THEMATIC REVIEW

Pituitary tumours: molecular and genetic aspects

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Abstract

'Pituitary tumours' is an umbrella term for various tumours originating from different regions of the hypothalamic–pituitary system. The vast majority of pituitary tumours are pituitary adenomas, also recently referred to as pituitary neuroendocrine tumours. The prevalence of clinically relevant pituitary adenomas is approximately 1 in 1000; other pituitary tumours such as craniopharyngioma and pituicytoma are comparatively very rare. This review addresses the molecular and genetic aspects of pituitary adenomas. We first discuss the germline genetic variants underlying familial pituitary tumours, which account for approximately 5% of all pituitary adenoma cases. This includes variants in established pituitary adenoma/hyperplasia predisposition genes (*MEN1*, *PRKAR1A*, *AIP*, *CDKN1B*, *GPR101*, *SDHA*, *SDHB*, *SDHC*, *SDHD*, *SDHAF2*) as well as emerging genetic associations. In addition, we discuss McCune–Albright syndrome which lies between the germline and somatic pituitary tumour genes as the causative *GNAS* mutations are postzygotic rather than being inherited, and the condition is associated with multiglandular features due to the involvement of different cell lines rather than being limited to the pituitary. By contrast, somatic *GNAS* mutations contribute to sporadic acromegaly. *USP8* is the only other gene where somatic driver mutations have been established in sporadic pituitary tumorigenesis. However, there are now known to be a variety of other somatic genetic and molecular changes underpinning sporadic pituitary adenomas which we review here, namely: copy number variation, molecular changes in signalling and hypoxia pathways, epithelial–mesenchymal transition, DNA repair, senescence, the immune microenvironment and epigenetics.

Key Words

- pituitary adenomas
- familial pituitary tumours
- sporadic pituitary tumours
- genetics

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Introduction

The pituitary gland, residing in the bony sella turcica, is composed of the anterior lobe (adenohypophysis) derived from oral ectoderm and the posterior lobe (neurohypophysis) derived from neuroectoderm. The anterior lobe contains five types of hormone-producing neuroendocrine cells: somatotrophs, lactotrophs, gonadotrophs, corticotrophs and thyrotrophs. Several other cell types are scattered through the pituitary: folliculostellate cells, follicular cells, marginal zone cells, degranulated hormonal cells, mesenchymal cells, endothelial cells and immune cells (Vankelecom 2007). The posterior lobe contains pituicytes, a type of glial cell, and axonal extensions from hypothalamic neurons that secrete oxytocin and vasopressin directly into the peripheral circulation (Pepe *et al.* 2019, Asa *et al.* 2022).

The latest World Health Organization (WHO) classification of tumours relating to the pituitary gland is as follows (Asa *et al.* 2022):
Anterior lobe tumours:

- Pituitary neuroendocrine tumour (also known as pituitary adenoma)
- Pituitary blastoma
- Craniopharyngioma

Posterior lobe tumours:

- Oncocytic pituicytoma (formerly, spindle cell oncocytoma)
- Granular cell pituicytoma (formerly, granular cell tumour)
- Ependymal pituicytoma (formerly, sellar ependymoma)

Hypothalamic tumours:

- Gangliocytoma
- Neurocytoma

The 2022 WHO classification refers to tumours of anterior pituitary origin as pituitary neuroendocrine tumours, whereas most of the existing literature uses the terms 'pituitary adenoma' (PA), 'aggressive pituitary tumour' (APT) and 'pituitary carcinoma' (PC) as employed in this review. We focus on the molecular and genetic aspects of PAs, which account for the vast majority of pituitary tumours. We do not cover the roles of genes in other pituitary tumours, such as *DICER1* in pituitary blastoma, *CTNNB1* in adamantinomatous craniopharyngioma and *BRAF* in papillary craniopharyngioma (De Sousa *et al.* 2018).

In this review, we first expand on the classification of pituitary tumours, which is integral to understanding the genetic and molecular pathogenesis of PAs. We then discuss familial pituitary tumours which relate to heritable germline defects in PA predisposition genes, followed by the various somatic aberrations found in sporadic PAs. Citations have been selected throughout the review to showcase Australian contributions to the pituitary tumour literature in this special issue.

Classification of pituitary tumours

Pituitary tumour classification has rapidly evolved in the past few decades (Table 1), reflecting an improved understanding of underlying biological processes. In 2004, the WHO classified tumours as 'typical' or 'atypical', based on the presence or absence of two of three proliferative markers: Ki67 index, mitotic count and p53 immunostaining (Delellis 2004). The 2017 WHO classification heralded a major paradigm shift, classifying tumours based on cellular lineage, as determined by transcription factor (TF) and hormonal immunohistochemistry. TFs drive maturation from pituitary stem cells: pituitary-specific POU-class homeodomain transcription factor (PIT-1) gives rise to acidophil cells; steroidogenic factor 1 (SF1) gives rise to gonadotroph cells and T-box family member TBX19 (T-PIT) gives rise to corticotroph cells (Lloyd 2017, Asa *et al.* 2022). Although the terms 'typical' and 'atypical' were omitted from the 2017 WHO classification, the prognostic value of Ki67 and mitotic count were retained but with emphasis placed on 'higher-risk' histological types: silent corticotroph adenoma, PIT-1 positive plurihormonal adenoma, sparsely granulated somatotroph adenoma, lactotroph adenoma in men and Crooke's cell adenoma. TF analysis has been associated with the refinement of diagnostic information, with the reclassification of 12% of tumours in a cohort of 171 patients (Lenders *et al.* 2021). In the same cohort, 'higher-risk' histological types were associated with reduced progression-free survival (Lenders *et al.* 2021). The 2022 WHO classification has included an additional category of 'no distinct cell lineage' and, within this, 'plurihormonal tumours', characterised by a monomorphous population of cells with multiple TF expressions (Asa *et al.* 2022). Although recent evolution in classification has marked significant progress in the diagnostic evaluation of pituitary tumours, there remain significant challenges for clinicians and pathologists. A major shortcoming of the WHO classifications has been a failure to define clear

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Table 1 Progressive changes in the WHO classification system of pituitary tumours.

	2004	2017		2022	
IHC	Hormonal	TF and hormonal		TF and hormonal	
Diagnosis	Typical adenoma	SF1 lineage	Gonadotroph adenoma	SF1 lineage	Gonadotroph tumour
		TPIT lineage	Corticotroph adenoma	TPIT lineage	Corticotroph tumour
	Atypical adenoma (proliferative markers present)	PIT1 lineage	Lactotroph adenoma (sparsely granulated, densely granulated, ASC)	PIT1 lineage	Lactotroph tumour (sparsely granulated, densely granulated)
	Carcinoma (craniospinal or distant metastases)		Somatotroph adenoma (sparsely granulated, densely granulated, mammosomatotroph, mixed somatotroph-lactotroph)		Somatotroph tumour (sparsely granulated, densely granulated)
			Thyrotroph adenoma		Mammosomatotroph tumour[b]
			Plurihormonal adenoma (PIT1 positive plurihormonal[a], unusual combinations)		Mixed somatotroph and lactotroph tumour[b]
					Thyrotroph tumour
					Mature plurihormonal PIT1 lineage tumour[c]
					Immature PIT1 lineage tumour[c]
					Acidophil stem cell tumour[b]
		No distinct cell lineage	Null-cell adenoma	No distinct cell lineage	Null-cell tumour
					Plurihormonal tumour[c]
Proliferative markers	Ki67 ≥ 3% Mitoses > 2/10 HPF P53 immuno-positivity	Ki67 ≥ 3% Mitoses > 2/10 HPF		Ki67 ≥ 3% Mitoses > 2/10 HPF	

[a]Newly defined in 2017 WHO classification; [b]newly described as separate 'type' rather than 'subtype' in 2022 WHO classification; [c]newly defined in 2022 WHO classification.
ASC, acidophilic stem cell; IHC, immunohistochemistry; TF, transcription factor.

diagnostic cut-offs for TF immunopositivity, leaving this open to interpretation (Lenders *et al.* 2021). Expansion of diagnostic histopathology has been associated with significant increases in cost, necessitating a streamlined algorithmic approach to tumour evaluation (Asa *et al.* 2022, Lenders *et al.* 2022). Future multicentre prospective studies should continue to explore tumour biology and develop streamlined diagnostic and prognostic approaches to evaluation.

Familial pituitary tumours

'Familial pituitary tumours' is an umbrella term for various clinical syndromes characterised by a heritable predisposition to PA formation, with or without an identifiable germline genetic defect. The predisposition to tumorigenesis may be limited to the pituitary, as in familial isolated pituitary adenoma (FIPA) syndrome, which is defined by PA development in two or more related individuals within a family (Daly *et al.* 2006), or extend beyond the pituitary to involve other endocrine and possibly non-endocrine tissues. Familial pituitary tumours account for approximately 5% of all PA cases (De Sousa *et al.* 2017).

Established familial pituitary tumour genes

The genes that have been definitively associated with familial pituitary tumours are outlined in Table 2. The first established familial pituitary tumour gene was the *MEN1* gene which was identified in 1997 through positional cloning. This involved initial linkage of the clinical MEN1 syndrome (defined as ≥2 of 3 tumour sites: parathyroid, pituitary and enteropancreatic endocrine tissue) to the q13 band of the long arm of chromosome 11, followed by finer mapping through meiotic recombination and tumour loss of heterozygosity (LOH) analysis, and finally DNA sequencing of resident genes revealing causative *MEN1* variants in 14/15 probands with MEN1 syndrome (Chandrasekharappa *et al.* 1997). DNA sequencing by Burgess *et al.* of 152 members of the Tasman 1 kindred in Tasmania, Australia – representing the largest MEN1 kindred globally – subsequently demonstrated near

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Table 2 Genetic, molecular and clinical features relating to established familial pituitary tumour genes as well as the *GNAS* gene implicated in the non-inherited condition, McCune–Albright syndrome (Pepe *et al.* 2019, Abboud *et al.* 2020, Barry & Korbonits 2020, Vasilev *et al.* 2020, Garcia-Rendueles *et al.* 2021, Franke *et al.* 2022)

Gene features	Protein features	Typical germline aberration	Somatic second Hit	Corresponding syndrome	Inheritance	Pituitary phenotype	Other typical manifestations	Pituitary penetrance
AIP Chr 11q13.2 Tumour suppressor gene in the pituitary but not all other organs OMIM *605555	Aryl hydrocarbon receptor-interacting protein 330 AA Co-chaperone molecule with several interacting partners, including aryl hydrocarbon receptor, PKA subunits including PRKAR1A, and RET tyrosine kinase receptor Widely expressed but co-expression with RET only in somatotrophs, AIP deficiency blocks the RET-apoptotic pathway thereby promoting the RET-survival pathway leading to somatotrophinomas Normally only expressed by somatotrophs and lactotrophs, but staining demonstrated across PA types	Inactivating mutations Mostly nonsense and frameshift mutations, less commonly missense, splicing and promoter mutations, deletions, insertions and segmental duplications Uncommonly, large deletions (<10%)	Frequent LOH	Familial isolated pituitary adenoma (FIPA)	AD	Typically somatrophinomas (usually sparsely granulated), followed by mixed somatotroph–lactotroph adenomas and less commonly prolactinomas, other PA types uncommon PAs typically large, invasive, clinically aggressive, treatment-resistant and with greater GH hypersecretion than sporadic cases Typical onset in 2nd decade of life Increased risk of pituitary apoplexy	Nil	15–30%
CDKN1B Chr 12p13.1 Tumour suppressor gene OMIM *600778	$p27^{Kip1}$ Cyclin-dependent kinase inhibitor Regulates cell cycle by preventing transition from G1 to S phase	Inactivating mutations Mostly missense mutations, less commonly frameshift and nonsense mutations, also 5′ UTR mutations that reduce p27 expression	Lack of somatic second hits in animal models, suggesting haploinsufficiency as mechanism of tumorigenesis	Multiple endocrine neoplasia type 4 (MEN4)	AD	Observed PA types include NFPA, somatotrophinoma, prolactinoma, corticotrophinoma[a] PA is the second most common manifestation after PHPT	MEN1-like phenotype[a]	37%[a]
GNAS Also referred to as *gsp* oncogene Chr 20q13.32 Oncogene Paternally imprinted in several tissues including pituitary OMIM *139320	Gs-alpha: alpha subunit of the stimulatory guanine nucleotide-binding protein (G protein) Component of cAMP pathway associated G protein	Postzygotic activating mutations, resulting in somatic mosaicism Causative variants only identified at two residues which are critical sites for GTPase activity: Arg201, rarely Gln227 Causative variants result in loss of GTPase activity, leading to constitutive activation of adenylate cyclase and hence the cAMP-dependent PKA pathway, causing hormone hypersecretion and cell proliferation Same activating mutations found in tumour DNA of sporadic cases of somatotroph PA (up to 50%), NFPA (10%) and corticotroph PA	Not applicable[c]	McCune–Albright syndrome (MAS)	Sporadic condition, not inherited	Typically GH excess, more commonly due to pituitary hyperplasia than PA, mean age of onset 24 years Frequent prolactin co-hypersecretion	Polyostotic fibrous dysplasia; café-au-lait skin macules; peripheral precocious puberty; hyperthyroidism; Leydig and/or Sertoli cell hyperplasia; rarely neonatal hypercortisolism Variable expressivity related to degree of mosaicism and expression of the mutant allele in different tissues	20%

(Continued)

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Table 2 Continued.

Gene features	Protein features	Typical germline aberration	Somatic second Hit	Corresponding syndrome	Inheritance	Pituitary phenotype	Other typical manifestations	Pituitary penetrance
GPR101 Chr Xq26.3 TADopathy region, with Xq26.3 duplications disrupting local chromatin architecture and forming a new topologically associating domain (TAD) including several pituitary active cis-regulatory elements OMIM *300393	GPR101 protein Orphan G-protein coupled receptor Stimulatory effects on cAMP-PKA pathway and GHRH secretion Not expressed in the adult pituitary or in sporadic somatotroph PAs but expressed in fetal and adolescent pituitary glands and in X-LAG-related PAs	Germline or somatic mosaic Xq26.3 microduplications (average ~500 kb) consistently involving the *GPR101* gene and resulting in GPR101 overexpression and downstream increases in cAMP No consistent evidence for *GPR101* point mutations as a cause of PA	Not applicable[c]	X-linked acrogigantism (X-LAG)	XLD, thus far only rare cases of mother-son transmission	Typically gigantism with prolactin co-hypersecretion due to large mixed somatotroph–lactotroph adenomas +/– hyperplasia (rarely hyperplasia alone) GHRH excess also shown in some cases Marked growth acceleration and other GH-mediated effects by 2–4 years of age Characteristic histopathology with sinusoidal and lobular architecture, frequent calcifications, follicle-like structures	Nil	100%
MEN1 Chr 11q13.1 Tumour suppressor gene OMIM *613733	Menin Scaffold protein with multiple functions, including regulation of p27 expression and DNA replication and repair	Inactivating mutations distributed throughout the gene Mostly frameshift, nonsense and missense mutations, less commonly splice site mutations, in-frame deletions Rarely whole gene deletions (1%) *De novo* in 10%	LOH at 11q13 in 90% of *MEN1*-related tumours	Multiple endocrine neoplasia type 1 (MEN1)	AD	Most commonly lactotroph PA, followed by NFPA and somatotroph PA, rarely corticotroph or thyrotroph PA PAs typically macroadenomas, sometimes multiple Younger onset, larger and more aggressive than sporadic cases Female predominance PA is the presenting MEN1 manifestation in 15–30%	PHPT; gastroenteropancreatic, bronchial and thymic NETs; facial angiofibroma; lipoma; collagenoma; adrenal cortical adenoma; meningioma	30–40%
PRKAR1A Chr 17q24.2 Tissue-dependent tumour suppressor gene and oncogene functions OMIM *188830	Type 1 alpha regulatory subunit of PKA PKA is a cAMP-dependent protein kinase comprising two regulatory and two catalytic subunits Involved in cell proliferation, transcription and apoptosis	Inactivating mutations, resulting in increased PKA activity Mostly nonsense and frameshift mutations Large deletions found in 20% of CNC cases testing negative for *PRKAR1A* mutations by Sanger sequencing *De novo* in 30%	LOH observed but not invariable, indicating haploinsufficiency in some tumours	Carney complex (CNC)	AD	Typically mammosomatotroph hyperplasia, sometimes with adenomatous transformation If PA, most commonly somatotroph or mixed somatotroph–lactotroph microadenoma Biochemical GH excess in two-thirds of CNC patients, but clinical presentation with acromegaly only in 10% of CNC pts, usually by 3rd decade of life Frequent prolactin co-hypersecretion Rarely, Cushing's disease	Spotty skin and mucosal pigmentation; cutaneous, cardiac and other myxomas; schwannoma; primary pigmented nodular adrenocortical disease (leading to ACTH-independent Cushing's syndrome); large-cell calcifying Sertoli cell tumours; benign and malignant thyroid lesions; other endocrine and non-endocrine neoplasms Female predominance	10–20%
SDHx: *SDHA* (Chr 5p15.33) *SDHB* (Chr 1p36.13) *SDHC* (Chr 1q23.3) *SDHD* (Chr 11q23.1) *SDHAF2* (Chr 11q12.2) Tumour suppressor genes encoding the SDH complex	SDH subunits A, B, C, D and SDH complex assembly factor 2 protein SDH enzymatic complex consists of two catalytic units (SDHA, SDHB) and two anchoring units (SDHC, SDHD) SDH complex is bound to mitochondrial inner membrane, converts succinate into fumarate in Krebs cycle, also involved in oxidative phosphorylation	Inactivating mutations, resulting in reduced SDH activity SDH dysfunction causes succinate accumulation and increased intracellular reactive oxygen species, both causing inhibition of prolyl-hydroxylases and leading to downstream stabilisation of hypoxia-inducible factor 1 and activation of hypoxia pathways including angiogenesis	Frequently unknown, LOH has been observed	3P association syndrome (3PAs; phaeochromocytoma, paraganglioma and PA)	AD	Most commonly lactotroph PA, followed by somatotroph and NFPA Typically macroadenomas, sometimes aggressive, rarely pituitary carcinoma Hallmark histopathological feature is intracytoplasmic vacuoles caused by autophagic bodies	Phaeochromocytoma, paraganglioma	<1%[d]

[a]Based on limited data, possibility of ascertainment bias; [c]Monoallelic activating aberrations in pituitary tissue are sufficient for disease manifestation; [d]PA observed in <1% of patients with *SDHx* mutations, but this may be an underestimate given the lack of pituitary surveillance to date.

AA, amino acid; ACTH, adrenocorticotrophic hormone; AD, autosomal dominant; Arg, arginine; cAMP, cyclic adenosine monophosphate; Chr, chromosone; CNC, Carney complex; GH, growth hormone; GHRH, growth hormone releasing hormone; Gln, glutamine; GPR101, G protein-coupled receptor 101; LOH, loss of heterozygosity; MEN1, multiple endocrine neoplasia type 1; NET, neuroendocrine tumour; NFPA, non-functioning pituitary adenoma; OMIM, Online Mendelian Inheritance in Man; PA, pituitary adenoma; PHPT, primary hyperparathyroidism; PKA, protein kinase A; PRKAR1A, protein kinase A, R1-alpha subunit; RET, rearranged during transfection; SDH, succinate dehydrogenase; X-LAG, X-linked acrogigantism; XLD, X-linked dominant.

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complete segregation of a novel *MEN1* splice site mutation, c.446-3C>G, with the exception of seven family members who were deemed to be phenocopies (Burgess *et al.* 2000). The same group recently demonstrated that intrafamilial variability in *MEN1* promoter methylation accounts at least in part for the variable expressivity that is characteristic of MEN1 syndrome (De Paoli-Iseppi *et al.* 2018).

In 2000, *PRKAR1A* was discovered as the gene responsible for most cases of Carney complex via linkage of the condition with Chr 17q22-24. This was followed by tumour studies of affected individuals showing LOH in the same area, and finally, *PRKAR1A* sequencing demonstrating mutations in both familial and sporadic cases of Carney complex, including a hot spot 2-bp deletion in exon 4B (Kirschner *et al.* 2000). Although pituitary hyperplasia – and PAs to a lesser degree – are frequent in Carney complex, *PRKAR1A* appears to rarely be the cause of PAs outside of the Carney complex phenotype, with no *PRKAR1A* mutations found in a subsequent study of 61 sporadic PAs and 6 patients with a MEN1-like phenotype (Sandrini *et al.* 2002). We too found no *PRKAR1A* variants in a study of 44 patients with PA and young onset and/or other personal or family history of endocrine neoplasia (De Sousa *et al.* 2017).

AIP was established as a familial pituitary tumour gene in 2006 through linkage analysis and blood expression profiles which guided targeted *AIP* gene sequencing showing a single nonsense *AIP* mutation perfectly segregating with growth hormone (GH) hypersecretion in two affected families in Northern Finland (Vierimaa *et al.* 2006). Inactivating *AIP* mutations have since been shown to account for 15–30% of FIPA kindreds (Pepe *et al.* 2019). *AIP* mutations most closely relate to young-onset PAs and especially GH-secreting PAs, with germline mutations detectable in approximately 30% of pituitary gigantism cases, and relative treatment resistance, with reduced response to somatostatin analogues (Jennings *et al.* 2009, Joshi *et al.* 2018).

CDKN1B was also implicated in familial pituitary tumours in 2006 through a process of linkage analysis, expression profiling and DNA sequencing of MENX rats (overlapping MEN1/MEN2 phenotype) showing a *Cdkn1b* frameshift mutation, followed by identification of a nonsense *CDKN1B* variant in a woman with a personal and family history of PA that was consistent with familial MEN1 syndrome but without an identifiable *MEN1* mutation (Pellegata *et al.* 2006). The phenotype associated with *CDKN1B* variants is slowly becoming clearer, with an Australian group recently highlighting an association with prolactinoma in addition to previous reports linking *CDKN1B* variants with non-functioning, GH-secreting and ACTH-secreting PAs (Seabrook *et al.* 2022).

GPR101 and its corresponding condition, X-linked acrogigantism (X-LAG), were described in 2014 when germline Xq26.3 microduplications were found in 13/43 (30.2%) patients with gigantism using array comparative genomic hybridisation, with *GPR101* being the only gene of four resident genes to be overexpressed in X-LAG pituitary lesions (Trivellin *et al.* 2014). A later study defined the smallest region of overlap of duplications to only contain *GPR101* (Iacovazzo *et al.* 2016*b*). Putative activating *GPR101* point mutations were also originally proposed to cause sporadic adult-onset acromegaly (Trivellin *et al.* 2014); however, this was not corroborated by subsequent studies (Iacovazzo *et al.* 2016*b*).

The *SDHx* genes (*SDHA*, *SDHB*, *SDHC*, *SDHD*; and *SDHAF2* in some references) are best known for their association with paraganglioma and phaeochromocytoma. Following case reports of PA in individuals with *SDHx* defects, the *SDHx* genes were formally implicated in pituitary tumorigenesis in 2015 with the naming of the 3P (phaeochromocytoma, paraganglioma and PA) association syndrome (3PAs). *SDHx* gene sequencing demonstrated pathogenic variants in three out of four patients with familial 3PAs, whilst *Sdhb* heterozygote mice demonstrated pituitary hyperplasia, particularly of lactotrophs and somatotrophs, with increased expression of hypoxia-inducible factor-1 alpha (HIF-1α) (Xekouki *et al.* 2015). A deep intronic *SDHC* variant causing aberrant splicing has also been found in a large Australian kindred affected by all four *SDHx*-related tumours, namely: paraganglioma, PA, renal cell carcinoma and gastrointestinal stromal tumour (De Sousa *et al.* 2020). However, isolated PAs are unlikely to be the product of *SDHx* defects as the original 3PAs study found no *SDHx* mutations in 164 patients with sporadic PA, familial PA or sporadic 3PAs (Xekouki *et al.* 2015). Furthermore, Gill *et al.* previously found abnormal succinate dehydrogenase staining signifying *SDHx* mutations in only 1/309 (0.3%) PA operative specimens, and even this case was attributable to somatic biallelic *SDHA* mutations rather than a germline *SDHx* mutation (Gill *et al.* 2014). In a separate case report, a patient with an isolated macroprolactinoma was found to have a germline *SDHB* mutation without any evidence of phaeochromocytoma or paraganglioma on subsequent investigations; however, there was still a family history of paraganglioma, with her father having a metastatic spinal paraganglioma (Maher *et al.* 2018).

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Emerging familial pituitary tumour genes

Table 3 outlines other genes with emerging evidence of a relationship between germline variants and pituitary tumorigenesis; further research is required for these genes to be regarded as definitive familial pituitary tumour genes.

Genetic testing for familial pituitary tumours

The genetic heterogeneity and overlapping clinical features of familial pituitary tumours highlight the utility of simultaneous gene testing via next-generation sequencing (NGS) in the assessment of suspected familial pituitary tumours. We previously demonstrated a high yield of rare variants in familial pituitary tumour genes using a custom NGS panel. In this nationwide study, we found rare variants in familial pituitary tumour genes in 11/44 (25%) PA patients with young onset PA and/or other personal or family history of endocrine neoplasia, including two patients with dual variants which would have been missed if single- or staged-gene sequencing was used (De Sousa *et al.* 2017). Notably, this study preceded the discovery of *GPR101* and hence this gene was not included in the panel design. Using a whole-exome sequencing (WES) or whole-genome sequencing (WGS) platform with limited analysis of only currently known familial pituitary tumour genes may allow laboratories to return to the raw sequencing data of tested patients as novel familial pituitary tumour genes emerge, whilst avoiding the large burden of incidental findings potentially created by WES/WGS with complete analysis (De Sousa *et al.* 2018).

Assessment for copy number variants (CNVs) is important in genes where large deletions or duplications are a known mechanism of disease (e.g. *AIP*, *PRKAR1A*). This may be achieved by multiplex ligation-dependant probe amplification or, in some cases, by CNV-tailored bioinformatic pipelines using WES or ideally WGS data (De Sousa *et al.* 2018, Pepe *et al.* 2019).

In the case of chromosomal-level variants as in the X chromosome microduplications underlying X-LAG, the definitive diagnostic test is chromosomal microarray (for example, via array comparative genomic hybridisation). Chromosomal microarray techniques, particularly modern high-density techniques, show the approximate start and stop points of deletions/duplications and can typically indicate mosaicism if present. Testing affected tissue is another strategy if mosaicism is suspected. CNV droplet digital PCR (ddPCR) is an additive tool as the especially high resolution of this test allows detection of small deletions/duplications which may be missed by standard array comparative genomic hybridisation platforms. ddPCR can also indicate gene dosages lying between normal and deletion/duplication, thereby highlighting somatic mosaicism. However, ddPCR requires a dedicated workflow for a given deletion/duplication and does not define breakpoints (Daly *et al.* 2016, Iacovazzo *et al.* 2016*b*).

McCune–Albright syndrome

Postzygotic activating *GNAS* mutations result in McCune–Albright syndrome (MAS), characterised by polyostotic fibrous dysplasia, café-au-lait macules and endocrinopathies, including GH excess in 20% and frequent thyroid lesions (Pepe *et al.* 2019). MAS should be considered separate from the conditions causing familial pituitary tumours as MAS occurs postzygotically rather than being inherited, and it is not transmitted to offspring as germline *GNAS* activation appears incompatible with life. *GNAS* is also distinct from the somatic mutations implicated in sporadic PA formation because of the multiglandular manifestations of MAS (De Sousa *et al.* 2018).

In 1991, the recent discovery of somatic activating *GNAS* mutations in somatrophinomas and thyroid tumours prompted a search for these same mutations in MAS. Amplification of the genomic DNA regions where the activating *GNAS* mutations reside (Arg201 in exon 8, Gln227 in exon 9) followed by denaturing gradient gel electrophoresis and allele-specific oligonucleotide hybridisation demonstrated *GNAS* mutations in 4/4 MAS patients, confirming *GNAS* as the gene responsible for MAS (Weinstein *et al.* 1991). A multicentric study of American, Italian and Australian cohorts has shown that the subset of MAS patients with GH–insulin-like growth factor 1 axis hyperactivity exhibits greater fibrous dysplasia morbidity, and that timely treatment of GH excess may lessen this (Tessaris *et al.* 2018).

As MAS is a mosaic condition by definition, genetic testing in clinical practice typically involves NGS rather than Sanger sequencing in order to visualise variants at allele frequencies below 0.5 (Pepe *et al.* 2019).

Somatic aspects of pituitary adenomas

Copy number variation

Large-scale copy number variation involving chromosomal segments is the predominant somatic genetic aberration

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in sporadic PAs (Bi *et al.* 2017*b*, De Sousa *et al.* 2019). CNVs are especially frequent in functioning PAs, particularly prolactinomas, as well as PAs with high proliferative indices (Bi *et al.* 2017*b*).

In an Australian WES study of prolactinomas, recurrent copy number gains and copy-neutral LOH were observed at several sites (De Sousa *et al.* 2019). Other NGS studies involving a mix of PA subtypes have demonstrated copy number losses, in addition to gains (Song *et al.* 2016, Bi *et al.* 2017*b*) and LOH (Bi *et al.* 2017*b*). The regions affected by gain, loss or copy-neutral LOH vary between these studies. This may be at least partly explained by the heterogenous mixes of different PA subtypes and clinical features. In prolactinomas, for example, DA resistance may lead to accumulated copy number variation or *vice versa* (De Sousa *et al.* 2019).

The landmark NGS studies by Bi *et al.* outlined two patterns of copy number variation in PAs. A highly genomically disrupted group – mostly comprising functional PAs (including prolactinomas) and atypical null-cell PAs – exhibited copy number variation involving a mean of 39% of the genome. A genomically quiet group – mostly consisting of non-functioning pituitary adenomas (NFPAs) – exhibited copy number variation involving only 0.5% of the genome (Bi *et al.* 2017*a*,*b*). Notably, this research predated the introduction of TFs in the classification of

Table 3 Genes with an emerging germline association with pituitary tumour development.

Gene	Association with pituitary adenomas
AHR	Following an association between germline *AHR* variants and acromegaly severity, a rare germline missense variant was found in a man with cyclical Cushing's disease, but no other reports of germline mutations in association with PA (De Sousa *et al.* 2020).
CABLES1	Potentially pathogenic variants found in a small minority of patients with Cushing's disease (4/182), but without evidence of LOH or loss of CABLES1 expression in corresponding tumours (Hernandez-Ramirez *et al.* 2017) and not repeated elsewhere.
CDH23	Variants with at least some evidence of pathogenicity found in 4/12 FIPA kindreds and 15/125 patients with sporadic PA, but a notably large gene with expected heterogeneity and no functional studies to provide a mechanistic basis for pituitary tumorigenesis (Zhang *et al.* 2017). Also, not repeated elsewhere.
CDKI genes	The CDKI genes, *CDKN1A*, *CDKN2B* and *CDKN2C*, have been implicated in rare cases of a MEN1-like phenotype with no identifiable germline *MEN1* mutation. This includes a kindred with two affected relatives with prolactinomas and a segregating variant in *CDKN1A* (encoding p21) that had some evidence of pathogenicity (Agarwal *et al.* 2009).
MAX	Several cases of PA (prolactinomas and somatotrophinomas) in individuals with germline variants and phaeochromocytomas in the setting of 3PAs or recently termed 'MEN5' syndrome including various endocrine and non-endocrine tumours, but nil tumour DNA studies from PAs to confirm expected two-hit tumour suppressor gene model in the pituitary (Seabrook *et al.* 2022).
MMR genes (*MSH2*, *MSH6*, *MLH1*, *EPCAM*)	PAs are overrepresented amongst patients with Lynch syndrome. PAs in these individuals appear more severe, including cases of pituitary carcinoma which is otherwise exceedingly rare. Some reports of somatic changes supporting a direct role of MMR gene defects in pituitary tumorigenesis – for example, pituitary MMR IHC patterns corresponding to the germline mutated gene and microsatellite instability (Bengtsson *et al.* 2017).
NF1, TSC1/2, VHL	Isolated reports of PA in patients with neurofibromatosis type 1, tuberous sclerosis complex and von Hippel-Lindau syndromes, respectively, but without evidence that the PAs are due to the corresponding germline mutation rather than being phenocopies (O'Toole *et al.* 2015, Vasilev *et al.* 2020).
PRKACB	A 1.6-Mb triplication of Chr 1p31.1, including *PRKACB*, was found in a woman with Carney complex, including acromegaly, in the absence of *PRKAR1A* mutations, but no other germline *PRKACB* defects were found in association with PA (Forlino *et al.* 2014).
PRLR	Germline rare variants overrepresented in a single study of 46 prolactinoma patients when compared to the Exome Aggregation Consortium (ExAC) database (Gorvin *et al.* 2019), but not repeated elsewhere.
RET	The most common germline *RET* mutation in MEN2A, c.1900T>C (p.Cys634Arg), was found in a man with an overlapping phenotype of MEN2A syndrome and pituitary Cushing's. A potential alternative explanation of ectopic ACTH syndrome was ruled out by demonstrating negative ACTH immunohistochemistry in the patient's medullary thyroid cancer (Naziat *et al.* 2013).
USP8	Somatic variants are well described in corticotrophinomas, but only a single case of a germline mutation. The *de novo* heterozygous variant in the critical 14-3-3 binding motif hot spot was found in a girl diagnosed with Cushing's disease at 14 years in association with a multisystem disorder including developmental delay, dysmorphic features, hyperkeratosis, chronic lung disease, chronic kidney disease, hyperglycemia, dilated cardiomyopathy, hyperinsulinism and partial GH deficiency (Cohen *et al.* 2019).

3PAs, 3P (phaeochromocytoma, paraganglioma and pituitary adenoma) association syndrome; CABLES1, cyclin-dependent kinase 5 and ABL enzyme substrate 1; CDKI, cyclin-dependent kinase inhibitor; Chr, chromosome; FIPA, familial isolated pituitary adenoma; GH, growth hormone; IHC, immunohistochemistry; LOH, loss of heterozygosity; MEN1, multiple endocrine neoplasia type 1; MEN5, multiple endocrine neoplasia type 5; MMR, mismatch repair; PA, pituitary adenoma.

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pituitary tumours by the WHO in 2017 and the majority of the historically defined null-cell PAs are likely to in fact be SF1-positive/gonadotroph tumours (Lopes 2017). In support of the disrupted vs quiet PA paradigm, Song *et al.* found that 32% of their mixed PA cohort had copy number variation involving >80% of the genome, whilst 18% had copy number variation involving <10% of the genome (Song *et al.* 2016).

The close relationship between the degree of genomic disruption in PAs and Ki67 index as a marker of PA aggressiveness suggests that copy number variation may be intimately involved in the progression of PA to APT and PC (Bi *et al.* 2017*b*). However, the large size and variable locations of CNVs argue against any single gene or locus within the regions of copy number variation being the driver of pituitary tumorigenesis.

A more recent study of 134 PAs found that chromosomal alterations were associated with lineage differentiation but not tumour aggressiveness. Chromosomal instability was most common in PIT1-lineage tumours, which also showed global DNA hypomethylation and higher expression of transposable elements, with transposable element activation speculated to be the cause of chromosomal alteration (Neou *et al.* 2020). An exception to the associations of PIT1-lineage and hormone hypersecretion with DNA hypomethylation and chromosomal instability is *GNAS*-mutated somatotrophinomas, which exhibit a low degree of genomic disruption despite still demonstrating DNA hypomethylation (Hage *et al.* 2018, Neou *et al.* 2020).

Driver mutations

Only two genes have been repeatedly shown to be mutated in sporadic PAs: *GNAS* (predominantly in somatotrophinomas) and *USP8* (in corticotrophinomas). *GNAS* was the first gene found to harbour driver mutations in PAs. In 1989, Landis *et al.* categorised eight PA operative specimens from acromegalic patients as having low or high adenylyl cyclase activity. The authors hypothesised that constitutive activation of the alpha subunit of G-protein could lead to GHRH-independent production of adenylyl cyclase and hence somatroph tumorigenesis. They accordingly found somatic heterozygous activating *GNAS* mutations in 4/4 somatrophinomas with high adenylyl cyclase activity: R201C (in two tumours), R201H and Q227R (Landis *et al.* 1989). *GNAS* mutations have since been associated with relatively milder acromegaly, with smaller tumours, lower GH levels and greater somatostatin analogue sensitivity exhibited by *GNAS*-mutated vs *GNAS* wildtype somatotrophinomas (Landis *et al.* 1990, Yang *et al.* 1996). Soon after the discovery of *GNAS* mutations in somatrophinomas, the same mutations were found to produce MAS when occurring at the postzygotic stage (Weinstein *et al.* 1991), as discussed above.

With the advent of NGS came the opportunity for hypothesis-free genetic investigations into tumorigenesis. In 2015, Reincke *et al.* employed WES in corticotrophinomas and found somatic mutations in the *USP8* deubiquitinase gene in 4/10 tumours. All mutations were in the 14-3-3 protein binding motif and enhanced the proteolytic cleavage and catalytic activity of USP8, leading to increased deubiquitination of the epidermal growth factor receptor (EGFR) and thereby increased EGF signalling, with increases in *POMC* gene transcription and ACTH secretion (Reincke *et al.* 2015).

Contemporary NGS studies show somatic mutations in *GNAS* and *USP8* in approximately half of somatotrophinomas and corticotrophinomas, respectively (Song *et al.* 2016). By contrast, WES studies of NFPAs (Newey *et al.* 2013), prolactinomas (De Sousa *et al.* 2019) and TSHomas (Sapkota *et al.* 2017, Shen *et al.* 2020) have failed to demonstrate recurrent sequence variants that could constitute driver mutations.

Genes with potential, but as of yet unproven, links to sporadic PA development through somatic mutation include *USP48*, *BRAF* (Chen *et al.* 2018) and *NR3C1* (encoding the glucocorticoid receptor) (Miao *et al.* 2021) in corticotrophinomas; *SF3B1* in prolactinomas (Li *et al.* 2020); *ATRX* (Casar-Borota *et al.* 2021) and *TP53* (Sbiera *et al.* 2019) in aggressive corticotrophinomas; *AHR* in somatotrophinomas (Re *et al.* 2020) and *PIK3CA* and *HRAS* across various PA types (Lin *et al.* 2009). More evidence is required before these genes can be considered to be definitive contributors to sporadic pituitary tumorigenesis. In some cases, subsequent studies have specifically interrogated the candidate genes in the relevant PA type and have not found any mutations, whilst other studies have used hypothesis-free pangenomic sequencing of PAs without noting mutations in the relevant genes.

Pathway dysregulation and hypoxia

Dysregulation of cell signalling pathways – including growth factors and their receptors, intracellular signalling pathways and the cell cycle – contributes to pituitary tumorigenesis.

The vascular endothelial growth factor (VEGF) signalling pathway promotes tumour growth via effects on cell proliferation, angiogenesis, vascular permeability and the tumour immune microenvironment. VEGF binds

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tyrosine kinase receptors on the cell membrane, triggering downstream PI3k-Akt, Ras, VEGFA, Mapk and HIF-1 signalling cascades. Overexpression of VEGF and VEGF receptor is associated with tumour invasiveness and recurrence, with increased expression in PAs with cavernous sinus invasion (Sato *et al.* 2019). VEGF is also induced by pituitary tumour transforming gene 1 (PTTG1), which has a dual role in modulating epithelial–mesenchymal transition (EMT) and angiogenesis (Liu *et al.* 2019). PTTG is overexpressed in up to 90% of PAs and correlates with Ki67, tumour invasiveness and aggression (Lamb *et al.* 2020*b*). EGFR is a transmembrane glycoprotein, the binding of which triggers downstream signalling via MAPK and P13K pathways. Increased EGFR expression has been associated with proliferation, angiogenesis, invasion, recurrence and transition of PAs to APTs and PC (Onguru *et al.* 2004). EGFR is overexpressed in invasive PAs (Leriche *et al.* 1996) and correlates with tumour recurrence and cortisol/ACTH levels in Cushing's disease (Liu *et al.* 2019).

The PI3K/Akt/mTOR and Raf/Mek/ERK intracellular signalling pathways are both upregulated in PAs. *PI3KCA* mutations are observed in invasive PAs and are associated with tumour recurrence. Abnormal expression of cell cycle regulators retinoblastoma protein (pRB) and p16, and cell cycle promotor cyclin D1 occurs in up to 80% of PAs.

Hypoxia is commonly associated with tumorigenesis and progression in solid organ malignancies, through its activation of adaptive survival pathways (Qian *et al.* 2020). HIF-1α regulates VEGF, both of which are overexpressed under hypoxic conditions and have been implicated in PA invasiveness, growth and aggression (Trott *et al.* 2019).

Increased understanding of cell signalling pathways in PA pathogenesis has identified therapeutic targets with a successful application of novel therapies such as VEGF inhibitors. Other potential targets are the subject of the ongoing investigation.

Epithelial–mesenchymal transition

EMT is a process by which immotile epithelial cells become migratory mesenchymal cells. In embryonic development, EMT enables dynamic cell transition from one state to another, facilitating plasticity and motility. In epithelial-derived tumours, EMT renders cells capable of invading the extracellular matrix; integral to invasion and metastatic disease. Epithelial cells lose polarity and cell adhesion molecules, particularly E-cadherin, and upregulate expression of mesenchymal markers, ZEB1, N-Cadherin and vimentin (Jia *et al.* 2015, Hosseinkhan *et al.* 2022). Loss of cytoplasmic E-cadherin is often observed in conjunction with increased nuclear expression, suggesting translocation of the protein is a critical step in tumour invasion (Gil *et al.* 2021). EMT is increasingly recognised to represent a spectrum of states, rather than a binary process, the gradient of which may provide prognostic information (Gil *et al.* 2021, Hosseinkhan *et al.* 2022). Pleiotropic factors regulate EMT, through the induction of expression of transcription factors (EMT-TFs), along with epigenetic and post-translational regulators. TFs SNAI1, SNAI2 (SLUG), TWIST and ZEB repress E-Cadherin, in turn promoting the development of EMT (Gil *et al.* 2021, Hosseinkhan *et al.* 2022). These EMT-TFs have also been associated with PA invasiveness and aggressiveness (Gil *et al.* 2021). Several studies have demonstrated overexpression of PTTG1 and cyclin B1 (CCNB1) and downregulation of cell surface E-Cadherin in invasive compared with non-invasive NFPA (Øystese *et al.* 2018, Trott *et al.* 2019, Hosseinkhan *et al.* 2022).

DNA repair

The DNA-damage response pathway (DDR) includes multiple distinct DNA repair systems, including 06-methylguanine DNA methyl transferase (MGMT), mismatch repair (MMR) and base excision repair. Oncogene-induced senescence is believed to contribute to the 'benign' nature of most pituitary tumours. In this process, initial tumour proliferation causes DNA replicative stress and activation of DDR, resulting in the upregulation of key cell cycle regulator genes such as *TP53* and *CDKN1A* (encoding p21) and cell cycle arrest (Chesnokova *et al.* 2008).

Loss of DDR machinery may contribute to PA recurrence and aggressive behaviour. Pituitary tumours with low MGMT expression are more frequently found among recurrent and aggressive subtypes, including PC (Salehi *et al.* 2011). A differential gene expression study found low MGMT-expressing pituitary tumours had significant upregulation of DDR and transcriptional activity which may drive mutagenic potential and tumour proliferation (Mccormack *et al.* 2013). In fact, Lynch syndrome, caused by a germline mutation in MMR genes and characterised by a heritable predisposition to multiple cancers, is associated with microsatellite instability and a high rate of somatic mutations. Lynch syndrome patients may develop pituitary tumours, particularly aggressive forms, at a higher rate than the general population

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(Bengtsson *et al.* 2017). A reduction in MSH6 and MSH2 expression has been shown to drive PA proliferation through interference with the cell regulatory ATR-Chk1 pathway (Uraki *et al.* 2018).

Expression of DDR may also provide molecular biomarkers of drug treatment response. Response to the oral alkylating agent temozolomide (TMZ), now first-line chemotherapy for APTs, is associated with low MGMT expression. On the other hand, the emergence of TMZ resistance has been seen in association with loss of MSH6 expression (despite low MGMT expression) and tumour progression. However, MMR deficiency and/or a high mutation burden (the latter being a frequent outcome of TMZ treatment) may exhibit a good response to immunotherapy, an emerging treatment option for patients with APT (Burman *et al.* 2020).

Senescence

Historically, senescence in cancer has been considered beneficial as a tumour suppressive mechanism. However, more recently, the complex nature of the pro- and anti-tumorigenic effects of the senescence-associated secretory phenotype (SASP) has been recognised. SASP components promote tumorigenesis through paracrine effects and tumour vascularisation via secretion of angiogenic factors and establish an immunosuppressive environment.

The senescence marker SA-β-GAL is overexpressed in PAs compared with normal pituitary tissue, and senescence may contribute to the typically benign natural history of PAs (Manojlovic-Gacic *et al.* 2016). PTTG has been implicated in the mechanism of senescence in PAs, with both *PTTG1* deletion and overexpression of PTTG demonstrated to play a role (Chesnokova & Melmed 2010).

Immune microenvironment

The immune microenvironment of PAs has received increasing attention with the expansion of immunotherapy and its potential application as a novel treatment option for APTs.

The prevalence of tumour infiltrating lymphocytes (TILs) is higher in PAs than normal pituitary, and TILs are associated with biochemical persistence and residual or recurrent tumour postoperatively (Lupi *et al.* 2010). Further examination of TILs including cytotoxic CD8 and regulatory T cell FoxP3 has demonstrated that FoxP3 is associated with Ki67 and the FoxP3/CD8 ratio is significantly higher in invasive PAs (Marques *et al.* 2019, Sato *et al.* 2019).

Macrophage infiltration correlates with PA size and invasiveness (Lu *et al.* 2015). Furthermore, tumour-associated macrophage recruitment and polarisation to pro-tumoural M2 subtype may drive NFPA proliferation and invasion. Increased M2 to M1 gene expression ratios are noted in invasive PAs, with *in vitro* evidence that M2-differentiated THP-1 monocyte cells promote increased proliferation and migration (Yagnik *et al.* 2019).

The invasive nature of *AIP*-mutation-positive PAs may be attributed to crosstalk between the tumour and its microenvironment. *AIP*-mutation-positive PAs are infiltrated by a large number of macrophages and the EMT pathway is significantly altered compared to sporadic adenomas. *In vitro* data suggests that macrophage-derived factors promote an EMT phenotype and increase migration and invasion of *AIP* knockdown GH3 cells. Furthermore, *in vitro* and *in vivo* data demonstrate that loss of AIP increases macrophage migration and tumour infiltrate due to increased tumour-derived cytokine CCL5 (Barry *et al.* 2019).

PAs express variable levels of the immune checkpoint protein programmed cell death ligand 1 (PD-L1). Its role in pituitary tumorigenesis remains unclear. PD-L1 expression has been associated with a higher Ki67 index (Wang *et al.* 2018), but the relationship to invasiveness varies between studies (Sato *et al.* 2019, Uraki *et al.* 2020). Clinical experience with immune checkpoint inhibitors (ICIs) in the management of APTs shows promise (Lamb *et al.* 2020*a*), but further investigation is required to define the precise role of ICIs.

Epigenetics

Epigenetic influences on gene expression include DNA methylation changes, histone modifications and altered expression of miRNA. Methylation changes affect genes involved in cell cycle regulation, apoptosis, tumour suppression, growth factor receptor and cell growth regulation (Yacqub-Usman *et al.* 2012). The correlation between methylation changes and gene expression or clinical characteristics such as invasiveness or recurrence is variable. Overexpression of DNA methyltransferase (DNMT) enzymes is associated with invasiveness. The effect of DNMT3B on the downregulation of tumour suppressor genes is due to both DNA methylation and histone modifications, suggesting an interdependence of these two mechanisms (Shariq & Lines 2019). Different PA types display different methylation profiles; hypomethylation of promoter regions is associated with increased expression of the *GH1* and *SSTR5* genes in

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GH-secreting PAs and the *POMC* gene in ACTH-secreting PAs (Salomon *et al.* 2018).

Altered microRNA expression influences a number of pathways in PA development, including transcription regulation, intracellular signalling, cell cycle regulation and apoptosis. The expression of specific microRNAs in PAs may be increased or decreased (Shariq & Lines 2019).

Epigenetic changes in PAs may be reversed with epidrugs, including DNMT and histone deacetylase (HDAC) inhibitors (Lamb *et al.* 2020*b*). Further research is required to determine the clinical implications of these findings.

Future directions

Progressive insights into the molecular and genetic aspects of PAs are paving the way for optimised patient care. Identifying the causative germline variant in patients with familial pituitary tumours allows personalised PA management, gene-specific tumour surveillance, cascade testing of at-risk relatives and tailored reproductive advice with respect to PA management around pregnancy and risk to offspring.

Gene-specific medical therapy does not yet exist in familial pituitary tumours, but gene-targeted treatments are rapidly evolving across medicine. PARP inhibitors, used in the treatment of patients with *BRCA1/2*-mutated cancers, have been shown to decrease cell survival in human cell cultures and human tumour xenografts with SDHB deficiency, holding promise for therapeutic use in patients with *SDHx*-related tumours in future (Sulkowski *et al.* 2018).

In the somatic arena, the downstream upregulation of EGFR caused by somatic gain-of-function *USP8* mutations suggests that existing EGFR inhibitors such as gefitinib may be a treatment option in corticotrophinomas that harbour such mutations, which account for around half of Cushing's disease (Vasilev *et al.* 2020).

On a molecular level, receptor profiling may help to guide treatment decisions across PA types. Current medical therapies for PAs target specific receptors expressed on tumour cells, such as cabergoline targeting the dopamine-2 receptor (D2R) and octreotide, lanreotide and pasireotide targeting somatostatin receptors (SSTR). There is considerable variability of both biochemical response and tumour shrinkage to these agents (Gatto *et al.* 2020), with the relative efficacy of different somatostatin analogues closely related to the differential expression of the SSTR2 and SSTR5 receptor subtypes (Iacovazzo *et al.* 2016*a*). The sensitivity of GH-secreting tumours to octreotide and lanreotide has been correlated with *SSTR2* mRNA expression (both via Northern blot and qPCR), immunohistochemistry and tumour positivity on ^{111}In-pentetreotide scintigraphy (Marazuela *et al.* 2018, Gatto *et al.* 2020). *AIP* status is also relevant as individuals with PAs related to germline *AIP* mutations frequently show resistance to these first-generation somatostatin analogues irrespective of the degree of SSTR2 expression (Chahal *et al.* 2012). GH-secreting tumours resistant to standard SSTR2 analogues may be managed using pasireotide, with activation of SSTR5 being more prominent in the setting of low SSTR2 expression (Gatto *et al.* 2020). T2-weighted magnetic resonance imaging signal intensity (T2SWI) may indicate SSTR5 status as hyperintense adenomas have been shown to exhibit greater SSTR5 staining; however, there are currently mixed data as to whether this can in turn predict pasireotide response (Ilie *et al.* 2022). Profiling of the D2R may not be as useful to predict tumour responsivity, as it is expressed in almost 90% of PAs (Marazuela *et al.* 2018). While dopamine agonist (DA)-sensitive prolactinomas do tend to have greater expression of *DRD2* mRNA (encoding D2R) than DA-resistant prolactinomas (Marazuela *et al.* 2018), D2R expression did not predict cabergoline-induced tumour shrinkage in a cohort of NFPAs (Greenman *et al.* 2016). Before receptor profiling becomes routine practice, standardisation of the techniques used to quantify receptor expression must occur. A detailed discussion of the pros and cons of receptor profiling has been put forward by Marazuela *et al.* (Marazuela *et al.* 2018).

As mentioned, *GNAS* status influences the degree of somatic CNV seen in PAs (Hage *et al.* 2018, Neou *et al.* 2020). SSTR expression is also linked with genetic status, with higher SSTR2 expression in *GNAS*-mutated somatotrophinomas (Taboada *et al.* 2011) and higher SSTR5 expression in PAs with somatic *USP8* mutations (Neou *et al.* 2020) and in PAs from individuals with germline *AIP* mutations (Chahal *et al.* 2012). Given the various genetic and molecular interplays, a multi-omic classification of PAs was recently proposed by Neou *et al.* to further characterise PAs beyond the current WHO histological classification and to guide the selection of currently available antitumour agents. By this pangenomic system, the investigators highlighted: the chromosomal instability of PIT1-lineage PAs; the subclassification of corticotrophinomas into *USP8*-mutated tumours with overt secretion and higher SSTR5 expression, *USP8*-wildtype tumours with increased invasiveness and

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increased EMT, and large silent tumours with gonadotroph transdifferentiation; and an unexpected finding of SF1 expression in *GNAS*-wildtype somatotrophinomas that contests the current definition of the SF1/gonadotroph lineage (Neou *et al.* 2020).

Conclusions

A variety of traditional genetic studies (gene mapping, expression profiling, direct gene sequencing and chromosome microarray) have helped to elucidate roles for the 10 genes that are clinically established in familial pituitary tumours (*MEN1*, *PRKAR1A*, *AIP*, *CDKN1B*, *GPR101*, *SDHA*, *SDHB*, *SDHC*, *SDHD SDHAF2*) as well as the role of postzygotic mutations in *GNAS* in MAS. Contemporary studies employing NGS are now improving clinical access to simultaneous gene testing, allowing increased identification of familial pituitary tumours and thereby personalised care of patients tailored to the culprit gene. There is also ongoing study into the somatic genetic basis of sporadic PAs, which relates more to copy number variation than driver mutations. This is in addition to molecular changes in signalling and hypoxia pathways, EMT, DNA repair, senescence, the immune microenvironment and epigenetics. Increasing focus on cell lineage rather than hormone production in the classification of pituitary tumours may help to better understand how these various processes contribute to different types of PA and the transition of PA to APT and PC.

Declaration of interest

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

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