (CREB) and the CREB-binding protein is responsible for the activation of the GH promoter mediated by PIT1, a transcription factor involved with pituitary development and pituitary hormone expression (Cohen et al. 1999). The cAMP-dependent PKA pathway and the effects of AIP on this pathway are summarised in Fig. 1. In GH3 mammosomatotroph cells, overexpression of AIP inhibits the cAMP response to forskolin, an adenylate cyclase activator, while AIP knockdown leads to enhanced cAMP production (Formosa et al. 2013). The exact mechanisms linking AIP with the cAMP-dependent PKA pathway remain to be fully elucidated. In human AIP mutation-positive PAs, the expression of the G inhibitory protein \( \Gamma_{i-2} \) was found to be reduced compared to AIP mutation-negative tumours (Tuominen et al. 2015), and the expression of AIP was found to be positively associated with that of \( \Gamma_{i-2} \) also in sporadic AIP mutation-negative PAs (Ritvonen et al. 2017). Moreover, while knockdown of \( \Gamma_{i-2} \) and \( \Gamma_{i-3} \) led to a significant increase in cAMP in AIP WT cells, this effect was not observed in AIP-knockout cell lines (Tuominen et al. 2015), suggesting that the loss of AIP can affect G inhibitory protein function. However, other mechanisms could also link AIP and the cAMP signalling pathway. For instance, AIP has been found to interact with both the catalytic (PRKACA) and the regulatory (PRKAR1A) subunits of PKA (Scherthaner-Reiter et al. 2018). An interaction between AIP and PRKACA was demonstrated in the presence of HSP90, and cytoplasmic co-localisation of AIP and PRAKACA was observed (Hernandez-Ramirez et al. 2018, Scherthaner-Reiter et al. 2018), suggesting that the AIP/HSP90 complex could regulate PKA localisation and potentially affect the interaction between the catalytic and regulatory subunits of PKA. Moreover, AIP has been shown to interact with members of the type 4 phosphodiesterase family.
such as PDE4A5 (Bolger et al. 2003) – enzymes involved in the degradation of cAMP. Interestingly, the expression of PDE4A4 (human homologue of PDE4A5) and PDE4A8 was found to be significantly reduced in AIP mutation-positive somatotroph adenomas (Bizzi et al. 2018), suggesting that reduced expression of PDE4 enzymes might contribute to the enhanced cAMP signalling observed as a consequence of the loss of AIP.

AIP mutation-positive PAs are often resistant to treatment with somatostatin analogues (SSAs), despite expressing somatostatin receptors at levels comparable to sporadic AIP mutation-negative PAs (Chahal et al. 2012). Moreover, SSA resistance has also been observed in sporadic tumours with reduced AIP protein expression independently of the expression of the somatostatin receptor subtype 2 (SSTR2) (Kasuki et al. 2012, Iacovazzo et al. 2016a, Ozkaya et al. 2018). Thus, mechanisms other than altered somatostatin receptor expression are likely to be involved in determining resistance to SSAs in patients harbouring an AIP mutation. Reduced Goi protein expression observed in AIP-related PAs could potentially underlie their resistance to SSAs, as Goi signalling is involved in mediating the anti-secretory effect of SSAs (Theodoropoulou & Stalla 2013). Moreover, AIP knockdown was found to reduce the mRNA expression of ZAC1, a putative tumour suppressor gene involved in the anti-proliferative and anti-secretory effects of SSAs (Chahal et al. 2012). Notably, a positive correlation was described between ZAC1 protein expression and IGF-1 normalisation and tumour shrinkage in a group of 45 patients with acromegaly (Theodoropoulou et al. 2009). While the mechanisms linking AIP and ZAC1 remain

Figure 1
The cAMP-dependent protein kinase A pathway in the somatotroph cell and genes involved in its regulation. Under normal conditions, GHRH released by the GHRH neurons in the arcuate nucleus (ARC) of the hypothalamus determines activation of the adenylate cyclase (AC) through its G stimulatory protein-coupled receptor (GHRH receptor, GHRH-R) in the somatotroph cell. The increased cAMP production causes the regulatory subunits (R) of protein kinase A (PKA) to dissociate from the catalytic subunits (C), which can translocate to the nucleus and phosphorylate its targets, including CREB. Phosphorylated CREB can bind to the promoter of PIT1. These events are required to promote the expression of GH and somatotroph cell proliferation. Loss of AIP has been shown to increase cAMP production via various possible mechanisms, including reduced expression of the G inhibitory protein Ga(i-2) (which exerts an inhibitory effect on AC and is also involved in mediating the inhibitory effects of somatostatin (SS) on GH secretion via somatostatin receptors (SSTR)), AIP interaction with phosphodiesterases type 4 (PDE4), as well as its interaction with members of the PKA complex. This pathway is also affected by other genetic conditions, including Carney complex (CNC) due, in most cases, to inactivating mutations in the regulatory type 1 α subunit of PKA, and McCune–Albright syndrome (MAS), caused by post-zygotic activating mutations of the G stimulatory protein Gsα. While GPR101 is not expressed in adult human somatotroph cells, the duplication of GPR101 causing X-linked acrogigantism (XLAG) could affect the cAMP-dependent PKA pathway, as GPR101 is a Gsα-coupled constitutively active receptor and is significantly overexpressed in the tumours of affected patients. Potentially, GPR101 could also play a role in regulating GHRH secretion in the arcuate nucleus, where GPR101 is physiologically expressed at high levels. 3V, third ventricle.
to be elucidated, the downregulation of ZAC1 observed following knockdown of AIP suggests that this could be one of the mechanisms underlying the SSA resistance often observed in AIP-related PAs.

While AIP-related PAs are often invasive and clinically aggressive, this is rarely observed in other monogenic conditions predisposing to PAs through dysregulation of the cAMP-PKA pathway, such as CNC or MAS. Considering the vast repertoire of AIP-interacting proteins, cAMP-independent mechanisms could contribute to the clinical phenotype of AIP-related PAs. AIP has been recently shown to interact with proteins involved in the organisation of the cytoskeleton (Hernandez-Ramirez et al. 2018), such as members of the tubulin family, and specifically TUBB and TUBB2A (Hernandez-Ramirez et al. 2018). Moreover, two isotypes of beta tubulin, TUBB1 and TUBB2B, were found to be significantly downregulated at the mRNA level in AIP-related PAs compared with the normal human pituitary gland (Hernandez-Ramirez et al. 2018).

A direct interaction was also demonstrated between AIP and NME1 (Hernandez-Ramirez et al. 2018), a protein with anti-metastatic properties involved in the regulation of cell migration and motility (Murakami et al. 2008). Interestingly, NME1 knockdown was found to disrupt E-cadherin-mediated cell adhesion in human hepatoma and colon cancer cell lines, suggesting a critical role for NME1 in the control of intercellular adhesions and cell migration (Boissan et al. 2010). In one study, an inverse relationship between NME1 expression and PA invasiveness was demonstrated (Pan et al. 2005). AIP-related somatotroph PAs are typically sparsely granulated (Hernandez-Ramirez et al. 2015) — a tumour subtype which is characterised by decreased E-cadherin expression and increased invasiveness (Nishioka et al. 2003, Sano et al. 2004) — suggesting that the loss of AIP could contribute, through the alteration of the cytoskeleton organisation, to the invasive and aggressive phenotype often observed in AIP-related PAs.

Aip-deficient mouse models generally recapitulate the human phenotype (Raitila et al. 2010, Gillam et al. 2017). While constitutional Aip-knockout animals die in utero and display severe cardiovascular defects (Lin et al. 2007), Aip<sup>−/−</sup> mice are viable and develop pituitary tumours with full penetrance by the age of 15 months (Raitila et al. 2010), although in the same mouse model, only pituitary hyperplasia without occurrence of PAs was observed in 3- and 12-month-old animals (Lecoq et al. 2016b). Aip<sup>−/−</sup> mice develop PAs at a higher rate compared to WT mice, where incidental pituitary tumours, mostly prolactinomas, are also frequently observed. The majority of the tumours found in Aip<sup>−/−</sup> mice produce GH, although prolactinomas and mixed somatotroph–lactotroph adenomas were also seen (Raitila et al. 2010). LOH of the WT Aip allele was observed in two available tumour samples, confirming the findings in human tumours. Moreover, Aip<sup>−/−</sup> mice had higher circulating IGF-1 levels, and their pituitary tumours showed increased cell proliferation, evaluated via immunohistochemistry for Ki-67, compared to PAs observed in WT mice (Raitila et al. 2010). More recently, another mouse model where Aip was deleted specifically in the somatotroph cells has been characterised (Gillam et al. 2017). Aip-knockout animals were found to be bigger than WT controls both in terms of body length and weight beginning at 12 weeks of age (Gillam et al. 2017). Visceral organs, including heart, liver and kidney, were found to be larger compared to those in WT mice, and both GH and IGF-1 levels were significantly increased by 18 weeks of age. Macroscopic tumours, evaluated by MRI, were visible in 80% of mutant mice by the age of 20 weeks. Histological examination showed somatotroph cell adenomas which were preceded by pituitary hyperplasia observed starting from the age of 18 weeks (Gillam et al. 2017). Interestingly, markedly reduced expression of the cyclin-dependent kinase inhibitor p27 was observed in the adenomatous tissue, suggesting that dysregulation of cell cycle regulators, similarly to what has been observed for sporadic human PAs (Bamberger et al. 1999), could contribute to the neoplastic transformation.

The disease penetrance in AIP mutation carriers is typically low. Studies on large families show a penetrance of 15–30% (Vierimaa et al. 2006, Naves et al. 2007, Chahal et al. 2011, Williams et al. 2014), suggesting that environmental or additional genetic factors could participate in determining the risk of developing AIP-related PAs. PAs in AIP mutation carriers arise at a younger age compared to their sporadic counterpart, presenting clinically in most cases between the second and third decades of life, are often macroadenomas and are frequently larger and more invasive compared to AIP mutation-negative PAs (Daly et al. 2007, 2010, Igreja et al. 2010, Hernandez-Ramirez et al. 2015). In some (Leontiou et al. 2008, Daly et al. 2010), albeit not all, studies (Hernandez-Ramirez et al. 2015), a male preponderance has been described. This finding could be potentially explained by an ascertainment bias due to the prevalent inclusion of patients with gigantism, a condition that is more common in males (Rostomyan et al. 2015). Owing to the young onset of the disease, about 30% of AIP-related PAs manifest clinically with gigantism, a manifestation of GH excess starting at an early age,
before the closure of the growth plates (Leontiou et al. 2008, Daly et al. 2010). Apoplexy is relatively frequent in AIP-related PAs (about 8–10% of all cases) and can represent the presenting feature of the disease (Xekouki et al. 2013). Poor responsiveness to SSAs is common in AIP-related somatotroph adenomas (Leontiou et al. 2008, Daly et al. 2010) and an increased prevalence of AIP mutations has been described among sporadic patients with acromegaly who are resistant to SSAs (Oriola et al. 2013).

The vast majority (80%) of AIP-related PAs are represented by somatotroph adenomas, followed by mixed somatotroph–lactotroph and more rarely prolactinomas (Stiles & Korbonits 2011), while non-functioning pituitary adenomas (NFPAs) are rare, accounting for less than 10% of cases (Daly et al. 2010, Igreja et al. 2010), although many of these tumours are silent somatotroph/lactotroph adenomas, as they can be found to express GH or prolactin (Daly et al. 2010, Villa et al. 2011). Corticotroph and thyrotroph PAs have been very rarely described in AIP mutation carriers (Daly et al. 2010, Cazabat et al. 2012).

Over 100 different mutations have been identified in the AIP gene (Daly et al. 2010, Hernandez-Ramirez et al. 2015), including nonsense, missense, frameshift, splicing and promoter mutations, deletions, insertions and segmental duplications. About 70% of these mutations lead to the loss of the C-terminal end of AIP, due to either nonsense or frameshift mutations resulting in premature stop codons (Hernandez-Ramirez et al. 2015). A minority of mutations is represented by large deletions (<10%) (Georgtsi et al. 2008), highlighting the need to employ dedicated techniques, such as multiplex ligation-dependent probe amplification, in order to correctly identify these mutations.

**X-linked acrogigantism**

XLAG is a condition of early-onset pituitary gigantism due to the germline or somatic mosaic duplication of the GPR101 gene (Trivellin et al. 2014, Iacovazzo et al. 2016b, Iacovazzo & Korbonits 2018). XLAG patients present with marked GH excess, in most cases with associated hyperprolactinaemia, caused by mixed somatotroph–lactotroph adenomas associated, in some patients, with pituitary hyperplasia. In a minority of patients, the disease is due to pituitary hyperplasia in the absence of a PA. XLAG is very rare, with only 33 confirmed cases described so far in the medical literature (Trivellin et al. 2014, Beckers et al. 2015, 2017, Gordon et al. 2016, Iacovazzo et al. 2016b, Rodd et al. 2016). XLAG accounted for approximately 10 and 8% of cases in two large independent series of patients with pituitary gigantism, respectively (Rostomyan et al. 2015, Iacovazzo et al. 2016b). Different from other forms of gigantism, including those linked with AIP mutations and those without a known genetic predisposing factor, where most affected patients are males, XLAG is characterised by a female preponderance, and 24/33 reported XLAG patients are females carrying germline duplications, while somatic mosaic mutations have been identified in the only four reported cases of male patients with sporadic disease (Daly et al. 2016b, Iacovazzo et al. 2016b, Rodd et al. 2016). In three independent families, mother-to-son transmission has been described, in all cases with full penetrance (Trivellin et al. 2014, Gordon et al. 2016). As no other clinical manifestations have been described in these kindreds, XLAG is considered as a rare cause of FIPA.

The GPR101 gene (Xq26.3) encodes a G-protein-coupled receptor whose ligand is unknown. In mice, Gpr101 mRNA was identified primarily in the central nervous system, and particularly in the hypothalamus and amygdala (Bates et al. 2006). In humans, GPR101 was found to be highly expressed at the mRNA level in the nucleus accumbens, as well as in the medulla and the occipital lobe (Trivellin et al. 2016a). Interestingly, Gpr101 was found to be expressed in about half of the neuronal cells expressing the anorexigenic neuropeptide pro-opiomelanocortin in mice (Nilaweera et al. 2007). In the same study, starvation was found to increase GPR101 expression in the posterior hypothalamus, while decreased expression was seen in obese mice carrying the ob gene mutation, suggesting a possible role for GPR101 in regulating appetite and energy metabolism. While GPR101 was found to be significantly overexpressed in the pituitary tumours of XLAG patients, it was not expressed in sporadic somatotroph PAs or in the adult human pituitary gland (Trivellin et al. 2014). On the contrary, GPR101 protein expression was described using immunohistochemistry in the foetal human pituitary and in pituitary samples obtained from adolescents, suggesting that its expression, at least in the pituitary gland, could be age dependent and induced during development and adolescence (Trivellin et al. 2016a). The expression pattern of GPR101 in the pituitary gland also seems to be species specific. In the pituitary of the rhesus monkey, for example, GPR101 was uniquely expressed, at the protein level, in gonadotroph cells, while in the rat pituitary gland, GPR101 was found to be expressed only in a subpopulation of somatotroph cells (Trivellin et al. 2016a).

The mechanisms underlying the pathogenesis of XLAG remain to be determined. GPR101 is coupled with the G stimulatory protein, and is constitutively active,

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as shown by the increased production of cAMP following its overexpression in HEK293 and GH3 cells (Bates et al. 2006, Trivellin et al. 2014). Thus, the activation of the cAMP-PKA pathway induced by the overexpression of GPR101 could potentially underlie the development of pituitary hyperplasia and PAs in XLAG patients. Interestingly, elevated circulating GHRH levels have been described in some, although not all, XLAG patients, suggesting that GPR101 could also have a role in the regulation of GHRH secretion (Glasker et al. 2011, Beckers et al. 2015, Daly et al. 2016a, Iacovazzo et al. 2016b). Notably, the GHRH receptor was found to be abundantly expressed in XLAG patients’ hyperplastic and tumour pituitary samples (Trivellin et al. 2014), and this could possibly relate to increased hypothalamic secretion of GHRH, as GHRH was shown, at least in vitro, to induce the expression of its own receptor (Horikawa et al. 1996). GHRH administration was found to concomitantly stimulate the release of both GH and prolactin in XLAG patients, both in vivo (Moran et al. 1990) and in vitro (Daly et al. 2016a), and this effect was abolished by concomitant treatment with a GHRH receptor antagonist in cells cultured from the PA of an XLAG patient (Daly et al. 2016a). The implication of GPR101 in the hypothalamic regulation of GHRH secretion is further supported by the finding that GPR101 was found to be expressed at higher levels in the arcuate nucleus where, among others, GHRH neurons are localised (Bates et al. 2006). Potentially, the duplication of GPR101 could affect pituitary somatotroph cells both directly, as a result of its constitutive activity and activation of the cAMP-PKA pathway, and indirectly through increased GHRH secretion by the hypothalamus (Fig. 1). In this latter scenario, GPR101 could be involved in the hypothalamic regulation of the GHRH–GH axis, similarly to the action of GPR54, a G protein-coupled receptor expressed in the GnRH neurons which mediates the stimulatory effects of kisspeptin on GnRH release (Franssen & Tena-Sempere 2018).

The clinical features of XLAG patients are strikingly uniform. The disease is characterised by early onset of accelerated growth, in most cases observed during the first 2 years of life, and in all patients before the age of 4 (Beckers et al. 2015, Iacovazzo et al. 2016b). XLAG patients present markedly elevated GH levels resulting in significantly increased IGF-1 and height SDS, which are higher compared to patients with gigantism due to AIP mutations or to genetically undetermined cases (Rostomyan et al. 2015, Iacovazzo et al. 2016b). Other frequently observed features at diagnosis include acral enlargement, coarse facial features, increased appetite and, less frequently, acanthosis nigricans, sleep apnoea/snoring and hyperhidrosis (Beckers et al. 2015, Iacovazzo et al. 2016b). The histopathological features of XLAG-related PAs are also peculiar: these tumours present a typical sinusoidal and lobular architecture with frequent calcifications and follicle-like structures (Iacovazzo et al. 2016b).

Most XLAG patients harbour microduplications of Xq26.3 (in average spanning a region of about 500 kb) involving the GPR101 gene as well as three other neighbouring genes (Trivellin et al. 2014). However, one patient with a typical clinical phenotype was found to carry a complex genomic rearrangement with two duplicated regions separated by a normal copy number segment (Iacovazzo et al. 2016b). The distal duplication in this patient has allowed to narrow down the genomic region shared by all patients to an area encompassing solely (in its entirety) the GPR101 gene, confirming its pathogenic role (Iacovazzo et al. 2016b).

A missense variant in the GPR101 gene (c.924G>G p.E308D; minor allele frequency in the GnomAD database 0.0036) was initially described in about 4% of a series of patients with acromegaly and was found to modestly increase cell proliferation and GH release when expressed in GH3 cells (Trivellin et al. 2014). However, further studies have failed to show an increased prevalence of this variant in patients with acromegaly (Ferrau et al. 2016, Iacovazzo et al. 2016b), suggesting it might not play a role in the pathogenesis of somatotroph PAs. A further variant (c.1098C>A p.D366E) was described in one patient with sporadic acromegaly (Kamenicky et al. 2015). Although no in vitro studies are available, this variant was not identified in a series of almost 400 patients with acromegaly (lacovazzo et al. 2016b). Other GPR101 missense variants have been detected in patients with other PA subtypes, although their impact on the function of GPR101 remains to be determined (Lecoq et al. 2016a, Trivellin et al. 2016b). No pathogenic GPR101 mutations or copy number variations were identified in patients with congenital isolated GH deficiency (Castinetti et al. 2016).

**Syndromic pituitary tumours**

**Multiple endocrine neoplasia type 1**

MEN1 is a tumour predisposition syndrome inherited with an autosomal dominant pattern occurring with a prevalence between 1:10,000 and 1:100,000 (Pardi et al. 2015). Affected individuals develop mainly parathyroid hyperplasia or parathyroid adenomas – causing primary
hyperparathyroidism (in over 90% of patients by the age of 50 years) – gastroenteropancreatic neuroendocrine tumours (NETs, in approximately 60% of patients) and PAs (30–40% of cases). Other endocrine and non-endocrine tumours may also occur in this syndrome, such as bronchial and thymic NETs, facial angiofibromas, lipomas, collagenomas, adrenal cortical adenomas, meningiomas, ependymomas, breast cancer and, rarely, phaeochromocytomas (Marini et al. 2006, Drejerink et al. 2014, Thakker 2014, Maxwell et al. 2016). A diagnosis of MEN1 can be established in (i) an individual carrying a pathogenic MEN1 mutation, (ii) a patient with two or more main MEN1 manifestations or (iii) a patient with one MEN1-associated manifestation and a first-degree relative affected with MEN1 (Thakker et al. 2012). In 90% of cases, MEN1 is due to germline heterozygous mutations in the MEN1 gene. Most MEN1 patients have a positive family history for MEN1-associated manifestations, while de novo mutations occur in approximately 10% of the patients (Chandrasekharappa et al. 1997, Bassett et al. 1998). The MEN1 gene is located on the long arm of chromosome 11 (11q13) and acts as a tumour suppressor gene: heterozygous inactivating mutations in this gene predispose to the occurrence of tumours and in about 90% of MEN1-related tumours LOH at 11q13 can be identified (Larsson et al. 1988, Dong et al. 1997).

MEN1 encodes a protein named menin, a scaffold protein located mostly in the nucleus, involved in several cellular processes, including transcriptional regulation, genome stability, cell division and proliferation (Thakker 2014). The first identified direct partner of menin was JunD, a component of the AP1 transcription factor complex (Agarwal et al. 1999) which acts as a negative regulator of RAS-dependent cell proliferation and protects cells from p53-dependent senescence and apoptosis (Pfarr et al. 1994, Weitzman et al. 2000). It has been reported that menin represses JunD-activated transcription via recruitment of histone deacetylases through association with the corepressor mDin3A, suggesting a role of menin as a repressor at the transcriptional level (Kim et al. 2003). Interestingly, patients carrying mutations in the JunD-interacting domain of menin present a higher mortality risk (Thevenon et al. 2013). It has also been shown that menin serves as a molecular adaptor to allow the interaction between the mixed lineage leukaemia (MLL) protein and the transcriptional coactivator lens epithelium-derived growth factor, which is needed for the association of the MLL complex with chromatin and the expression of MLL target genes (Yokoyama & Cleary 2008). Menin recruits MLL to the promoters of the cyclin-dependent kinase inhibitor 1B (CDKN1B) and 1C (CDKN1C) (Milne et al. 2005, Wu & Hua 2011), promoting the transcription of these genes coding, respectively, for p27 and p57. The predominant expression of these genes, which control cell cycle progression at the G1 phase, in endocrine tissues might explain the selectivity of MEN1 tumorigenesis for endocrine organs. Moreover, cyclin-dependent kinase 4 (CDK4) has also been described as a target of MEN1 (Gillam et al. 2015). CDK4 regulates the cell cycle during G1/S transition, and its activation may be related to tumorigenesis in pituitary and pancreatic tissues (Gillam et al. 2015), as shown by the evidence that mice with heterozygous deletion of the 

Murine Men1 heterozygous knockout models develop a phenotype similar to that of MEN1 patients, with hyperplasia and tumours mainly of the parathyroids, pancreatic islets and anterior pituitary (Crabtree et al. 2001). Constitutional homozygous Men1-knockout mice die at an early embryonic stage (Crabtree et al. 2001), while conditional tissue-specific disruption of menin leads to pancreatic and pituitary tumorigenesis (Biondi et al. 2004).

To date, more than 1500 MEN1 mutations have been described (Lemos & Thakker 2008, Concolino et al. 2016). Most of these are represented by frameshift, missense and nonsense mutations (Lemos & Thakker 2008, Concolino et al. 2016), and they are distributed throughout the whole gene. A clear genotype–phenotype correlation has not been demonstrated (Kouvaraki et al. 2002, Verges et al. 2002, Horiiuchi et al. 2013, de Laat et al. 2015). Approximately 30–40% of MEN1 patients develop PAs (Verges et al. 2002, Trouillas et al. 2008, de Laat et al. 2015), which can represent the first manifestation of the disease in about 15–30% of all patients. PAs are more commonly diagnosed in female patients. Lactotroph PAs are the most common PA subtype in MEN1 (40–60%), followed by NFPAs (15–40%), somatotroph PAs (5–10%) and, rarely, corticotroph or thyrotroph adenomas (Verges et al. 2002, Trouillas et al. 2008, de Laat et al. 2015). PAs in MEN1 are frequently macroadenomas, and they arise at a younger age compared to sporadic PAs and can be multiple.

Considering that PAs can represent the first disease manifestation and the rate of de novo mutations, screening for MEN1 should be considered in patients with childhood-onset pituitary macroadenomas, especially prolactinomas, as a relatively high frequency (6%) of MEN1 mutations has been shown in paediatric PA patients (Cuny et al. 2013).
Multiple endocrine neoplasia type 4

A small percentage of patients showing MEN1 clinical features do not harbour mutations in the MEN1 gene. The characterisation of the phenotype (named MENX) of a rat strain harbouring a spontaneous Cdkn1b mutation prompted studies in patients with an MEN1-like phenotype. Cdkn1b mutations have been identified in rare cases of such patients without identifiable MEN1 mutations, and this condition has been named MEN4 (Pellegata et al. 2006). To date, 19 cases have been reported (reviewed in Alrezk et al. 2017): most of these patients developed primary hyperparathyroidism, either isolated or associated with NETs, mostly gastroenteropancreatic. Seven of the reported patients developed PAs, including four with a somatotroph PA, one with an NFPA, one with a corticotroph PA and one with a prolactinoma. One of the patients with a somatotroph tumour presented with gigantism due to a somatotroph macroadenoma diagnosed at the age of 5 years (Sambugaro et al. 2015). It should be noted that, in the case of two AIP mutation-negative FIPA kindreds harbouring two distinct Cdkn1b variants (Tichomirowa et al. 2012), for one of the identified variants segregation with the PA phenotype could not be assessed, while for the second, only one of the two affected family members carried the variant, therefore making it an unlikely cause for their familial PA.

Cdkn1b is located on chromosome 12q13 and encodes for p27, a tumour suppressor gene involved in cell cycle regulation (Chu et al. 2008). p27 is a member of the cyclin-dependent kinase inhibitors family and negatively regulates the cyclin E/cyclin-dependent kinase 2 complex preventing transition from the G1 to the S phase of the cell cycle (Sheaff et al. 1997). Interestingly, Cdkn1b-knockout mice develop hyperplasia of the intermediate lobe of the pituitary gland, and about 50% these animals develop pituitary tumours originating from the intermediate lobe (Nakayama et al. 1996). Development of pituitary tumours was also observed in Cdkn1b+/− animals challenged with either irradiation or carcinogens, although no deletions or mutations of the WT allele were detected in these tumours, suggesting that p27 does not conform to the two-hit inactivation hypothesis and that tumorigenesis depends on haploinsufficiency rather than complete loss of the gene product (Fero et al. 1998). Reduced p27 protein expression was detected in all human PA subtypes (Bamberger et al. 1999), and especially in corticotroph PAs and pituitary carcinomas (Lidhar et al. 1999). Interestingly, p27 expression was significantly reduced in PAs compared to the normal pituitary cells of the same subtype (Lidhar et al. 1999). The mechanisms underlying downregulation of p27 in human PAs remain to be determined; in a study including 48 PA patients, no differences were observed among the various PA subtypes and the normal pituitary in terms of expression of Cdkn1b transcriptional regulators and specific miRNAs (Martins et al. 2016).


Carney complex

CNC is a rare multiple neoplasia syndrome inherited with an autosomal dominant manner. CNC is characterised by the presence of pigmented lesions of the skin, cardiac and cutaneous myxomas and multiple nonendocrine and endocrine neoplasms, including pituitary hyperplasia and PAs (Carney et al. 1985). The most common endocrine manifestation observed in CNC patients is ACTH-independent Cushing’s syndrome due to primary pigmented nodular adenocortical disease (Stratakis et al. 1993, Bertherat et al. 2009, Rothenbuhler & Stratakis 2010, Courcoutsakis et al. 2013). This condition is observed in about 25% of CNC patients and occurs more often in females (Stratakis et al. 1993). Other endocrine manifestations observed in CNC include testicular tumours, especially large-cell calcifying Sertoli cell tumours, observed in about one-third of affected males at presentation, thyroid nodules (mostly follicular adenomas) which occur in up to 75% of CNC patients and, occasionally, also differentiated thyroid cancer (both papillary and follicular). About two-thirds of CNC patients show elevation of GH or IGF-1, often with associated hyperprolactinaemia, although symptomatic acromegaly occurs only in about 10% of CNC patients, usually by the third decade of life (Bertherat et al. 2009, Correa et al. 2015). Most CNC patients with acromegaly present with pituitary hyperplasia, typically affecting the mammosomatotroph cells, expressing both GH and prolactin (Stergiopoulos et al. 2004) which can be accompanied, in some patients, by one or multiple areas of adenomatous transformation. Most PAs observed in CNC...
are represented by somatotroph or mixed somatotroph–lactotroph microadenomas (Stergiopoulos et al. 2004, Stratakis et al. 2004), although large macroadenomas have also been described. While most cases of Cushing’s syndrome in CNC patients are ACTH independent, two cases of ACTH-dependent Cushing’s disease have been described in patients with CNC harbouring a PRKAR1A mutation (Hernandez-Ramirez et al. 2017b, Kiifer et al. 2017). In both cases, LOH at the PRKAR1A locus has been described in the pituitary tumour, supporting a pathogenic role for the PRKAR1A mutation in causing Cushing’s disease in these patients.

The genetic background of CNC is heterogeneous. About 70% of cases are due to heterozygous inactivating mutations in the PRKAR1A gene (17q24.2), coding for the regulatory subunit type 1 alpha of PKA. PKA is a cAMP-dependent protein kinase implicated in several cellular processes including hormone release, transcriptional regulation, cell cycle progression, cell proliferation and apoptosis (Bossis & Stratakis 2004). The PKA enzyme complex is a tetramer formed of two catalytic and two regulatory components. In the presence of cAMP, the enzymatic complex dissociates releasing the two catalytically active subunits (McKnight et al. 1988) (Fig. 1). To date, four regulatory subunits (R1α, R1β, R2α and R2β) and three catalytic subunits (Ca, Cβ and Cγ) have been identified and, depending on the tissue availability of each subunits, several combinational PKA configurations exist (Skalhegg & Tasken 2000). Two major enzymic complexes have been identified, named PKA type I and II. Type I PKA contains either R1α or R1β regulatory subunits and is considered the main subtype that mediates response to cAMP in mammalian cells (Gamm et al. 1996). Loss-of-function PRKAR1A mutations lead to increased cAMP-dependent PKA activity which drives tumour formation in tissues affected by CNC (Casey et al. 2000, Salpea et al. 2014). Interestingly, PRKAR1A does not seem to behave like a ‘classical’ tumour suppressor gene (Bossis & Stratakis 2004). First, while LOH at the 17q24 locus has been shown in many CNC-related tumours (Kirschner et al. 2000a), in some cases LOH was not detected (Groussin et al. 2002), suggesting that haploinsufficiency might be sufficient for tumour development. Moreover, PRKAR1A seems to behave like an oncogene in selected tissues. For instance, increased expression was described in several human malignancies, including renal and breast cancer (Fossberg et al. 1978, Handschin & Eppenberger 1979). Overexpression of PRKAR1A was also shown to promote growth advantages in different cell lines, including Chinese hamster ovary cells and in breast epithelial cell lines (Tortora et al. 1994a,b). Thus, PRKAR1A could function as both an oncogene and tumour suppressor gene in a cell context-dependent way.

Homozygous deletion of Prkar1a is lethal in mice during embryogenesis (Amieux et al. 2002), while Prkar1a+/− mice were found to develop CNC-related tumours, including Schwannomas and thyroid neoplasms, although no PAs were observed in these animals (Kirschner et al. 2005). In contrast, mice with pituitary-specific homozygous deletion of Prkar1a under the GHRH receptor promoter developed pituitary tumours of the Pit1 lineage expressing GH, prolactin and TSH, and had higher circulating levels of GH compared to WT mice (Yin et al. 2008).

To date, more than 125 PRKAR1A mutations have been described (Correa et al. 2015), most of which are represented by nonsense and frameshift mutations. In the majority of cases, the predicted mutant protein products are not identified as a result of nonsense mRNA-mediated decay (Kirschner et al. 2000a,b). Large deletions can be found in about 20% of the cases where PRKAR1A mutations cannot be detected by Sanger sequencing, and are often associated with a more severe phenotype (Horvath et al. 2008, Salpea et al. 2014). About 70% of CNCs arise in a familial setting, while 30% present sporadically, due to de novo mutations (Correa et al. 2015).

A second genetic locus at 2p16 has been associated with CNC; however, the responsible gene is not yet known (Stratakis 2016). There are no phenotypic differences between CNC patients with mutations at either locus. A single case of a CNC patient harbouring a triplication of the catalytic beta subunit of PKA (PRKACB) has been described (Forlino et al. 2014). This patient presented at the age of 19 years with acromegaly, spotty skin hyperpigmentation and multiple myxomas. Interestingly, PKA activity measured in the patient’s lymphocytes was found to be increased to levels comparable to those seen in two PRKAR1A mutated patients (Forlino et al. 2014), suggesting that overexpression of the Cβ catalytic subunit can affect PKA activity in a way similar to that observed in case of PRKAR1A mutations.

McCune–Albright syndrome

Somatic activating mutations in the GNAS gene (20q13.32), encoding the cAMP pathway associated G protein Gsα, represent the only recurrent mutation found in somatotroph adenomas (Valimaki et al. 2015, Ronchi et al. 2016) and can be identified at a rate of 10–50% (Peverelli et al. 2014). These missense mutations are known to occur at only one of two residues, Arg201 (more commonly) or,
rarely, Gln227, which represent critical sites for GTPase activity. Mutations at these sites cause loss of the GTPase activity with consequent permanent activation of the adenylate cyclase and constitutive activation of the cAMP-dependent PKA pathway (Fig. 1). This results in increased cell proliferation in cAMP-responsive tissue, including the pituitary gland. As such, GNAS is considered a proto-oncogene, activated by these point mutations into the gsp oncogene. When these mutations occur at an early postzygotic stage, the resulting somatic mosaicism underlies a syndromic condition known as MAS. MAS is a rare disorder with an estimated prevalence between 1:100,000 and a 1:1,000,000 (Boyce & Collins 1993) and is defined by the occurrence of polyostotic fibrous dysplasia, café-au-lait skin macules and endocrinopathies, including precocious puberty (especially in females), hyperthyroidism, testicular lesions (Leydig and/or Sertoli cell hyperplasia), growth hormone excess or, more rarely, neonatal hypercortisolism. The clinical manifestations of MAS are extremely variable and depend on the degree of mosaicism. The probability of detecting a GNAS mutation by standard PCR is high in affected tissues, while it can be very low in leukocyte-derived DNA, especially in subjects with only one manifestation of MAS (Lumbroso et al. 2004), although the use of next-generation sequencing can increase the mutation detection rates (Narumi et al. 2013). The GNAS gene is paternally imprinted in several tissues, including the pituitary gland, and most somatotroph adenomas have been found to occur in patients harbouring the mutation on the maternal allele (Hayward et al. 2001, Mantovani et al. 2004).

Pituitary involvement in MAS manifests with GH excess which can be present in about 20% of patients (Salenave et al. 2014). This is in most cases associated with hyperprolactinaemia. PAs can be found in 30–50% of affected patients, with the other patients most likely having pituitary hyperplasia without an adenoma (Galland et al. 2006). The age at onset is variable with a mean age of 24 years (Salenave et al. 2014). While cases of young onset disease have been described, the final stature in MAS patients is often normal, and this might be explained by the high prevalence of associated precocious puberty and increased levels of sex steroids.

**Phaeochromocytoma/paraganglioma with pituitary adenoma**

Germine heterozygous mutations in genes encoding succinate dehydrogenase subunits (SDHx) and the SDH complex assembly factor 2 protein (SDHAF2) have been described in patients with hereditary phaeochromocytoma and paraganglioma (PPGL) (Baysal et al. 2000, Niemann & Muller 2000, Astuti et al. 2001, Hao et al. 2009, Bayley et al. 2010, Burnichon et al. 2010).

The first description of PPGL coexisting with a PA dates back to 1952 (Iversen 1952), but only recently a causative link between genes predisposing to PPGL and PAs has been established, following the description of a patient carrying an SDHD mutation having bilateral phaeochromocytomas and concomitant acromegaly due to a somatotroph PA. This patient’s pituitary tumour showed loss of heterozygosity at the SDHD locus and reduced protein expression of both SDHD and SDHB (Xekouki et al. 2012). Other reports (Benn et al. 2006, Dwight et al. 2013, Varsavsky et al. 2013, Gill et al. 2014, Papathomas et al. 2014, Denes et al. 2015, Xekouki et al. 2015, Tufton et al. 2017, Maher et al. 2018), including a study showing that Sdhb−/+ mice develop pituitary lactotroph hyperplasia (Xekouki et al. 2015), have further strengthened the link between germline SDHx mutations and PAs, and this has allowed the definition of a novel clinical entity called 3PAs (phaeochromocytoma/paraganglioma with PAs). Notably, only one case of a double somatic mutation (detected by loss of SDHB and SDHA immunostaining and confirmed by sequencing) was described in 1/309 sporadic PAs, implying that this is an extremely rare event (Gill et al. 2014).

The SDH enzymatic complex is composed of two subunits which form the catalytic core (SDHA and SDHB) and two subunits which are responsible for anchoring the complex to the mitochondrial membrane (SDHC and SDHD). The SDH complex is responsible for the reversible enzymatic conversion of succinate into fumarate within the citric acid cycle (Bardella et al. 2011). The mechanisms linking loss of SDH function with tumorigenesis remain to be fully determined but are likely to be multifactorial (Fig. 2). Disruption of the SDH complex as a result of loss-of-function SDHx mutations leads to the accumulation of succinate, which in turn causes an inhibition of prolyl-hydroxylases, leading to stabilisation of the hypoxia-inducible factor 1α (HIF1α) and transcription of HIF-responsive genes, some of which are involved in tumorigenesis, including, among others, VEGF and TGF (Selak et al. 2005, Cervera et al. 2008, Guzy et al. 2008). These findings are corroborated by the evidence that gene expression profiling in SDHx-related paragangliomas overlaps with that observed in tumours due to VHL (encoding a component of the ubiquitin ligase complex that mediates the degradation of HIFs) and EPAS1 (HIF2A) mutations (Comino-Mendez et al. 2013). Interestingly, HIF1α was found to exert an anti-apoptotic role in a
human PA cell line in hypoxic conditions (Yoshida et al. 2006) and hypoxia was found to induce invasiveness of the same cell line in vitro (Yoshida & Teramoto 2007). HIF1α knockdown and HIF1α inhibition were shown to increase the sensitivity of human PA cells to temozolomide, an alkylating agent employed for the treatment of aggressive PAs and pituitary carcinomas, both in vitro and in PA xenografts (Chen et al. 2013). Altogether, these data support a role for the HIF1α pathway in pituitary tumorigenesis and might provide a mechanistic link between SDHx mutations and PAs.

In addition, loss of SDH activity leads to increased intracellular production of reactive oxygen species, which can also contribute to the inhibition of prolyl-hydroxylases and to the stabilisation of HIF1α (Niecknig et al. 2012). Moreover, reactive oxygen species promote a condition of chronic metabolic oxidative stress and genomic instability (Ishii et al. 2005, Slane et al. 2006). Whether this could as well contribute to the SDH-related tumorigenesis is yet to be determined, also considering that SDH-mutated paragangliomas were found to harbour a low rate of somatic mutations or copy number alterations (Castro-Vega et al. 2015). Interestingly, SDH-deficient tumours present with a significantly greater genomic methylation level compared to SDH-proficient neoplasms, as it was shown in gastrointestinal stromal tumours (Killian et al. 2013). Similar findings were shown in paragangliomas, where SDHx- and particularly SDHB-related tumours showed a hypermethylator phenotype (Letouze et al. 2013). Hypermethylated tumours were characterised by younger age at diagnosis and a worse prognosis. Sdhb knockout chromaffin cells displayed increased 5-methylcytosine and increased H3K9 and H3K27 methylation (Letouze et al. 2013). These methylome changes were associated with downregulation of several genes, including genes associated with neuroendocrine differentiation, the tumour suppressor gene RBP1, known to be downregulated in several human malignancies (Esteller et al. 2002, Mendoza-Rodriguez et al. 2013) and KRT19 (encoding cytokeratin-19), a marker of epithelial-to-mesenchymal transition. Moreover, Sdhb-knockout mouse chromaffin
cells presented mesenchymal changes reminiscent of epithelial-to-mesenchymal transition (Loriot et al. 2015) with increased invasiveness and enhanced cell migration, and expression of KRT19 by lentiviral transduction partially rescued the invasive phenotype of these cells and enhanced cell adherence (Loriot et al. 2015). While the hypermethylation secondary to SDHx mutations seem to play a pivotal role in mediating tumorigenesis in paragangliomas, no data are available whether this mechanism could also be involved in SDHx-associated PAs.

The penetrance of pituitary tumours in patients carrying SDHx mutations is estimated to be low (<1%). However, this might be an underestimation, considering that subjects carrying SDHx mutations are not routinely screened for pituitary tumours. Among 18 cases of SDHx-related PAs which were confirmed by genetic testing (Benn et al. 2006, Xekouki et al. 2012, 2015, Dwight et al. 2013, Varsavsky et al. 2013, Papathomas et al. 2014, Denes et al. 2015, Tufton et al. 2017, Maher et al. 2018), data regarding family history are available from 16 patients. Among these, 14 patients had a positive family history of PPGL (and PAs in two kindreds), while only two patients presented with sporadic disease. Most patients with SDHx-related PAs were diagnosed with PPGL (in most instances, PPGL were diagnosed first or simultaneously to the pituitary tumour), while five PAs occurred in patients without a personal history of PPGL. Among the 16 patients with available clinical data, most (10) were affected by prolactinomas, while somatotroph or NFPAs occurred in three cases each. Most of the reported cases were macroadenomas, in some cases displaying an aggressive behaviour, including a non-functioning pituitary carcinoma in a patient harbouring an SDHB mutation (Tufton et al. 2017). Notably, SDHx-related PAs showed peculiar histopathology features, with typical intracytoplasmic vacuoles (Denes et al. 2015, Tufton et al. 2017, Maher et al. 2018). As SDHx mutations are extremely rare in patients with sporadic PAs (Gill et al. 2014, Xekouki et al. 2015), sequencing of SDHx genes should be reserved for patients with a personal or family history of paraganglioma or phaeochromocytoma. Considering the aggressive phenotype of SDHx-related PAs and the potential risk of malignancy, patients carrying SDHx mutations should be screened for pituitary tumours, although, owing to the small number of reported cases, frequency and modalities of screening remain to be established.

PAs have been recently reported in patients with phaeochromocytomas harbouring mutations in the MAX gene (14q23.3) (Roszko et al. 2017, Daly et al. 2018, Kobza et al. 2018), including three patients with prolactinomas and two affected with acromegaly. Interestingly, three of these reported cases carried large deletions that were missed by Sanger sequencing. MAX is one of several genes causing predisposition to familial PPGL (Comino-Mendez et al. 2011) and encodes a protein which acts as an interacting partner for MYC and MXD1, transcription factors involved in the regulation of cell proliferation and apoptosis (Atchley & Fitch 1995). While the role of MAX in PA pathogenesis has not been investigated, these reports expand the knowledge on the genetic background of the 3PAs association and suggest that genes other than SDHx could be involved in its pathogenesis.

**DICER1 syndrome**

The DICER1 syndrome, or pleuropulmonary blastoma (PPB)-familial tumour and dysplasia syndrome, is a rare autosomal dominant disorder due to germline heterozygous mutations in the DICER1 gene.

DICER1 syndrome is characterised by a variety of cancerous and benign tumours, including pleuropulmonary blastoma, ovarian sex cord-stromal tumours (mostly Sertoli-Leydig cell tumour), cystic nephroma, nodular hyperplasia of the thyroid, differentiated thyroid cancer, pituitary blastoma, nasal chondromesenchymal hamartoma, ciliary body medulloepithelioma, renal sarcoma, genitourinary embryonal rhabdomyosarcoma and pinealoblastoma (Doros et al. 1993, Schultz et al. 2018).

The first case of pituitary blastoma was characterised in 2008 in a 13-month-old female with ACTH-dependent Cushing’s disease (Scheithauer et al. 2008), although a link with DICER1 mutations was only established more recently (de Kock et al. 2014). The term blastoma was employed as these neoplasms presented the appearances of pituitary embryonic tissue with an aggressive clinical behaviour (Scheithauer et al. 2008). The histopathological features of pituitary blastomas are typical and include Rathke-like epithelial cells forming rosettes or gland-like structures admixed with secretory cells disposed in lobules rather than acini and positive for ACTH and, less frequently, also for GH. Pituitary blastomas are very rare in the setting of DICER1 syndrome (<1%). These aggressive tumours usually arise in young children (median age at presentation is 8 months with a range from 7 to 24 months), and they present clinically with severe ACTH-dependent Cushing’s disease and, in some cases, ophthalmoplegia. The condition can be fatal in about 40% of the cases (Scheithauer et al. 2008, de Kock et al. 2018).
Germline and mosaic mutations in pituitary tumours

2014). Out of 12 pituitary blastoma patients available for genetic testing, Dicer1 mutations were identified in 11 patients (de Kock et al. 2014), suggesting that these rare tumours represent a pathognomonic feature of the Dicer1 syndrome.

The Dicer1 gene is located on the long arm of chromosome 14 (14q32.13). This gene encodes a cytoplasmic endoribonuclease responsible for processing precursor into mature miRNAs, which modulate mRNA expression at the post-transcriptional level (Krol et al. 2010). The pathogenesis in Dicer1 syndrome normally relies on a germline loss-of-function mutation (more often represented by a nonsense or frameshift mutation) followed by a second somatic ‘hit’, often involving the Rnase IIib catalytic domain of Dicer1 (Heravi-Moussavi et al. 2012, de Kock et al. 2014). Somatic mosaic mutations, often affecting the Rnase IIib catalytic domain, have also been identified using high-sensitivity detection systems (Brenneman et al. 2015, de Kock et al. 2016). Interestingly, these mutations appeared to be accompanied by second somatic mutations represented by truncating Dicer1 mutations outside the Rnase IIib domain or by LOH. Mutations affecting the Rnase IIib domain of Dicer1 lead to loss of its enzymatic activity and loss of miRNAs generated from the 5p strand of miRNA precursors (Gurtan et al. 2012, Heravi-Moussavi et al. 2012, Anglesio et al. 2013). In vitro, Dicer1 mutations were shown to lead to a reduction of 5p-derived miRNAs in ovarian Sertoli-Leydig cell tumours and to promote cell proliferation in a granulosa cell line via deregulation of the let-7 miRNA family (Wang et al. 2015), miRNAs with important roles in cell differentiation and proliferation (Bussing et al. 2008). Interestingly, in mice lacking epithelial Dicer1, increased Fgf9 expression in the lung epithelium, possibly mediated by downregulation of miR-140, resulted in hyperplastic changes resembling those observed in pleuropulmonary blastoma, the primary manifestation of Dicer1 syndrome (Yin et al. 2015). The occurrence of pituitary blastomas in Dicer1 syndrome is likely related to miRNA deregulation. For instance, let-7 miRNAs have been shown to be downregulated in PAs (Bottoni et al. 2007, Amaral et al. 2009, Qian et al. 2009). Among its targets, these miRNAs regulate the expression of HmgA2, which is often overexpressed in prolactinomas (Finelli et al. 2002). Moreover, mice transgenic for HmgA2 develop PAs, especially lactotroph and somatotroph tumours, supporting a role for this oncogene in PA pathogenesis (Fedele et al. 2002). However, the molecular mechanisms linking Dicer1 with pituitary blastomas still remain to be determined.

Other germline mutations linked with pituitary tumours

Recently, novel genes have been implicated with the occurrence of both sporadic and familial PAs. In one Fipa kindred with two cases of acromegaly and two NFpas, exome sequencing revealed a heterozygous missense mutation in the CDH23 gene (10q22.1) (Zhang et al. 2017), encoding a cadherin member previously implicated in the pathogenesis of a subtype of Usher syndrome (Usher syndrome type 1D), an autosomal recessive condition of hearing impairment, vestibular dysfunction and retinitis pigmentosa (Bolz et al. 2001). The CDH23 c.4136G>T p.R1379L missense variant was found to segregate with the PA phenotype and was predicted to alter the formation of hydrogen bonds and impair the calcium-binding ability and stability of one of the extracellular cadherin domains. In 3 of 11 other Fipa families, three CDH23 missense variants were detected and found to co-segregate with the phenotype. All these variants were rare (minor allele frequency <0.05%) and predicted to be pathogenic by at least one in silico prediction tool employed. Out of 125 patients with sporadic PAs of different subtypes, 15 harboured rare CDH23 variants predicted to be potentially pathogenic. All potentially pathogenic CDH23 variants identified in this study were found to affect extracellular cadherin domains, and the frequency of these variants in the PA cohort was significantly higher compared to 260 local healthy control individuals (Zhang et al. 2017). No in vitro functional studies have been performed, and the mechanisms how CDH23 mutations could lead to PA remain unclear. Of note, Usher syndrome patients or unaffected heterozygous mutation carriers are not known to be at increased risk of developing PAs, and the highly polymorphic nature of CDH23 (and other Usher syndrome-related genes) can make the interpretation of genetic variants in this gene challenging (Le Quesne Stabej et al. 2012). Thus, further studies will be needed to confirm the role of CDH23 in PA pathogenesis.

The Cables1 gene (18q11.2) is a cell cycle regulator involved in the negative regulation of cell cycle progression in corticotroph cells in response to glucocorticoids (Roussel-Gervais et al. 2016). Cables1 knockdown was found to stimulate the growth of a corticotroph cell line (AtT-20 cells) and counteracted the inhibitory effects of glucocorticoids on cell growth. Interestingly, Cables1 expression was lost in about 50% of a series of 31 corticotroph adenomas, and this was strongly associated with loss of p27 expression (Roussel-Gervais et al. 2016). Cables1 was previously shown to maintain p21 protein...
stability by antagonising its proteasomal degradation (Shi et al. 2015), while Cables1-knockout mouse embryonic fibroblasts displayed reduced p27 and p16 expression (Kirley et al. 2005), supporting a potential broader role for CABLES1 as a cell cycle regulator. Recently, four patients harbouring potentially pathogenic missense CABLES1 variants were found in a cohort of 181 sporadic patients (2.2%) with Cushing’s disease (Hernandez-Ramirez et al. 2017a). These variants affected residues located within or in proximity with the predicted cyclin-dependent kinase 3-binding domains of CABLES1. All four patients carrying these variants had corticotroph macroadenomas (one patient harboured a silent corticotroph PA) with high proliferation index and an aggressive behaviour, with two patients requiring more than one operation. None of these patients had a family history of Cushing’s disease or other PAs. Tamoxifen-inducible chimeric CABLES1 proteins were produced and, while WT CABLES1 inhibited cell growth when expressed in AtT-20 corticotroph cells in the presence of tamoxifen, this effect was lost in cells expressing the four mutant forms of CABLES1, supporting their pathogenic role. Further confirmatory studies will be necessary to assess the occurrence of CABLES1 mutations in patients with Cushing’s disease or other PAs.

Concluding remarks

While most PAs occur sporadically, about 5% of all PAs occur in a familial setting as a result of a genetic predisposing mutation. More commonly, familial PAs occur without other associated manifestations as FIPA – two genes, AIP and very rarely GPR101, are known to be responsible for this condition, while the causative gene(s) in the majority of FIPA kindreds are yet to be identified. PAs can also occur as part of syndromic conditions and can sometimes represent the first manifestation of the disease. Remarkably, somatotroph PAs and prolactinomas represent the most common PA subtypes associated with a predisposing genetic mutation. While the molecular mechanisms linking these mutations with pituitary tumorigenesis have not always been uncovered, several lines of evidence confirm the involvement of the CAMP-dependent PKA pathway, which plays a central role in regulating hormone secretion and proliferation in cells of the PIT1 lineage. In some cases, more than one pathway might be affected, as seems to be the case for AIP-related PAs.

Significant advances have been achieved in the field of pituitary genetics in recent years, although further studies will be needed to better elucidate the molecular mechanisms linking genetic mutations and pituitary tumours. With the use of pan-genomic techniques, novel genes involved in PA pathogenesis are expected to be discovered, and this will broaden our understanding of the mechanisms underlying PA formation.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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