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The roles of *AIP* and *GPR101* in Familial Isolated Pituitary Adenomas (FIPA)

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Abstract

Familial isolated pituitary adenomas (FIPA) is one of the most frequent conditions associated with an inherited presentation of pituitary tumors. FIPA can present with pituitary adenomas of any secretory/non-secretory type. Mutations in the gene for the *aryl-hydrocarbon receptor interacting protein* (*AIP*) have been identified in approximately 20 % of FIPA families and are the most frequent cause (29%) of pituitary gigantism. Pituitary tumors in FIPA are larger, occur at a younger age and display more aggressive characteristics and evolution than sporadic adenomas. This aggressiveness is especially marked in FIPA kindreds with *AIP* mutations. Special attention should be paid to young patients with pituitary gigantism and/or macroadenomas as *AIP* mutations are prevalent in these groups. Duplications on chromosome Xq26.3 involving the gene *GPR101* lead to X-linked acrogigantism (X-LAG), a syndrome of pituitary gigantism beginning in early childhood; three kindreds with X-LAG have presented in the setting of FIPA. Management of pituitary adenomas in the setting of FIPA, *AIP* mutations and *GPR101* duplications is often more complex than in sporadic disease due to early onset disease, aggressive tumor growth and resistance to medical therapy.

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Introduction

Pituitary adenomas are intracranial tumors that occur in 10-15 % of individuals in autopsy series and in 20-38% of those undergoing CT/MRI (Ezzat, et al. 2004; Molitch 2009) (Freda, et al. 2011). The vast majority of these adenomas, however, are incidentally-discovered, very small, non-functioning pituitary microadenomas with no clinical impact. The medical significance of pituitary adenomas lies in the group of tumors that are sufficiently large and/or hormonally active to cause signs and symptoms. The prevalence of these clinically-relevant pituitary adenomas is of the order of 1 case per 1000 of the general population (Daly, et al. 2006b). The management of clinically relevant pituitary adenomas is often challenging and involves a multidisciplinary team of endocrinologists, neurosurgeons, ophthalmologists and radiologists. Despite their benign nature, some pituitary adenomas may have aggressive behavior and pose additional morbidity for patients.

To date, the molecular mechanisms behind the development of the majority of pituitary adenomas are not well understood. Usually, these tumors are sporadic, but approximately 5 % have a known genetic or familial background (Daly, et al. 2009). Pituitary pathology has been described in several syndromes of multiple endocrine neoplasia (MEN). Among them, MEN1 is the most common and accounts for about 3% of all pituitary adenomas (Scheithauer, et al. 1987). MEN1 is caused by germline inactivating mutations in *MEN1* gene on chromosome 11q13 (Chandrasekharappa, et al. 1997). Carney complex (CNC) is another inherited syndrome that can include familial pituitary adenomas (usually acromegaly) and is predominantly caused by inactivating mutations in the gene encoding the type 1A regulatory subunit of protein kinase A (*PRKAR1A*) (Carney, et al. 1985; Casey, et al. 1998). Inherited pituitary adenoma risk is also associated with the recently characterized syndromes of multiple endocrine neoplasia type 4 (MEN4) caused by inactivating mutations of *CDKN1B* gene (Pellegata, et al. 2006), or in the setting of the pheochromocytoma-paraganglioma with pituitary adenoma association (3PA) that is due to mutations in succinate dehydrogenase (*SDH*) or *MAX* genes (Daly, et al. 2018; Xekouki, et al. 2015).

The emergence of FIPA

Individual cases of familial acromegaly and gigantism arising in a non-syndromic setting have been reported in literature ever since the first description of the disease by Pierre Marie (Beckers, et al. 2018; de Herder 2009). Although these first records concerned only acromegaly, by the 1990s several kindreds with familial corticotropinomas, prolactinomas and non-functioning pituitary adenomas (NFPA) were also reported (Berezin and Karasik 1995; Salti and Mufarrij 1981; Yuasa, et al. 1990). The concept of familial isolated pituitary adenomas (FIPA) emerged in the late 1990s with the publication of an initial cohort of 27 families with pituitary adenomas, including acromegaly as well as other secretory phenotypes, in the absence of signs and symptoms of MEN1 and CNC (Beckers 2004; Valdes Socin, et al. 2000; Verloes, et al. 1999). Currently, FIPA is defined as the presence of pituitary adenomas of any type in at least two related members of the same family in the absence of clinical and genetic evidence of other known syndromic diseases ((Daly, et al. 2006a); Table 1)). It is considered to account for approximately 2 % of all clinically relevant pituitary adenomas and up to 3.8 % when considering only hormonally active tumors (Daly et al. 2006a; Marques, et al. 2017). Genealogical data indicate that FIPA is inherited in autosomal dominant manner with variable penetrance that can reach 33 % in some kindreds (Daly et al. 2006a; Daly, et al. 2007; Naves, et al. 2007). Depending on the functional type of the adenoma, FIPA can be divided into homogeneous – when pituitary tumors of the same type are present in all affected family members, and heterogeneous – with different pituitary adenomas within the kindred. Compared to sporadic pituitary adenomas, FIPA patients are diagnosed significantly earlier (on average 4 years). In multigenerational families descendants are diagnosed at considerably younger age than their parents or grandparents, especially in homogeneous FIPA (Daly et al. 2006a). In the overall cohort of FIPA, prolactinomas are the most frequent secretory subtype (37.5%) (Beckers, et al. 2013). When prolactinomas occur in heterogeneous FIPA, they present with more aggressive characteristics, like higher rates of suprasellar expansion and invasion of the cavernous sinus. Somatotropinomas are the second most common pituitary tumors in FIPA accounting for 35 % of FIPA and somatoprolactinomas are observed in another 6.4 % of

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cases. They are almost equally divided between homogeneous and heterogeneous families but, unlike FIPA prolactinomas, GH-secreting adenomas are more aggressive when occurring in a homogeneous setting. In homogeneous FIPA, acromegaly can be diagnosed up to 10 years earlier with tumors more frequently displaying extrasellar growth as compared to heterogeneous kindreds and sporadic populations (Beckers et al. 2013). Non-secreting adenomas occur predominantly in heterogeneous families and account for 14.5 % of FIPA patients. They are diagnosed earlier and are more frequently invasive than their sporadic counterparts. Corticotropinomas, gonadotropinomas and thyrotropinomas are rare in FIPA (2.9%; 2.0%, 0.5 % respectively). They are usually associated with other adenoma types in heterogeneous kindreds (Beckers et al. 2013).

Molecular genetics of FIPA

Original linkage studies in familial somatotropinomas detected loss of heterozygosity on chromosome 11q13 in a region different to the MEN 1 locus (Gadelha, et al. 1999; Yamada, et al. 1997). Genetic analyses in this genomic region in large Finnish kindreds with heterogeneous FIPA identified inactivating mutation in the gene for *aryl hydrocarbon receptor interacting protein* (*AIP*) on chromosome 11q (Vierimaa, et al. 2006). Its causative role in FIPA was later confirmed in other affected families and by now over 100 different mutations of *AIP* have been described (Beckers et al. 2013; Daly, et al. 2010; Hernandez-Ramirez, et al. 2015; Pepe, et al. 2019).

AIP is a tumor suppressor gene but the exact molecular mechanisms leading to pituitary tumorigenesis have not been completely elucidated. It is widely expressed in various tissues and in normal pituitary it is associated with secretory granules in somatotrope and lactotrope cells (Leontiou, et al. 2008). Homozygous $AIP^{-/-}$ knockout mice develop lethal cardiovascular defects early in the embryonic period suggesting that AIP plays a role in cardiovascular development (Lin, et al. 2007). Heterozygous $AIP^{+/-}$ animals, however, develop a phenotype that is very similar to human pituitary disease with the majority of the mice presenting with aggressive somatotropinomas (Raitila, et al. 2010), although some variability exists in relation to older age at onset in mice versus humans (Lecoq, et al. 2016).

The *AIP* gene consists of six exons and encodes a 37kDa cytoplasmic protein of 330 amino-acid residues with highly conserved sequence among different species (Trivellin and Korbonits 2011). Its N-terminal end displays significant homology with immunophilins but appears not to possess such functional activity. The carboxy terminal half of the protein contains three tetratricopeptide repeats (TPR) and a terminal α7-helix which mediate numerous protein-protein interactions (Morgan, et al. 2012). AIP was initially described as negative regulator of X antigen of hepatitis B virus (Kuzhandaivelu, et al. 1996). Furthermore, it was shown to interact also with Epstein-Barr virus-encoded nuclear antigen 3 (EBNA-3) suggesting that AIP may play a role in virus induced pathology (Kashuba, et al. 2006). One of the most thoroughly characterized interactive partners of AIP is the aryl hydrocarbon receptor (AhR). It belongs to the family of ligand activated transcription factors and modulates cellular responses to various xenobiotic toxins, such as dioxins, as well as some endogenous compounds such as cAMP (Trivellin and Korbonits 2011). In the absence of ligands AhR binds to two molecules of the 90kDa heat shock protein (HSP90) and the co-chaperones AIP and p23 to form a multiprotein complex in the cytoplasm. The activation of the complex by its xenobiotic ligand leads to dissociation of the HSP90 dimer and conformational change of AhR that exposes its nuclear localization sequence. In the subsequent nuclear translocation, the complex AhR-AIP binds to the aryl hydrocarbon receptor nuclear translocator (ARNT), also known as HIF1β, and promotes the transcription of specific genes coding various drug metabolizing enzymes (Ramadoss and Perdew 2005). Ligand activated AhR may also modulate the activity of other transcription factors such as estrogen and androgen receptors (Beckers et al. 2013; Trivellin and Korbonits 2011). AIP has been shown to maintain the stability of the complex by protecting AhR from ubiquitin-dependant degradation (Morales and Perdew 2007). AhR itself is constitutively active and it was recently shown that AhR may act as tumor suppressor in the pituitary even in the absence of exogenous ligands (Formosa, et al. 2017). Reduced AIP expression in pituitary adenomas that are positive for *AIP* mutations is associated with decreased AhR activity, suggesting an inhibitory function of AhR in pituitary tumorigenesis (Jaffrain-Rea, et al. 2009). Furthermore, AIP overexpression in cell cultures -including pituitary cell lines- slows down cell proliferation rates (Leontiou et al. 2008). Apart from stabilizing the AhR complex, AIP may also be

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involved in several other nuclear receptor pathways by being able to bind to the peroxisome proliferator-activated receptor α, the glucocorticoid receptor, and β-thyroid hormone receptor 1 (Beckers et al. 2013). AIP can also interact with the transcript of the RET protooncogene - a tyrosine kinase receptor that is involved in cell growth and regulation of apoptosis. The domain responsible for the pro-apoptotic activity is the same as that responsible for the AIP interaction (Vargiolu, et al. 2009). This RET-AIP binding presumably prevents the formation of a complex between AIP and survivin - an inhibitor of apoptosis and cell cycle regulator. In the absence of AIP, survivin is subject to proteosomal degradation with consequent increase in apoptosis (Kang and Altieri 2006). However, this interplay of AIP, RET and survivin probably does not have a role in pituitary tumorigenesis. AIP has been shown to participate in the transfer of mitochondrial preproteins from the cytosol by interacting with the translocator of outer mitochondrial membrane 20 (TOMM20) (Yano, et al. 2003). The list of interacting partners of AIP has recently been updated with some cytoskeletal proteins, especially TUBB and TUBB2A, and it may be suggested that loss of AIP impairs the organisation of the cytoskeleton and contributes to the more invasive nature of *AIP*-mutant pituitary adenomas (Hernandez-Ramirez, et al. 2018a).

One of the most attractive links between *AIP* mutations and pituitary tumorigenesis is the interaction with the cAMP-dependent signaling pathway. Responsiveness to hypothalamic, peripheral and paracrine ligands is mediated through either stimulatory or inhibitory G-protein coupled receptors which use cAMP as second intracellular messenger. In the pituitary, the activation of the cAMP pathway stimulates cell proliferation and hormonal secretion (Hernandez-Ramirez, et al. 2018b). In pituitary cell lines in which *AIP* is knocked down, there is an increase in stimulated cAMP levels (Formosa, et al. 2013). Loss of AIP has been shown to affect inhibitory G-protein function as *AIP*-mutant pituitary tumors are associated with reduced expression of $G\alpha_{i-2}$ (Tuominen, et al. 2015). In the anterior pituitary, $G\alpha_i$ proteins mediate somatostatin-related inhibition of GH and prolactin secretion through somatostatin receptors. Thus, a defect $G\alpha_{i-2}$ function could explain the resistance to somatostatin analogues that is usually observed in *AIP*-mutant somatotropinomas (Daly et al. 2010). It has recently been demonstrated that AIP physically

interacts with both the catalytic (PRKACA) and the regulatory (PRKAR1A) subunits of PKA and likely regulates its downstream effects (Schernthaner-Reiter, et al. 2018). Another cAMP pathway interaction of AIP occurs at the level of phosphodiesterases – the enzymes that inactivate cAMP. It has recently been shown that *AIP*-mutant somatotropinomas exhibit reduced expression of PDE4A4 and PDE4A8 possibly resulting in enhanced cAMP signaling (Bizzi, et al. 2018).

Despite the large and increasing number of molecular interactions of AIP with different regulatory and functional cascades, none has been proven critical for pituitary tumor development to date. Although AIP is ubiquitously expressed in the body, no other tumor types except pituitary adenomas have been consistently associated with *AIP* mutations suggesting the presence of heavily pituitary-specific tumorigenic pathways. Also, somatic *AIP* mutations are not a major cause of pituitary tumorigenesis (Beckers et al. 2013). Almost 70 % of mutations affect the C-terminal end of the protein. Nonsense and frameshift mutations lead to premature stop codons with a resulting truncated protein. A genotype-phenotype correlation has been recently suggested as patients with truncating mutations may develop pituitary disease at younger age than patients with non-truncating mutations (Hernandez-Ramirez et al. 2015). Pathogenic missense mutations alter protein stability and lead to rapid proteosomal degradation with a significantly reduced half-life of the mutated protein. Moreover, a direct correlation between half-life of mutant AIP and age at diagnosis has been documented in patients (Hernandez-Ramirez, et al. 2016). N-terminal mutations have also been proven pathogenic probably by reducing the ability to inhibit cAMP signalling (Formosa and Vassallo 2017). Apart from point mutations and small insertions and deletions, in minority of cases larger genomic alterations like exon or whole gene deletions may be present. Such genomic rearrangements cannot be detected by direct sequencing and require the use of multiple ligation-dependant probe amplification (MLPA) (Beckers et al. 2013; Georgitsi, et al. 2008).

Clinical implications of *AIP* mutations

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AIP mutations explain the pathology of about 20% of FIPA families (Beckers et al. 2013) and they are associated with some specific clinical characteristics that differentiate them from patients with wild type *AIP* (Table 1). Somatotropinomas are the most frequent secretory phenotype representing more than 70% of all tumors in *AIP*-mutated FIPA kindreds and half of them exhibit co-secretion of prolactin. In total, acromegaly and prolactinoma families make up almost 90 % of all cases, the rest are mainly NFPA. Single cases of thyrotropinomas and Cushing's disease have also been described in association with *AIP* mutations (Beckers et al. 2013). *AIP*-mutation related somatotropinomas develop at significantly younger age and experience a more aggressive evolution that their non-mutated counterparts. Mutated tumors are also significantly larger and they are often already macroadenomas with invasive characteristics and frequent extrasellar expansion at presentation (Daly et al. 2010). Due to the young onset of disease, 29-30% of *AIP* positive GH-secreting adenomas manifest clinically with gigantism (Daly et al. 2010; Rostomyan, et al. 2015). Control of GH excess and tumor growth is also difficult to achieve and maintain in *AIP* mutation carriers because of poorer responsiveness to first generation somatostatin analogues in these patients. In the long term, *AIP* mutant tumors often require multiple surgeries and radiotherapy (Daly et al. 2010). A recent report, however, indicates that pasireotide, a second-generation somatostatin analogue, may be effective in providing hormonal control and tumor shrinkage in AIP-positive acromegaly patients (Daly, et al. 2019b). *AIP*–mutated prolactinomas also present with large tumors size and invasive features. Resistance to dopamine agonists may be observed in 50 % of them, resulting in the need for surgery and/or radiotherapy (Daly et al. 2010). Due to rapid growth, *AIP* mutation related pituitary adenomas can undergo apoplexy (Figure 1), so there should be particular vigilance for *AIP* mutations among young pituitary apoplexy patients or in those with a familial history of pituitary apoplexy (Villa, et al. 2011; Xekouki, et al. 2013).

Screening for AIP mutations

The identification of *AIP* mutations may be beneficial for patients and their relatives by potentially providing early diagnosis and higher likelihood of successful treatment, although no formal outcome studies of screening have been performed. Currently, genetic screening in unselected pituitary adenoma patients is

not justified because the prevalence of *AIP* mutations in such population is very low – 0-4 % (Barlier, et al. 2007; Cazabat, et al. 2012). However, focused screening may be recommended in some specific high-risk groups (Figure 2). The highest probability of *AIP* mutation is in patients with pituitary gigantism where such genetic alterations are found in 29 % (Rostomyan et al. 2015). *AIP* mutations are present in almost 12 % of patients younger than 30 years with early onset large macroadenomas (Tichomirowa, et al. 2011) and more than 20% in pediatric patients with macroadenomas (Stratakis, et al. 2010) making these populations good candidates for genetic screening. FIPA patients represent another readily identifiable group in which testing for *AIP* mutations is warranted, as they are present in about 20% of kindreds. Development of risk stratification models to inform genetic testing guidelines remains a work in progress, and the most informative criteria continue to be those identified above (i.e. gigantism, young onset, FIPA kindreds, large macroadenomas). A recent study suggests that somatostatin analog resistance is not a helpful additional criterion to identify potential *AIP* mutation-related acromegaly cases (Daly, et al. 2019a).

There are no guidelines for the management of *AIP* mutation positive and familial pituitary adenomas and their treatment largely follows the current guidelines for their sporadic counterparts in terms of indications and therapeutic approaches. Detailed physical examination for extra-pituitary involvement and comprehensive family history should be initially performed and any suspicion for syndromic conditions such as MEN1 and Carney complex should be confirmed when considering patients and their families for genetic testing. Unaffected *AIP* mutation carriers should be offered complete clinical, biochemical and MRI evaluation at baseline and regular endocrinological surveillance after that. More challenging is the situation in FIPA families without identifiable *AIP* mutations. In such cases it may be appropriate to inform unaffected subjects about signs and symptoms of pituitary disease and encourage them to seek endocrinological consultation if such symptomatology occurs. The common presence of pituitary gigantism in patients with *AIP* mutations means that particular attention needs to be paid to pediatric mutation carriers. Early diagnosis and a decreased delay before effective treatment have been shown to

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improve final height in patients with pituitary gigantism (Rostomyan et al. 2015) and efficient management of *AIP* mutation carriers with incipient somatotropinomas could help to limit long term overgrowth.

GPR101 and FIPA

We recently described a novel syndrome of early childhood onset pituitary gigantism due to chromosome Xq26.3 duplications called X-linked acrogigantism (X-LAG) (Beckers, et al. 2015; Trivellin, et al. 2014). Patients with X-LAG are typically born at normal size following unremarkable pregnancies, but over the first 12-36 months of life develop rapid increases in length and weight due to GH/IGF-1 hypersecretion from pituitary somatotrope-somatomammotrope macroadenomas and/or hyperplasia (Beckers et al. 2015; Trivellin et al. 2014). Growth hormone releasing hormone (GHRH) excess in some cases studied indicates that X-LAG may be a multi-level disease involving both the hypothalamus and anterior pituitary (Daly, et al. 2016a). Treatment of the pituitary tumor in X-LAG is complex due to the young age of affected children and relatively large tumor size. In addition, first-line medical therapy with somatostatin analogs like octreotide and lanreotide is usually ineffective to control GH/IGF-1 excess and height gain. The GH receptor antagonist, pegvisomant, has proven effective in IGF-1 and growth control in X-LAG cases. The chromosome Xq26.3 duplication includes the gene *GPR101*, that encodes an orphan G-protein coupled receptor whose expression is highly elevated in tumors of X-LAG patients (Trivellin et al. 2014). This duplication usually occurs spontaneously either as a constitutive duplication or in a somatic mosaic state, the latter occurring in sporadic males with X-LAG (Daly, et al. 2016b). The chromosome Xq26.3 duplication involving *GPR101* can also be inherited in an X-linked dominant manner, and all carriers of the duplication are affected by pituitary gigantism. To date, three FIPA kindreds have been described in which the underlying cause of isolated somatotropinomas was proven to be X-LAG. These all involved transmission of the Xq26.3 duplication from affected mother to affected son (two sons in one kindred and one son each in the other two kindreds). In two of the families, pregnancy occurred following assisted reproduction techniques that were necessary due to extensive pituitary tumor surgery and secondary

gonadotropin deficiency. In one familial case the Xq26.3 duplication was diagnosed prenatally on chorionic villus sampling and led to a perinatal diagnosis of a pituitary adenoma secreting high levels of GH and prolactin within the first month of life (Gordon, et al. 2016; Wise-Oringer, et al. 2019). Further details about *GPR101* and X-LAG are discussed by Trivellin et al in the current issue of Endocrine related Cancer (Trivellin et al. 2020).

Conclusions

The definition of FIPA almost 20 years ago and the discovery of *AIP* as its causative gene represent important advances in our expanding knowledge about the genetic background of pituitary adenoma development. The more recent description of X-LAG due to *GPR101* duplications provides a novel mechanism by which families with pituitary gigantism can rarely present in the FIPA setting. However, much remains to be explored in FIPA as in almost 80 % of FIPA kindreds the genetic causes remain unknown. Besides the wide spectrum of functions that have been ascribed to *AIP* the precise molecular pathways that lead to pituitary tumorigenesis are yet to be fully explained. Consensus guidelines for genetic testing, management and follow-up of FIPA patients are still to emerge, and require long term monitoring studies to arrive at the most clinically relevant recommendations.

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References

Barlier A, Vanbellinghen JF, Daly AF, Silvy M, Jaffrain-Rea ML, Trouillas J, Tamagno G, Cazabat L, Bours V, Brue T, et al. 2007 Mutations in the aryl hydrocarbon receptor interacting protein gene are not highly prevalent among subjects with sporadic pituitary adenomas. *J Clin Endocrinol Metab* **92** 1952-1955.

Beckers A 2004 Familial isolated pituitary adenomas. The Ninth International Workshop on multiple endocrine neoplasia (MEN2004). *Journal of Internal Medicine* **255** 696-730.

Beckers A, Aaltonen LA, Daly AF & Karhu A 2013 Familial isolated pituitary adenomas (FIPA) and the pituitary adenoma predisposition due to mutations in the aryl hydrocarbon receptor interacting protein (AIP) gene. *Endocr Rev* **34** 239-277.

Beckers A, Lodish MB, Trivellin G, Rostomyan L, Lee M, Faucz FR, Yuan B, Choong CS, Caberg JH, Verrua E, et al. 2015 X-linked acrogigantism syndrome: clinical profile and therapeutic responses. *Endocr Relat Cancer* **22** 353-367.

Beckers A, Petrossians P, Hanson J & Daly AF 2018 The causes and consequences of pituitary gigantism. *Nat Rev Endocrinol* **14** 705-720.

Berezin M & Karasik A 1995 Familial prolactinoma. *Clin Endocrinol (Oxf)* **42** 483-486.

Bizzi MF, Pinheiro SVB, Bolger GB, Schweizer J, Giannetti AV, Dang MN, Ribeiro-Oliveira A, Jr. & Korbonits M 2018 Reduced protein expression of the phosphodiesterases PDE4A4 and PDE4A8 in AIP mutation positive somatotroph adenomas. *Mol Cell Endocrinol* **476** 103-109.

Carney JA, Gordon H, Carpenter PC, Shenoy BV & Go VL 1985 The complex of myxomas, spotty pigmentation, and endocrine overactivity. *Medicine (Baltimore)* **64** 270-283.

Casey M, Mah C, Merliss AD, Kirschner LS, Taymans SE, Denio AE, Korf B, Irvine AD, Hughes A, Carney JA, et al. 1998 Identification of a novel genetic locus for familial cardiac myxomas and Carney complex. *Circulation* **98** 2560-2566.

Cazabat L, Bouligand J, Salenave S, Bernier M, Gaillard S, Parker F, Young J, Guiochon-Mantel A & Chanson P 2012 Germline AIP mutations in apparently sporadic pituitary adenomas: prevalence in a prospective single-center cohort of 443 patients. *J Clin Endocrinol Metab* **97** E663-670.

Chandrasekharappa SC, Guru SC, Manickam P, Olufemi SE, Collins FS, Emmert-Buck MR, Debelenko LV, Zhuang Z, Lubensky IA, Liotta LA, et al. 1997 Positional cloning of the gene for multiple endocrine neoplasia-type 1. *Science* **276** 404-407.

Daly A, Cano DA, Venegas E, Petrossians P, Dios E, Castermans E, Flores-Martinez A, Bours V, Beckers A & Soto A 2019a AIP and MEN1 mutations and AIP immunohistochemistry in pituitary adenomas in a tertiary referral center. *Endocr Connect*.

Daly A, Rostomyan L, Betea D, Bonneville JF, Villa C, Pellegata NS, Waser B, Reubi JC, Waeber Stephan C, Christ E, et al. 2019b AIP-mutated acromegaly resistant to first-generation somatostatin analogs: long-term control with pasireotide LAR in two patients. *Endocr Connect*.

Daly AF, Castermans E, Oudijk L, Guitelman MA, Beckers P, Potorac I, Neggers S, Sacre N, van der Lely AJ, Bours V, et al. 2018 Pheochromocytomas and pituitary adenomas in three patients with MAX exon deletions. *Endocr Relat Cancer* **25** L37-L42.

Daly AF, Jaffrain-Rea ML, Ciccarelli A, Valdes-Socin H, Rohmer V, Tamburrano G, Borson-Chazot C, Estour B, Ciccarelli E, Brue T, et al. 2006a Clinical characterization of familial isolated pituitary adenomas. *J Clin Endocrinol Metab* **91** 3316-3323.

Daly AF, Lysy PA, Desfilles C, Rostomyan L, Mohamed A, Caberg JH, Raverot V, Castermans E, Marbaix E, Maiter D, et al. 2016a GHRH excess and blockade in X-LAG syndrome. *Endocr Relat Cancer* **23** 161-170.

Daly AF, Rixhon M, Adam C, Dempegioti A, Tichomirowa MA & Beckers A 2006b High prevalence of pituitary adenomas: a cross-sectional study in the province of Liege, Belgium. *J Clin Endocrinol Metab* **91** 4769-4775.

Daly AF, Tichomirowa MA & Beckers A 2009 The epidemiology and genetics of pituitary adenomas. *Best Pract Res Clin Endocrinol Metab* **23** 543-554.

Daly AF, Tichomirowa MA, Petrossians P, Heliovaara E, Jaffrain-Rea ML, Barlier A, Naves LA, Ebeling T, Karhu A, Raappana A, et al. 2010 Clinical Characteristics and Therapeutic Responses in Patients with Germ-Line AIP Mutations and Pituitary Adenomas: An International Collaborative Study. *J Clin Endocrinol Metab* **95** E373-383.

Daly AF, Vanbellinghen JF, Khoo SK, Jaffrain-Rea ML, Naves LA, Guitelman MA, Murat A, Emy P, Gimenez-Roqueplo AP, Tamburrano G, et al. 2007 Aryl hydrocarbon receptor-interacting protein gene mutations in familial isolated pituitary adenomas: analysis in 73 families. *J Clin Endocrinol Metab* **92** 1891-1896.

Daly AF, Yuan B, Fina F, Caberg JH, Trivellin G, Rostomyan L, de Herder WW, Naves LA, Metzger D, Cuny T, et al. 2016b Somatic mosaicism underlies X-linked acrogigantism syndrome in sporadic male subjects. *Endocr Relat Cancer* **23** 221-233.

de Herder WW 2009 Acromegaly and gigantism in the medical literature. Case descriptions in the era before and the early years after the initial publication of Pierre Marie (1886). *Pituitary* **12** 236-244.

Ezzat S, Asa SL, Couldwell WT, Barr CE, Dodge WE, Vance ML & McCutcheon IE 2004 The prevalence of pituitary adenomas: a systematic review. *Cancer* **101** 613-619.

Formosa R, Borg J & Vassallo J 2017 Aryl hydrocarbon receptor (AHR) is a potential tumour suppressor in pituitary adenomas. *Endocr Relat Cancer* **24** 445-457.

Formosa R & Vassallo J 2017 Aryl Hydrocarbon Receptor-Interacting Protein (AIP) N-Terminus Gene Mutations Identified in Pituitary Adenoma Patients Alter Protein Stability and Function. *Horm Cancer* **8** 174-184.

Formosa R, Xuereb-Anastasi A & Vassallo J 2013 Aip regulates cAMP signalling and GH secretion in GH3 cells. *Endocr Relat Cancer* **20** 495-505.

Freda PU, Beckers AM, Katznelson L, Molitch ME, Montori VM, Post KD & Vance ML 2011 Pituitary incidentaloma: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab* **96** 894-904.

Gadelha MR, Prezant TR, Une KN, Glick RP, Moskal SF, 2nd, Vaisman M, Melmed S, Kineman RD & Frohman LA 1999 Loss of heterozygosity on chromosome 11q13 in two families with acromegaly/gigantism is independent of mutations of the multiple endocrine neoplasia type I gene. *J Clin Endocrinol Metab* **84** 249-256.

Georgitsi M, Heliovaara E, Paschke R, Kumar AV, Tischkowitz M, Vierimaa O, Salmela P, Sane T, De Menis E, Cannavo S, et al. 2008 Large genomic deletions in AIP in pituitary adenoma predisposition. *J Clin Endocrinol Metab* **93** 4146-4151.

Gordon RJ, Bell J, Chung WK, David R, Oberfield SE & Wardlaw SL 2016 Childhood acromegaly due to X-linked acrogigantism: long term follow-up. *Pituitary* **19** 560-564.

Hernandez-Ramirez LC, Gabrovska P, Denes J, Stals K, Trivellin G, Tilley D, Ferrau F, Evanson J, Ellard S, Grossman AB, et al. 2015 Landscape of Familial Isolated and Young-Onset Pituitary Adenomas: Prospective Diagnosis in AIP Mutation Carriers. *J Clin Endocrinol Metab* **100** E1242-1254.

Hernandez-Ramirez LC, Martucci F, Morgan RM, Trivellin G, Tilley D, Ramos-Guajardo N, Iacovazzo D, D'Acquisto F, Prodromou C & Korbonits M 2016 Rapid Proteasomal Degradation of Mutant Proteins Is the Primary Mechanism Leading to Tumorigenesis in Patients With Missense AIP Mutations. *J Clin Endocrinol Metab* **101** 3144-3154.

Hernandez-Ramirez LC, Morgan RML, Barry S, D'Acquisto F, Prodromou C & Korbonits M 2018a Multi-chaperone function modulation and association with cytoskeletal proteins are key features of the function of AIP in the pituitary gland. *Oncotarget* **9** 9177-9198.

Hernandez-Ramirez LC, Trivellin G & Stratakis CA 2018b Cyclic 3',5'-adenosine monophosphate (cAMP) signaling in the anterior pituitary gland in health and disease. *Mol Cell Endocrinol* **463** 72-86.

Jaffrain-Rea ML, Angelini M, Gargano D, Tichomirowa MA, Daly AF, Vanbellinghen JF, D'Innocenzo E, Barlier A, Giangaspero F, Esposito V, et al. 2009 Expression of aryl hydrocarbon receptor (AHR) and AHR-

Accepted Manuscript published as ERC-20-0015. Accepted for publication: 18-Feb-2020

interacting protein in pituitary adenomas: pathological and clinical implications. *Endocr Relat Cancer* **16** 1029-1043.
Kang BH & Altieri DC 2006 Regulation of survivin stability by the aryl hydrocarbon receptor-interacting protein. *J Biol Chem* **281** 24721-24727.
Kashuba EV, Gradin K, Isaguliants M, Szekely L, Poellinger L, Klein G & Kazlauskas A 2006 Regulation of transactivation function of the aryl hydrocarbon receptor by the Epstein-Barr virus-encoded EBNA-3 protein. *J Biol Chem* **281** 1215-1223.
Kuzhandaivelu N, Cong YS, Inouye C, Yang WM & Seto E 1996 XAP2, a novel hepatitis B virus X-associated protein that inhibits X transactivation. *Nucleic Acids Res* **24** 4741-4750.
Lecoq AL, Zizzari P, Hage M, Decourtye L, Adam C, Viengchareun S, Veldhuis JD, Geoffroy V, Lombes M, Tolle V, et al. 2016 Mild pituitary phenotype in 3- and 12-month-old Aip-deficient male mice. *J Endocrinol* **231** 59-69.
Leontiou CA, Gueorguiev M, van der Spuy J, Quinton R, Lolli F, Hassan S, Chahal HS, Igreja SC, Jordan S, Rowe J, et al. 2008 The role of the aryl hydrocarbon receptor-interacting protein gene in familial and sporadic pituitary adenomas. *J Clin Endocrinol Metab* **93** 2390-2401.
Lin BC, Sullivan R, Lee Y, Moran S, Glover E & Bradfield CA 2007 Deletion of the aryl hydrocarbon receptor-associated protein 9 leads to cardiac malformation and embryonic lethality. *J Biol Chem* **282** 35924-35932.
Marques NV, Kasuki L, Coelho MC, Lima CHA, Wildemberg LE & Gadelha MR 2017 Frequency of familial pituitary adenoma syndromes among patients with functioning pituitary adenomas in a reference outpatient clinic. *Journal of Endocrinological Investigation* **40** 1381-1387.
Molitch ME 2009 Pituitary tumours: pituitary incidentalomas. *Best Pract Res Clin Endocrinol Metab* **23** 667-675.
Morales JL & Perdew GH 2007 Carboxyl terminus of hsc70-interacting protein (CHIP) can remodel mature aryl hydrocarbon receptor (AhR) complexes and mediate ubiquitination of both the AhR and the 90 kDa heat-shock protein (hsp90) in vitro. *Biochemistry* **46** 610-621.
Morgan RM, Hernandez-Ramirez LC, Trivellin G, Zhou L, Roe SM, Korbonits M & Prodromou C 2012 Structure of the TPR domain of AIP: lack of client protein interaction with the C-terminal alpha-7 helix of the TPR domain of AIP is sufficient for pituitary adenoma predisposition. *PLoS One* **7** e53339.
Naves LA, Daly AF, Vanbellinghen JF, Casulari LA, Spilioti C, Magalhaes AV, Azevedo MF, Giacomini LA, Nascimento PP, Nunes RO, et al. 2007 Variable pathological and clinical features of a large Brazilian family harboring a mutation in the aryl hydrocarbon receptor-interacting protein gene. *Eur J Endocrinol* **157** 383-391.
Pellegata NS, Quintanilla-Martinez L, Siggelkow H, Samson E, Bink K, Hofler H, Fend F, Graw J & Atkinson MJ 2006 Germ-line mutations in p27Kip1 cause a multiple endocrine neoplasia syndrome in rats and humans. *Proc Natl Acad Sci U S A* **103** 15558-15563.
Pepe S, Korbonits M & Iacovazzo D 2019 Germline and mosaic mutations causing pituitary tumours: genetic and molecular aspects. *J Endocrinol* **240** R21-R45.
Raitila A, Lehtonen HJ, Arola J, Heliovaara E, Ahlsten M, Georgitsi M, Jalanko A, Paetau A, Aaltonen LA & Karhu A 2010 Mice with inactivation of aryl hydrocarbon receptor-interacting protein (Aip) display complete penetrance of pituitary adenomas with aberrant ARNT expression. *Am J Pathol* **177** 1969-1976.
Ramadoss P & Perdew GH 2005 The transactivation domain of the Ah receptor is a key determinant of cellular localization and ligand-independent nucleocytoplasmic shuttling properties. *Biochemistry* **44** 11148-11159.
Rostomyan L, Daly AF, Petrossians P, Nachev E, Lila AR, Lecoq AL, Lecumberri B, Trivellin G, Salvatori R, Moraitis AG, et al. 2015 Clinical and genetic characterization of pituitary gigantism: an international collaborative study in 208 patients. *Endocr Relat Cancer* **22** 745-757.
Salti IS & Mufarrij IS 1981 Familial Cushing Disease. *Am J Med Genet* **8** 91-94.

Scheithauer BW, Laws ER, Jr., Kovacs K, Horvath E, Randall RV & Carney JA 1987 Pituitary adenomas of the multiple endocrine neoplasia type I syndrome. *Semin Diagn Pathol* **4** 205-211.

Schernthaner-Reiter MH, Trivellin G & Stratakis CA 2018 Interaction of AIP with protein kinase A (cAMP-dependent protein kinase). *Hum Mol Genet*.

Stratakis CA, Tichomirowa MA, Boikos S, Azevedo MF, Lodish M, Martari M, Verma S, Daly AF, Raygada M, Keil MF, et al. 2010 The role of germline AIP, MEN1, PRKAR1A, CDKN1B and CDKN2C mutations in causing pituitary adenomas in a large cohort of children, adolescents, and patients with genetic syndromes. *Clin Genet* **78** 457-463.

Tichomirowa MA, Barlier A, Daly AF, Jaffrain-Rea ML, Ronchi C, Yaneva M, Urban JD, Petrossians P, Elenkova A, Tabarin A, et al. 2011 High prevalence of AIP gene mutations following focused screening in young patients with sporadic pituitary macroadenomas. *Eur J Endocrinol* **165** 509-515.

Trivellin G, Daly AF, Faucz FR, Yuan B, Rostomyan L, Larco DO, Schernthaner-Reiter MH, Szarek E, Leal LF, Caberg JH, et al. 2014 Gigantism and acromegaly due to Xq26 microduplications and GPR101 mutation. *N Engl J Med* **371** 2363-2374.

Trivellin G & Korbonits M 2011 AIP and its interacting partners. *J Endocrinol* **210** 137-155.

Trivellin G, Faucz FR, Daly AF, Beckers A & Stratakis CA 2020 HEREDITARY ENDOCRINE TUMORS: CURRENT STATE-OF-THE-ART AND RESEARCH OPPORTUNITIES: GPR101, an orphan GPCR with roles in growth, puberty, and possibly appetite regulation. *Endocrine-Related Cancer* **27** [in press]. (https://doi.org/10.1530/ERC-20-0025)

Tuominen I, Heliovaara E, Raitila A, Rautiainen MR, Mehine M, Katainen R, Donner I, Aittomaki V, Lehtonen HJ, Ahlsten M, et al. 2015 AIP inactivation leads to pituitary tumorigenesis through defective Galphai-cAMP signaling. *Oncogene* **34** 1174-1184.

Valdes Socin H, Poncin J, Stevens V, Stevenaert A & Beckers A 2000 Adenomes hypophysaires familiaux isoles non lies avec la mutation somatique NEM-1. Siuvi de 27 patients. *Annales D Endocrinologie* **61** 301.

Vargiolu M, Fusco D, Kurelac I, Dirnberger D, Baumeister R, Morra I, Melcarne A, Rimondini R, Romeo G & Bonora E 2009 The tyrosine kinase receptor RET interacts in vivo with aryl hydrocarbon receptor-interacting protein to alter survivin availability. *J Clin Endocrinol Metab* **94** 2571-2578.

Verloes A, Stevenaert A, Teh BT, Petrossians P & Beckers A 1999 Familial acromegaly: case report and review of the literature. *Pituitary* **1** 273-277.

Vierimaa O, Georgitsi M, Lehtonen R, Vahteristo P, Kokko A, Raitila A, Tuppurainen K, Ebeling TM, Salmela PI, Paschke R, et al. 2006 Pituitary adenoma predisposition caused by germline mutations in the AIP gene. *Science* **312** 1228-1230.

Villa C, Lagonigro MS, Magri F, Koziak M, Jaffrain-Rea ML, Brauner R, Bouligand J, Junier MP, Di Rocco F, Sainte-Rose C, et al. 2011 Hyperplasia-adenoma sequence in pituitary tumorigenesis related to aryl hydrocarbon receptor interacting protein gene mutation. *Endocr Relat Cancer* **18** 347-356.

Wise-Oringer BK, Zanazzi GJ, Gordon RJ, Wardlaw SL, William C, Anyane-Yeboa K, Chung WK, Kohn B, Wisoff JH, David R, et al. 2019 Familial X-Linked Acrogigantism: Postnatal Outcomes and Tumor Pathology in a Prenatally Diagnosed Infant and His Mother. *J Clin Endocrinol Metab* **104** 4667-4675.

Xekouki P, Mastroyiannis SA, Avgeropoulos D, de la Luz Sierra M, Trivellin G, Gourgari EA, Lyssikatos C, Quezado M, Patronas N, Kanaka-Gantenbein C, et al. 2013 Familial pituitary apoplexy as the only presentation of a novel AIP mutation. *Endocr Relat Cancer* **20** L11-14.

Xekouki P, Szarek E, Bullova P, Giubellino A, Quezado M, Mastroyannis SA, Mastorakos P, Wassif CA, Raygada M, Rentia N, et al. 2015 Pituitary adenoma with paraganglioma/pheochromocytoma (3PAs) and succinate dehydrogenase defects in humans and mice. *J Clin Endocrinol Metab* **100** E710-719.

Yamada S, Yoshimoto K, Sano T, Takada K, Itakura M, Usui M & Teramoto A 1997 Inactivation of the tumor suppressor gene on 11q13 in brothers with familial acrogigantism without multiple endocrine neoplasia type 1. *J Clin Endocrinol Metab* **82** 239-242.

Accepted Manuscript published as ERC-20-0015. Accepted for publication: 18-Feb-2020

Yano M, Terada K & Mori M 2003 AIP is a mitochondrial import mediator that binds to both import receptor Tom20 and preproteins. *J Cell Biol* **163** 45-56.
Yuasa H, Tokito S, Nakagaki H & Kitamura K 1990 Familial pituitary adenoma--report of four cases from two unrelated families. *Neurol Med Chir (Tokyo)* **30** 1016-1019.

Legends

Figure 1. Contrast enhanced MRI images of showing pituitary macroadenomas in two sisters from an *AIP* mutation positive FIPA family. Both patients presented at a young age (12 and 17 years) with symptoms related to tumor size (headache and visual loss); and on imaging and on surgery there was evidence of incipient pituitary apoplexy. Reproduced with permission from Villa et al.(Villa et al. 2011)

Figure 2. Potential at-risk groups for pituitary adenomas related to *AIP* germline mutations. The highest prevalence of *AIP* mutations (green boxes) occurs in pituitary gigantism (29%) and FIPA (20% of kindreds). An intermediate risk (yellow boxes) occurs among pediatric pituitary adenoma patients (particularly GH secreting adenomas) and in patients with aggressive, large or invasive macroadenomas with disease/symptom onset during adolescence and early adulthood. *AIP* mutation patients with aggressively growing pituitary adenomas can develop apoplexy, so a history of symptoms or radiologic findings suggestive of apoplexy should be considered. Groups of pituitary adenoma patients that are unselected for age at onset, tumor characteristics or family history have a low level of *AIP* mutations in international studies (red box). Similarly, the presence of SSA resistance in acromegaly or DA resistance in prolactinomas alone (without other aggressive/early onset tumor features) does not help in identifying patients with *AIP* mutations (red box). DA: dopamine agonist; SSA: somatostatin analog.

Figure 1

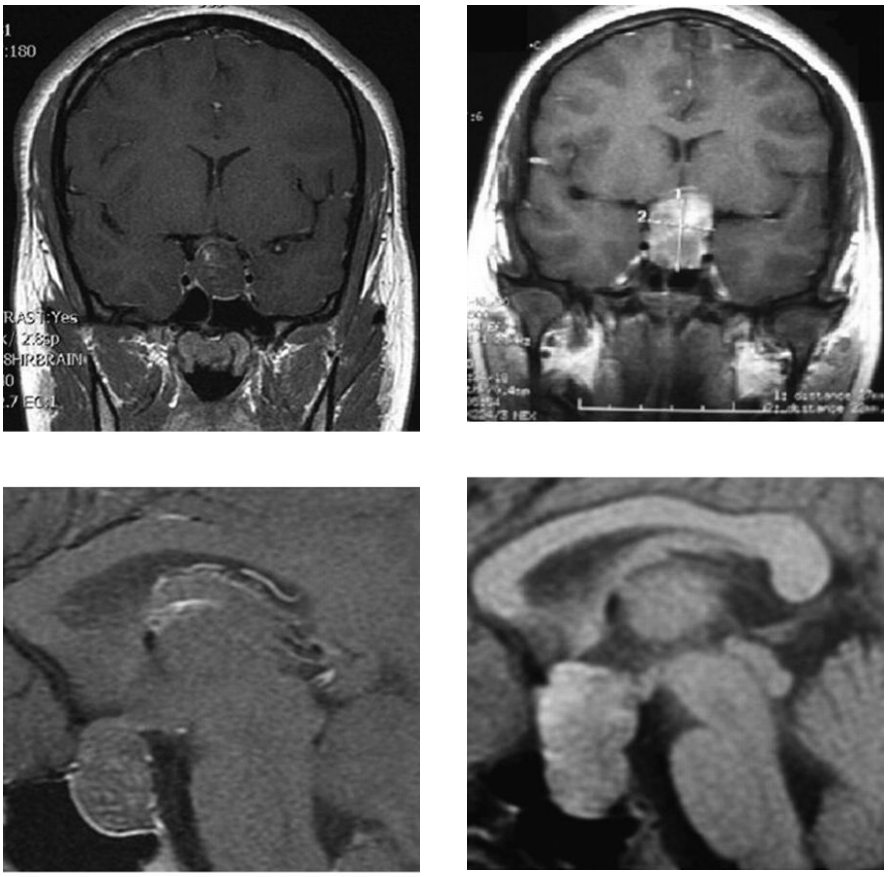

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Figure 2

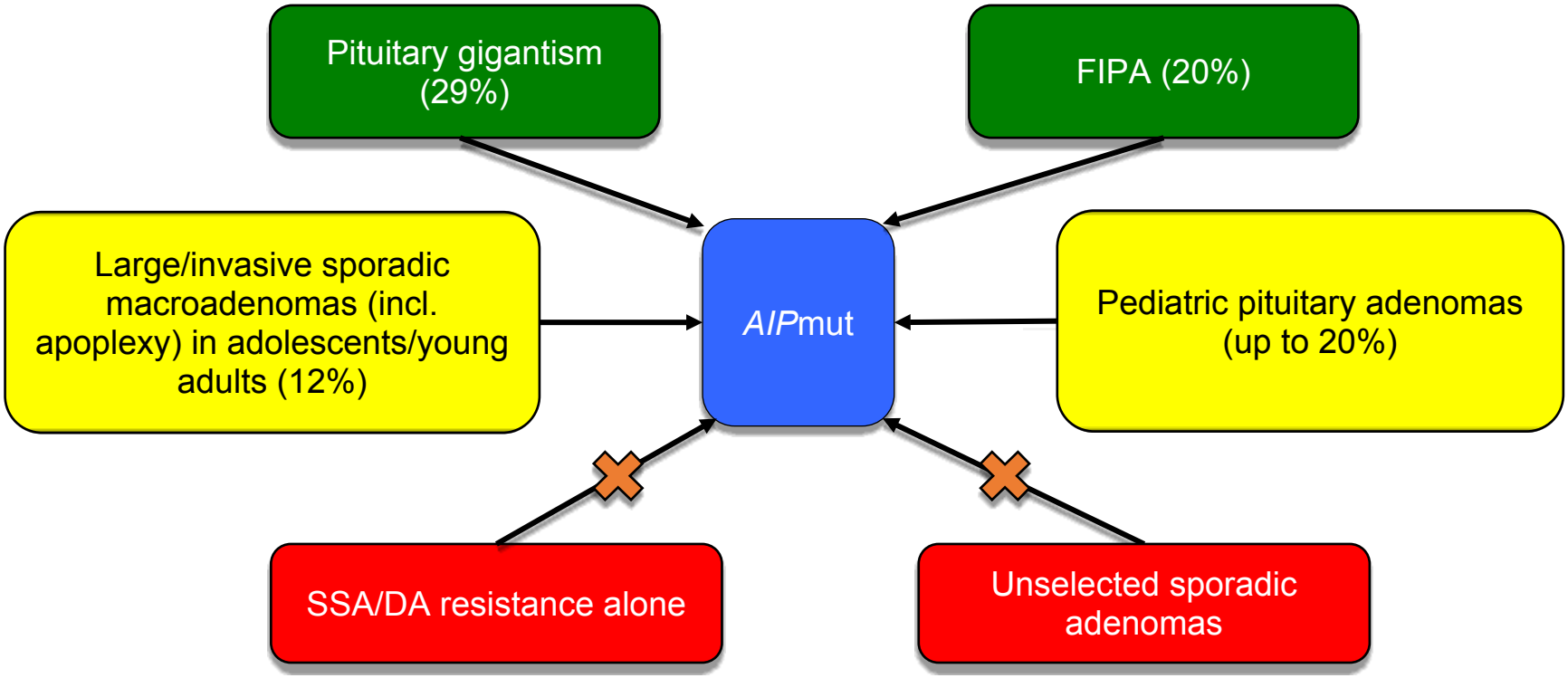

Table 1. Summary of clinical features in patients with FIPA, *AIP* mutations and *GPR101* duplications.

Familial isolated pituitary adenomas (FIPA)

- Occurs when at least two related members of the same kindred have isolated pituitary adenomas (i.e. syndromic conditions affecting other endocrine organs like *MEN1* are not present)
- Can present homogeneously (all affected members of the same kindred have the same pituitary adenoma subtype) or heterogeneously (different pituitary adenoma subtypes across the kindred).
- Generally FIPA cases have an earlier onset and have a larger tumor size than sporadic non-FIPA pituitary adenoma cases
- Genetic causes are present in about 20% of kindreds
    - *AIP* mutations
    - *GPR101* duplications (rare presentation)

***AIP* mutations**

- Autosomal dominant disease with incomplete penetrance
- About 20% of *AIP* mutation carrier will develop a pituitary adenoma
- All secretory and non-secretory pituitary adenoma subtypes can occur
    - Predominantly (>90%) somatotropinomas, mixed GH and prolactin secreting tumors and prolactinomas
- Prolactin co-secretion is common in *AIP* mutated somatotropinomas
    - Cushing's disease very rarely associated with *AIP* mutations
- Early onset tumors affecting children, adolescents, young adults (median age at diagnosis 21 years)
- Aggressive growth potential leads to large and expansive macroadenomas
- Pituitary apoplexy is a feature of sporadic and familial *AIP* mutation related pituitary adenomas
- Rare cases of whole or partial *AIP* gene deletions can be missed on genetic sequencing and require multiplex ligand specific probe amplification (MLPA) to identify
- Disease control in *AIP* mutation related acromegaly requires greater cumulative use of treatment than non-mutated acromegaly
    - Patients with acromegaly and *AIP* mutation have a decreased responsiveness to first-generation somatostatin analogs
        - There are significantly decreased GH and IGF-1 responses in *AIP* mutated acromegaly
        - Tumor shrinkage is significantly lower in *AIP* mutated acromegaly
    - Decreased somatostatin receptor subtype 2 (SST2) can occur in *AIP* mutated somatotropinomas
        - Octreotide/lanreotide resistant *AIP* mutated acromegaly can be controlled hormonally and undergo marked tumor shrinkage with to pasireotide treatment

***GPR101* duplications**

- X-linked dominant inheritance (100% penetrance)
- Familial early childhood onset pituitary acrogigantism due to chromosome Xq26.3 duplications can present as FIPA in rare cases (n=3 kindreds)

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- Inherited duplications involving *GPR101* can be transmitted from affected mother to affected son
- Lead to early onset (12-36 months of age) pituitary GH and prolactin positive macroadenomas with or without hyperplasia
- GHRH levels can be elevated (suggests a hypothalamic-pituitary pathological axis)
- Hyperprolactinemia usually present (responsive to dopamine agonists)
- Patients often require radical neurosurgery as part of a multimodal management scheme
- Somatostatin analogs are typically ineffective in controlling GH/IGF-1 secretion and overgrowth
- GH receptor antagonist (pegvisomant) can lower IGF-1 to normal range and control growth