REVIEW

Succinate dehydrogenase and MYC-associated factor X mutations in pituitary neuroendocrine tumours

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Abstract

Pituitary neuroendocrine tumours (PitNETs) associated with paragangliomas or phaeochromocytomas are rare. SDHx variants are estimated to be associated with 0.3–1.8% of PitNETs. Only a few case reports have documented the association with MAX variants. Prolactinomas are the most common PitNETs occurring in patients with SDHx variants, followed by somatotrophinomas, clinically non-functioning tumours and corticotrophinomas. One pituitary carcinoma has been described. SDHC, SDHB and SDHA mutations are inherited in an autosomal dominant fashion and tumorigenesis seems to adhere to Knudson’s two-hit hypothesis. SDHD and SDHAF2 mutations most commonly have paternal inheritance. Immunohistochemistry for SDHB or MAX and loss of heterozygosity analysis can support the assessment of pathogenicity of the variants. Metabolomics is promising in the diagnosis of SDHx-related disease. Future research should aim to further clarify the role of SDHx and MAX variants or other genes in the molecular pathogenesis of PitNETs, including pseudohypoxic and kinase signalling pathways along with elucidating epigenetic mechanisms to predict tumour behaviour.

Key Words
- succinate dehydrogenase
- SDH
- MAX
- pituitary neuroendocrine tumour
- paraganglioma
- phaeochromocytoma

Introduction

Primary tumours of adenohypophyseal cells recently suggested to be redefined as pituitary neuroendocrine tumours (PitNETs) can rarely occur in association with paraganglioma (PGL) or phaeochromocytoma. These tumours may develop in patients with or without identifiable germline variants. The combination of PitNET and phaeochromocytoma/PGL (PPGL) is also uncommon but well-described in the setting of multiple endocrine neoplasia (MEN) type 1 whilst the association in MEN2 is probably coincidental. Succinate dehydrogenase (SDH) gene variants (collectively known as SDHx) can associate with PPGL. (Baysal et al. 2000). The association of PitNET
and PPGL in the setting of SDHx variant was established at the molecular level in 2012 (Xekouki et al. 2012) and has since been known as the 3P (pituitary, parangangioma, phaeochromocytoma) association (3PA) syndrome (Xekouki et al. 2015). In some cases, no genetic alteration can be identified (Denes et al. 2015). In addition to PPGL and PitNETs, SDHx variants may also result in renal cell carcinoma and gastrointestinal stromal tumour (Carney & Stratatakis 2002, Malinoc et al. 2012). The lifetime PPGL-related penetrance of SDHA, SDHB and SDHC genes is 1.7, 8.3 and 22.0%, respectively (Benn et al. 2018), while the penetrance of a paternally inherited SDHD pathogenic variant is 43.2% by age 60 years (Andrews et al. 2015). In decreasing order of frequency, germline mutations of SDHx genes have been found in PPGL, gastrointestinal stromal tumours, renal cell carcinoma and PitNETs, seemingly making PitNETs the least frequent of these SDHx-associated tumours (Evenepoel et al. 2015). Exceptional reports of SDHx variants in a pancreatic neuroendocrine tumour and lymphoid malignancy have been documented (Renella et al. 2014, Niemeijer et al. 2015). In unselected PitNET cohorts, the prevalence of SDHx variants is 0.3–1.8% (Gill et al. 2014, Xekouki et al. 2015, MacFarlane et al. 2020, Mougel et al. 2020).

Germline MAX variants have been implicated in PPGL and renal oncocytoma, and somatic variants have been identified in small cell carcinoma of the lung (Romero et al. 2014, Kurschner et al. 2017). Other tumours reported in association with MAX variants include endometrial carcinoma, ganglioneuromas, neuroblastoma, pancreatic cancer, lung adenocarcinoma and breast cancer (Walker et al. 2018, Seabrook et al. 2021). In one study, germline MAX variants accounted for approximately 1% of PPGLs in patients with a negative RET, VHL, SDHB, SDHC, SDHD and TMEM127 genetic screen, thus making it a very rare cause of PPGL (Burnichon et al. 2012). Data would tend to suggest that the presence of young onset bilateral PPGL or multifocal unilagular phaeochromocytoma should raise the suspicion of a pathogenic MAX variant (Burnichon et al. 2012, Korpershoek et al. 2016, Seabrook et al. 2021). The 3PA syndrome has now also been described in patients with MYC-associated factor X gene (MAX) variants (Roscko et al. 2017, Daly et al. 2018, Mamedova et al. 2021). Two families with PPGL and multiple endocrine and non-endocrine tumours in the setting of MAX variants have raised the suggestion of naming this syndrome as multiple endocrine neoplasia type 5 (Seabrook et al. 2021).

This review summarises the inheritance and pathophysiology of SDHx and MAX variants, considers the clinical manifestations and discusses the evidence in reported cases of SDHx and MAX-associated PitNETs to date in order to provide an overview of the investigative strategy for these rare tumours.

**SDH: pathophysiology**

The SDH complex is located on the inner mitochondrial membrane and consists of four subunits: A, B, C and D, each coded by one of the SDHx genes (Fig. 1). The SDH complex is accompanied by an associated assembly factor, SDHAF2, which facilitates flavination of SDHA (Fig. 1).

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**Figure 1**

The SDH complex and its relationship to the mitochondrial membranes and mitochondrial cristae. Together the SDH subunits make up respiratory complex II. The hydrophobic SDHD and SDHC subunits anchor the complex within the inner mitochondrial membrane, while the hydrophilic SDHA and SDHB subunits catalyse the oxidation of succinate to fumarate as part of the tricarboxylic cycle. SDHAF2, also known as SDH5, is known to have roles in the flavination of SDHA and research into its roles and structure is ongoing (Sharma et al. 2020). Electrons generated by the tricarboxylic acid cycle (e-): reduce FAD to FADH2 in SDHA before proceeding through Fe-S clusters in SDHB. These electrons then reduce ubiquinone (Q) to ubiquinol (QH2) before being transported to the adjacent respiratory complex III. Mitochondrion image created with Biorender.com
The hydrophobic C and D subunits act to anchor the SDH complex, and the hydrophilic A and B subunits are the sites for enzymatic activity (Fig. 1). SDHA and SDHB catalyse the oxidation of succinate to fumarate in the tricarboxylic acid cycle (also known as Krebs cycle or citric acid cycle) and transfer electrons from carbon oxidation within the cycle to ubiquinone within the electron transport chain (Fig. 1) (Rutter et al. 2010). With its roles in both the tricarboxylic acid cycle and the electron transport chain, the SDH complex is a linchpin of aerobic respiration.

The malfunctioning of the SDH complex secondary to SDHx mutations results in accumulation of succinate. Accumulated succinate can then enter the cytosol via the inner mitochondrial membrane dicarboxylic acid translocator followed by the outer mitochondrial membrane voltage-dependent anion channel (Selak et al. 2005). This excess of succinate can disrupt prolyl hydroxylases within the cytosol resulting in the von Hippel–Lindau (VHL) protein dissociating from hypoxia-inducible factor (HIF) (Selak et al. 2005). The stabilisation and subsequent accumulation of HIF results in a state of pseudohypoxia, which may contribute to tumorigenesis via epigenetic modifications, such as disruption of RNA networks (Puissegur et al. 2011, Zhang et al. 2019). For example, a HIF-1α-dependent increase of miR-210 and subsequent mitochondrial dysfunction in A549 human lung adenocarcinoma cells has been demonstrated (Puissegur et al. 2011). Furthermore, SDHD is a miR-210 target and SDHD knockdown in A549 cells replicated miR-210-induced mitochondrial dysfunction and mitochondrial structural abnormalities (Puissegur et al. 2011). In another lung adenocarcinoma cell model (EGFR-mutated H1975 cells), miR-147b repressed SDHD activity, which is known to result in HIF accumulation (Zhang et al. 2019). Hypermethylation also appears to be an important epigenetic mechanism. In a cohort of 145 PPGL, only one hypermethylyated tumour did not have an SDHx variant. Hypermethylation was higher in SDHB-mutated PPGL when compared to SDHA, SDHC and SDHD cases, which may explain the greater metastatic potential of SDHB-mutated tumours (Letouze et al. 2013). In this study, the authors hypothesised that succinate may limit demethylation by TET proteins and more recently it has been shown that inhibition of TET results in SDHB-related hypermethylation, which acts in concert with HIF-2α-induced pseudohypoxia to promote a mesenchymal phenotype in Sdhb−/− cells in vitro and in vivo (Morin et al. 2020). Additionally, elevated HIF-1α levels have been shown in an SDHD-mutated somatotrophinoma and the cytoplasm of Sdhb−/− mouse pituitary cells (Xekouki et al. 2012, 2015).

The role of SDHx variants in pituitary tumorigenesis is supported by a double knockout animal model (Xekouki et al. 2015). Sdhb−/− mice have hypercellular pituitary glands with increased number of prolactin and growth hormone-positive cells (Xekouki et al. 2015). Tumour cells in this model show large mitochondria with dysmorphic and/or absent mitochondrial cristae that are the site of SDH subunits (Fig. 1). It is hypothesised that pituitary hyperplasia could be one of the first steps in the development of SDHx-related PitNETs (Xekouki et al. 2015).

A transcriptomic analysis of 76 inherited and sporadic PPGLs identified 2 tumour clusters, one including SDHB, SDHD and VHL-mutated tumours (pseudohypoxic signalling cluster), and one comprising RET and NF1-mutated (kinase signalling cluster) tumours (Dahia et al. 2005). MAX falls within the kinase signalling cluster. A third cluster driven by Wnt signalling including CSDE1 and UBTF-MAML3 genes has also been recognised (Fishbein et al. 2017).

SDHx variants are well established in PitNETs while one highly proliferative macro somatotroph-lactotroph PitNET has been described in a 15-year-old with a germline VHL variant c.340G>C (p.Gly114ser); the patient later developed a pheochromocytoma (Tudorancea et al. 2012). It will be interesting to see if other genes identified in the pseudohypoxic cluster, such as SUCLG2, are also implicated in PitNET pathogenesis (Hadrava Novova et al. 2022). Growth hormone excess in association with optic glioma and germline NF1 variants has been reported, but a pathogenic role for NF1 and RET germline variants is yet to be elucidated in PitNETs. Germline Wnt-signalling gene variants are yet to be described in PitNETs, although beta-catenin mutations are well established in the pathology of adamantinomatous craniopharyngioma.

The majority of SDHx-associated PitNETs reported to date have been tumours of the PIT1 lineage. This may be because PIT1 lineage PitNETs are simply more common, or alternatively, there may be a mechanistic explanation for this. For example, HIF-1 has many binding partners, one of these being the pituitary transcription factor PITX1 (Mudie et al. 2014). PITX1 has been found to regulate HIF-dependent cellular survival in hypoxia and depletion of PITX1 in U2OS and HeLa cells resulted in increased apoptosis in hypoxic conditions (Mudie et al. 2014). Whether the elevated HIF levels arising from SDHx pathogenic variants may also inhibit apoptosis of PIT1-derived pituitary cells resulting in hyperplasia progressing to overt tumorigenesis is an interesting consideration, and a recent study has established a link between HIF-1α excess and protein kinase A, CREB and downstream excess...
growth hormone secretion via repression of PRKAR2B transcription (Lucia et al. 2020).

**SDHx variants**

SDHB, SDHA and SDHC mutations are commonly inherited in an autosomal dominant fashion. Tumorigenesis in PPGL adheres to Knudson’s two-hit hypothesis (Fig. 2A). Patients with PPGL most commonly develop their disease from paternally transmitted mutations in SDHD and SDHAF2; however, a few cases of maternal transmission of SDHD mutations resulting in PPGL do exist (Kunst et al. 2011). Two different mechanisms have been suggested (Fig. 2B) (Hensen et al. 2004, Baysal et al. 2011). Proposed candidates for the unknown imprinted SDHD modifier gene shown in Fig. 2B include CDKN1C, SLC22A18 and H19 (Hoekstra et al. 2016, Björklund & Backman 2018).

More recently, a further hypothesis for the parent-of-origin effects of SDHD expression suggested maternal imprinting at a promoter for a large intergenic ncRNA, designated the name UPGL (untranslated in paraangioma locus) downstream of SDHD on chromosome 11 (Fig. 3) (Baysal et al. 2011). It is hypothesised that methylation of this locus controls long-range enhancer–promoter contacts, alteration of chromatin structures and subsequent downregulation of transcriptional activity of the SDHD gene (Baysal et al. 2011).

**SDHB variants in PitNETs**

SDHB (OMIM*185470) is located on chromosome 1p36.13 and codes for the catalytic SDHB subunit of the SDH complex (Fig. 1). SDHB mutations manifest as familial PGL type 4. To date, there are 19 cases of SDHB-associated PitNETs reported. Five have had LOH analysis undertaken (three showed LOH). Evidence is inconclusive in the remainder of SDHB-related PitNETs analysed (LOH not present/not evaluated, heterogeneous/positive immunohistochemistry (IHC)). In 13 patients, no tissue analysis has been undertaken (Fig. 4 and Table 1). Cases with tumour analysis are summarised in this subsection.

A 33-year-old male with the SDHB c.298T>C (p.Ser100Pro) variant was reported to have a macroprolactinoma managed with dopamine agonist
and surgery. LOH at the SDHB locus was confirmed in the tumour tissue, suggesting a pathogenic role of the SDHB variant (Denes et al. 2015). Furthermore, vacuoles were observed in neoplastic cells by microscopy. The patient’s mother carried the same variant and had been diagnosed with a macroprolactinoma aged 35 years. Her prolactinoma tissue also showed vacuolated cells (Denes et al. 2015).

A 31-year-old female with family history of PGL was diagnosed with macroprolactinoma requiring 2 surgeries, cabergoline and radiotherapy (Denes et al. 2015). She had a germline deletion of exon 6–8 of SDHB. The pituitary tissue showed loss of the whole gene on the other allele and negative SDHB IHC (Denes et al. 2015).

SDHB-associated pituitary carcinoma has been described in a 53-year-old patient bearing the c.587G>A (p.Cys196Tyr) variant (Tufton et al. 2017). The lesion was clinically non-functioning (NF). Tumour cells expressed the steroidogenic factor 1 (SF1) but lacked the expression of pituitary hormones. The patient also had a history of PGL. Vacuoles typical of SDHB-mutated PitNETs were identified and again LOH was confirmed in the pituitary carcinoma tissue (Tufton et al. 2017). After three cycles of temozolomide, the patient showed dramatic clinical improvement with stable MRI appearances. A slight reduction in the size of primary and metastatic lesions was noted after a total of ten cycles of chemotherapy.

Figure 3
Long-range enhancer–promoter contacts in SDHD gene expression. On the paternal allele, an enhancer can influence an SDHD promoter and thereby increase SDHD transcription. This occurs via a UPGL promoter, which remains unmethylated due to the competitive binding of a transcription factor (TF) preventing cohesin from engaging with a CpG island (CPI). On the maternal allele, the UPGL promoter is methylated (CH3), preventing the TF binding, which enables cohesin to bind to the CpG island and block the enhancer–promoter activity on SDHD. Consequently, the enhancer binds to an alternative promoter and there is downregulation of SDHD transcription.

Figure 4
(A) All cases of PitNETs reported in association with SDHx variants are summarised. Prolactinomas account for a significant proportion (59%). It is notable that there have only been eight cases where evidence consistent with a causative role for SDHx variant reported in the literature and all are macro PitNETs (B). The average age at diagnosis in this sub-cohort is 44 years (range 31–60). One patient had a mixed somatotroph–lactotroph tumour with clinical acromegaly (Xekouki et al. 2012); 75% of this subgroup had prolactin-expressing tumours. It is also possible that prolactinomas will be under-represented in B as they are not routinely managed with surgery.

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Table 1  PitNETs reported in setting of SDHx variants.

| Gene | SDHx variant | Sex | Age at diagnosis (years) | Phenotype | VarSome prediction | Reference |
|------|--------------|-----|-------------------------|-----------|--------------------|-----------|
| SDHB | c.761insC (p.254fs*255) | M | 15 | Unknown | Pathogenic | (Benn et al. 2006) |
| SDHB | c.18C>A (p.Ala6Ala) | F | 43 | Microprolactinoma | Benign | (Efstathiadou et al. 2014) |
| SDHB | c.423+1G>A (Splicesite) | F | 60 | Macroprolactinoma | Pathogenic | (Denes et al. 2015) |
| SDHB | c.770dupT (p.Asn258Glufs*17) | F | 50 | Micro NF PitNET | Pathogenic | (Denes et al. 2015) |
| SDHB | c.298T>C (p.Ser100Pro) | M | 33 | Macroprolactinoma | Pathogenic | (Denes et al. 2015) |
| SDHB | c.298T>C (p.Ser100Pro) | F | 35 | Macroprolactinoma | Pathogenic | (Denes et al. 2015) |
| SDHB | Deletion exon 6–8 | F | 31 | Macroprolactinoma | Pathogenic | (Denes et al. 2015) |
| SDHB | c.587G>A (p.Cys196Tyr) | F | 53 | Gonadotroph carcinoma | Pathogenic | (Tufton et al. 2017) |
| SDHB | c.298T>C (p.Ser100Pro) | F | 56 | Macroprolactinoma | Pathogenic | (Maher et al. 2018) |
| SDHB | c.587-591DelC (Intronic) | F | 74 | Macro somatotrophinoma | Uncertain significance | (Saavedra et al. 2019) |
| SDHB | c.689G>A (p.Arg230His) | M | 72 | Somatotrophinoma | Pathogenic | (Xekouki et al. 2015) |
| SDHB | c.642+1G>T (Splicesite) | F | 50 | Microprolactinoma | Pathogenic | (Xekouki et al. 2015) |
| SDHB | c.487T>C (p.Ser163Pro) | F | 14 | Micro corticotrophinoma | Benign | (Xekouki et al. 2015) |
| SDHB | c.487T>C (p.Ser163Pro) | M | 10 | Micro corticotrophinoma | Benign | (Xekouki et al. 2015) |
| SDHB | Large deletion exon 1 | F | 38 | Macroprolactinoma | Pathogenic | (Guerrero Pérez et al. 2016) |
| SDHB | c.5C>T (p.Ala2Val) | F | 49 | Microprolactinoma | Uncertain significance | (De Sousa et al. 2017) |
| SDHB | c.24C>T (p.Ser8Ser) | M | 70 | Prolactinoma | Benign | (De Sousa et al. 2017) |
| SDHB | Unknown | F | 38 | Macroprolactinoma | Unknown | (Gorospe et al. 2017) |
| SDHB | c.166-170delCCTA (p.Ala6Leu) | M | 45 | Macro NF PitNET* | Pathogenic | (Guerrero-Perez et al. 2019) |
| SDHD | c.298_301del (p.Thr100fs) | M | 37 | Macro somatotrophinoma | Pathogenic | (Kekouki et al. 2012) |
| SDHD | c.242C>T (p.Pro81Leu) | F | 33 | Macroprolactinoma | Pathogenic | (Varvasvky et al. 2013) |
| SDHD | c.274G>T (p.Asp92Tyr) | M | 60 | Macroprolactinoma | Pathogenic | (Papathomas et al. 2014) |
| SDHD | c.274G>T (p.Asp92Tyr) | F | 56 | Macro somatotrophinoma | Pathogenic | (Papathomas et al. 2014) |
| SDHD | c.149A>G (p.His50Arg) | F | 16 | Micro corticotrophinoma | Pathogenic | (Kekouki et al. 2015) |
| SDHD | c.242C>T (p.Pro81Leu) | M | 23 | Macroprolactinoma | Likely pathogenic | (Kekouki et al. 2015) |
| SDHA | c.53C>T (p.Ala18Val) | M | 12 | Micro corticotrophinoma | Benign | (Kekouki et al. 2015) |
| SDHA | c.315?-480+?del | M | 31 | Macro NF PitNET | Pathogenic | (Kekouki et al. 2015) |
| SDHA | c.256–257insTTT (p.Phe85dup) | M | 60 | Macroprolactinoma | Pathogenic | (Kekouki et al. 2015) |
| SDHA | c.380A>G (p.His127Arg) | M | 53 | Macroprolactinoma | Likely pathogenic | (Lemelin et al. 2019) |
| SDHA | c.403G>C (p.Glu110Gln) | F | 34 | Microprolactinoma | Benign | (Lopez-Jimenez et al. 2008) |
| SDHA | c.20+74A>G (Intronic) | M | 41 | Microprolactinoma | Benign | (Hussein et al. 2021) |
| SDHA | c.405+1G>T (Splicesite) | M | 17 | Macroprolactinoma | Uncertain significance | (De Sousa et al. 2021) |
| SDHA | c.1873C>T (p.His625Tyr) | M | 30 | Macro NF PitNET | Pathogenic | (De Sousa et al. 2021) |
| SDHA | c.725_736del (p.Ser243_Arg246del) and c.989_990insTA (p.Ala331ThrfsTer18) | M | 62 | Macro Silent Lactotroph PitNET | Likely pathogenic | (Gill et al. 2014) |
| SDHA | c.969C>T (p.Gly323Gly) | M | 53 | NF PitNET | Benign | (Denes et al. 2015) |

(Continued)
Table 1 Continued.

| Gene | SDHx variant | Sex | Age at diagnosis (years) | Phenotype | VarSome prediction | Reference |
|------|--------------|-----|-------------------------|-----------|--------------------|-----------|
| SDHA | c.91C>T (p.Arg31*) | F | 27 | Prolactinoma | Pathogenic | (Denes et al. 2015)** |
| SDHA | c.91C>T (p.Arg31*) | F | 49 | Macroprolactinoma | Pathogenic | (Niemeyer et al. 2015) |
| SDHA | c.757_758del(p.Val253Cys*67) | M | 42 | Macroprolactinoma | Pathogenic | (Mougel et al. 2020) |
| SDHA | c.1753C>T (p.Arg585Trp) | M | 37 | Macroprolactinoma | Uncertain significance | (Mougel et al. 2020) |
| SDHAF2 | c.-52T>C (Intronic) | M | 84 | Macromatrotrophinoma | Uncertain significance | (Denes et al. 2015) |

*This PitNET had focal positivity for prolactin and FSH. **The 27-year-old female with SDHA variant also had a concomitant VHL c.589G>A (p.Asp197Asn) variant.

In two further cases, the evidence for causation is considered inconclusive. One 56-year-old female patient bearing the SDHB c.298T>C (p.Ser100Pro) variant was diagnosed with macroprolactinoma (Maher et al. 2018). She had no syndromic disease. Her initial response to cabergoline was unsatisfactory. Surgical resection was undertaken. Histologically, the tumour cells showed considerable vacuolisation of the cytoplasm. The immunoreaction for SDHB showed normal expression suggesting that the SDHB variant might not have been causative and that a phenocopy was plausible. The most recent PitNET reported in association with an SDHB variant (c.587-591DelC frameshift) occurred in a 74-year-old female diagnosed with a macro somatotrophinoma on a background of metastatic PGL (Saavedra et al. 2019). Some neoplastic cells showed vacuoles. SDHA staining was retained whilst SDHB expression was reportedly heterogeneous from intensely positive immunostaining in some tumour cells to absent protein expression in others; no LOH was identified. The authors hypothesised this was a phenocopy, or alternatively, that partial loss of SDHB expression could have been pathogenic (Saavedra et al. 2019).

**SDHD variants in PitNETs**

SDHD (OMIM*602690) is located on chromosome 11q23 and encodes the anchoring SDHD subunit (Fig. 1) (Baysal et al. 2000). Mutations in SDHD are responsible for familial PGL type 1. Maternal imprinting of this gene was presumed for some time due to the apparent exclusive paternal transmission of SDHD mutations. More recently, maternal transmission of SDHx mutations has been recognised to result in PGL (Figs 2B and 3) (Hensen et al. 2004, Yeap et al. 2011, Burnichon et al. 2017). The occurrence of a maternally inherited SDHD variant associated with PitNET has yet to be reported. There are currently eight cases of SDHD-related PitNETs in the literature, with an additional case described in this review. Of these nine patients, one had LOH, two had heterogeneous IHC and the majority (56%) did not have any analysis undertaken in tumour tissue (Table 1). The evidence for those cases subjected to a more in-depth analysis is discussed later.

The first SDHD variant-linked PitNET was reported in 2012 in a 37-year-old male diagnosed with somatotrophinoma and the c.298_301del (p.Thr100fs) variant (Xekouki et al. 2012). SDHD IHC showed reduced and patchy SDHD expression. LOH was identified. Two other patients were reported in 2014. A 60-year-old male with macroprolactinoma had the c.274G>T (p.Asp92Tyr) variant. Tumour cells lacked SDHB staining at IHC but expressed SDHA; preserved SDHA IHC being a recognised phenomenon in SDHB, SDHC and SDHD pathogenic variants (Oudijk et al. 2019). LOH was present. The evidence thus suggests a causative role of the SDHD variant (Papathomas et al. 2014). The second patient was a 56-year-old female with the same SDHD c.274G>T (p.Asp92Tyr) variant. She was diagnosed with macromatrotrophinoma. SDHA and SDHB expression was retained in tumour cells. No LOH was identified in the PitNET (Papathomas et al. 2014).

**SDHC variants in PitNETs**

SDHC is one of the anchoring subunits of the SDH complex. The gene (OMIM*602413) is mapped on chromosome 1q23.3 (Fig. 1). SDHC-associated PitNETs are less frequently reported (n=5) than those associated with SDHB and SDHD variants. To date, there has been no comprehensive report of PitNET secondary to a pathogenic SDHC variant (Fig. 4). The first PitNET associated with SDHC variant was reported in 2008. No IHC or LOH analysis was undertaken in tumour tissue (Table 1) (Lopez-Jimenez et al. 2008).
Two cases were reported in 2017 (De Sousa et al. 2017). A 34-year-old female with a microprolactinoma and a 63-year-old female with pituitary gangliocytoma and primary hyperparathyroidism (De Sousa et al. 2017). Both cases carried an SDHC variant of unknown significance c.403G>C (p.Glu110Gln, VarSome predicted benign). However, no GH expression was identified in the gangliocytoma by De Sousa and colleagues and no somatotrophinoma was present in the tissue submitted for pathological assessment. In addition, the expression of growth hormone-releasing hormone was not evaluated in tumour tissue. The microprolactinoma expressed SDHB by IHC, reinforcing the prediction that this is a benign variant (De Sousa et al. 2017). The most recently described patient was a 17-year-old male with cystic macroprolactinoma and the pathogenic variant c.405+1G>T (splicesite) (Mougel et al. 2020). Tumour cells expressed SDHB. No cytoplasmic vacuoles were present and no LOH was proven suggesting this case might be a phenocopy (Mougel et al. 2020). Other cases of SDHC-associated PitNETs have been described but without supportive tissue analysis (Table 1).

### SDHA variants in PitNETs

SDHA (OMIM*600857) is located on chromosome 5p15.33. To our knowledge, seven cases of PitNET in setting of SDHA variants have been reported (Dwight et al. 2013, Gill et al. 2014, Denes et al. 2015, Niemeijer et al. 2015). Of these cases, two had LOH and three had no SDHA and SDHB expression in neoplastic cells.

The patient described by Gill and colleagues was a 62-year-old male with a 30 mm cystic, clinically NF-PitNET (Gill et al. 2014). Neoplastic cells were positive for pro lactin and SDHA, whilst no staining for SDHB was present. No cytoplasmic vacuoles were described. Further analysis identified two inactivating somatic variants; a deletion on exon 6 (c.725_736del) and an insertion on exon 8 (c.989_990insTA) (Gill et al. 2014).

Another SDHA variant c.969C>T (p.Gly323Gly) variant (synonymous variant, predicted benign) was reported in a 53-year-old patient with an NF-PitNET and family history of NF-PitNET (father). The same patient had a history of nephroblastoma at the age of 1 year, 2 liposarcomas at 32 and 40 years, retroperitoneal PGL and renal oncocytoma both at the age of 50 years (Denes et al. 2015). The tissue from his PitNET did not show LOH or loss of SDHA and SDHB expression, suggesting that the SDHA variant was not causative. The variant c.969C>T was absent in his father’s NF-PitNET (Denes et al. 2015).

A male with SDHA c.1873C>T (p.His625Tyr) variant (VarSome uncertain significance, likely pathogenic) was diagnosed with NF-PitNET at the age 30 years (Dwight et al. 2013). SDHA and SDHB IHC showed no expression in the PitNET tissue. Paradoxically, the WT allele was retained; however, the authors suggested this might have been due to insufficient DNA to complete the analysis therefore missing the presence of an additional somatic second hit or alternatively failing to detect an epigenetic modification of the WT allele (Dwight et al. 2013). A 49-year-old female with SDHA c.91C>T (p.Arg31Ter) variant and macroprolactinoma was reported by Niemeijer and colleagues. Tumour tissue showed no SDHB and SDHA expression alongside LOH, suggesting the SDHA variant was contributory (Niemeijer et al. 2015).

The most recent case of SDHA variant was reported in a 37-year-old male with SDHA c.1753C>T (p.Arg585Trp) variant and macroprolactinoma. Surgery was undertaken due to poor compliance with medical therapy. Analysis of the tissue revealed SDHB staining, no vacuoles and no LOH, suggesting a phenocopy (Mougel et al. 2020).

### SDHAF2 variants in PitNETs

The gene encoding the SDH assembly factor 2 (OMIM*613019) is mapped on chromosome 11q12.2. To our knowledge, no evidence supportive of a causative SDHAF2 variant in PitNETs has been reported (Fig. 4).

### PitNET and PPGL in the setting of SDHX variant without tumour analysis

Many other reports have described PitNETs with PPGL associated with SDHX variant without tissue-based analysis to support a causative role for an SDHX variant. It is therefore possible that a considerable proportion of these cases could be phenocopies. A summary of SDHX-associated PitNET and PPGL including such cases is outlined in Table 1.

### MAX: pathophysiology

MAX codes for the MAX protein, a component of the MYC signalling pathway. The protein forms heterodimers with C-MYC via basic-helix-loop-helix zipper (bHLHZ) domain interactions. These heterodimers can then bind...
to target DNA sequences or E-BOX sequences to regulate transcription of genes involved in cell proliferation and cell growth (Fig. 5). Like SDHx, epigenetics may play a role in MAX-associated tumorigenesis. Notably, the same microRNA (miR-210) implicated in SDHx-related disease has roles in MNT/MAX/MYC-mediated cellular proliferation (Walker et al. 2005, Zhang et al. 2009).

**MAX variants in PitNETs**

MAX is located on chromosome 14q23.3 and appears to behave as a tumour suppressor gene with inactivating mutations resulting in a failure of dimerisation with MYC and unchecked downstream gene transcription. Germline and somatic MAX variants can result in familial and sporadic PPGL, respectively (Burnichon et al. 2012). MAX variants have been reported in the setting of uniparental disomy, with a tendency towards paternal transmission, like that seen in SDHD and SDHAF2 (Comino-Méndez et al. 2011, Burnichon et al. 2012).

PitNETs have been reported in the setting of germline MAX variants. The possibility of MAX-associated syndromic disease being defined as multiple endocrine neoplasia type 5 has been mooted (Seabrook et al. 2021). Sporadic isolated or familial isolated pituitary adenoma in association with MAX variant has not yet been reported. One possible case of familial acromegaly with germline MAX variant (c.223C>T (p.Arg75Ter), VarSome pathogenic) has been documented (Mamedova et al. 2021), but the details on transmission are limited as the proband’s father was deceased. Based on old photographs showing acromegalic features, a history of receiving pituitary radiotherapy and sudden death (classical presentation of undiagnosed phaeochromocytoma), a familial syndromic disease with pituitary involvement in MAX germline variant seems possible (Mamedova et al. 2021).

Although microscopic features have not been reported for any MAX-associated PitNET, it appears they belong to the PIT1 lineage (prolactinomas and somatotrophinomas) (Roszko et al. 2017, Daly et al. 2018, Kobza et al. 2018, Seabrook et al. 2021). A report documented a 25-year-old presenting with hyperprolactinaemia responsive to cabergoline and a large PitNET. The patient re-presented at the age of 38 years with acromegaly. It is possible that the lesion was a mammosomatotroph or mixed somatotroph–lactotroph PitNET with growth hormone excess only becoming clinically evident later in life. The distinction
between primary and secondary acromegaly has been a challenge in a kindred with a phaeochromocytoma expressing growth hormone-releasing hormone (Seabrook et al. 2021).

Investigative strategy for tumours in the setting of SDHx and MAX variants

Histopathological analysis

Cytoplasmic vacuoles and/or nuclear pseudo-inclusions and inclusions are a feature of SDHx-associated PitNETs (Denes et al. 2015, Tufton et al. 2017). Optically clear pseudo-inclusions are cytoplasmic invaginations into the nucleoplasm whilst inclusions result from the accumulations of proteins within the nucleus (Ip et al. 2010). The exact nature of nuclear inclusions can be difficult to establish, but they have been observed in the pituitaries of Sdhh⁻/− mice (Xekouki et al. 2015). There is some evidence that SDHx variants can have structural and functional consequences on the mitochondrial assembly complex and mitochondrial cristae (Gimenez-Roqueplo et al. 2001, Kim et al. 2015, Xekouki et al. 2015) and that fragmented mitochondria can be engulfed by cytoplasmic vacuoles before being extruded (Nakajima et al. 2008). Whether damage to mitochondria causes vacuoles and nuclear pseudo-inclusions/inclusions is yet to be proven (Tufton et al. 2017, MacFarlane et al. 2020). Neuropathologists should be aware of these morphological appearances and report tumours with prominent cytoplasm vacuolisation, raising the possibility of a germline SDHx variant as such a diagnosis has repercussions on genetic screening and familial counselling (Tufton et al. 2017). PitNET types and subtypes reported in association with SDHx variants include mainly prolactinomas, somatotrophinomas and clinically NF-PitNETs (Fig. 4). Five corticotroph PitNETs have been reported. Two in patients with a likely pathogenic variant and three in patients with likely non-pathogenic variants. Thyroid-stimulating hormone-secreting tumours are yet to be reported.

The introduction of lineage restricted pituitary transcription factors (PIT1, TPIT and SF1) and of GATA3 by immunohistochemistry will improve the identification of cell lineages of SDHx-associated NF-PitNET. There is little evidence to suggest that standard proliferative markers such as Ki-67 or mitotic count are increased in SDHx-mutated PitNETs. As mentioned, the light microscopic features of PitNETs in the setting of a germline MAX variants have never been documented. Clinically, MAX-associated PitNETs are similar to SDHx with a predominance of tumours causing hyperprolactinaemia and acromegaly.

SDHA, SDHB and MAX IHC

Immunostains for SDHB and SDHA show positive granular cytoplasmic staining in non-SDHx-mutated cells (van Nederveen et al. 2009). Bi-allelic inactivation of any SDHx genes can result in degradation of SDHB. Absence or weak SDHB staining can therefore be supportive of SDHx variants being contributory to disease (Gill 2018). In one study, lack of SDHB expression at IHC demonstrated a sensitivity of 100% and specificity of 84% (van Nederveen et al. 2009). Studies have suggested that SDHB IHC can be positive in the setting of SDHA and SDHD variants. This finding is interesting and requires further investigation (Ugarte-Camara et al. 2019, Sato & Inomoto 2020, Snezhkina et al. 2020). False positive staining may account for this, but other possibilities include haploinsufficiency or a somatic mutation that may result in a dysfunctional SDH complex, which is still detectable by IHC (Ugarte-Camara et al. 2019). SDHB IHC is a cheap, reliable, readily available and quick test to screen tumours with vacuolar changes. However, a diagnostic algorithm suggested considering confirmatory functional tests (LOH or metabolomics) regardless of the SDHB IHC results, which can be employed as a screening step (MacFarlane et al. 2020).

The immunostain for MAX can also be used to assess its involvement in the pathogenesis. Expression in tumour cells theoretically refutes variant pathogenicity; however, in one of the studies, positive MAX IHC was seen in 3 out of 16 phaeochromocytomas with pathogenic MAX variant in the presence of LOH (Buchinon et al. 2012). This suggests, similarly to SDHB staining, a cautious and thorough approach to interpretation of MAX IHC should be considered.

Loss of heterozygosity

LOH can support the tumorigenic role of a variant, but LOH is not confirmatory. In a small series of phaeochromocytomas, four of five SDHB-mutated and two of four SDHD-mutated cases demonstrated LOH, suggesting alternative genetic mechanisms (Weber et al. 2012). Methylation has been heavily implicated in SDHx-related disease as alternative mechanism causing silencing of the WT allele. Other possible mechanisms include haploinsufficiency or an additional variant in an alternative gene. Searching for LOH may hold more weight in tumours with MAX variants, with 16/18 tumours in one study demonstrating LOH and the 2 without LOH carrying
MAX variants of unknown significance (Burnichon et al. 2012, Seabrook et al. 2021).

Metabolomics

Metabolomics is a technique used to assess the biochemical functional status of cells/tissue samples via analysis of small molecule metabolites using NMR spectroscopy or mass spectroscopy and can be performed *ex vivo* or *in vivo*. The technique can be used in targeted and non-targeted approaches. SDHx mutations result in disruption of the SDH complex, leading to a break in Krebs cycle and accumulation of succinate. Therefore, succinate can be measured as a surrogate marker for defective SDH. Metabolomics with *in vivo* magnetic resonance spectroscopy has been utilised in assessing PitNET tissue of an SDHB variant carrier in one previous instance. Results did not show any accumulation of succinate (Casey et al. 2018). The following case description highlights the contribution of metabolomics in assessing the pathogenic function of SDHx variants.

Case description

A 32-year-old male with maternally inherited pathogenic SDHD c.242C>T (p.Pro81Leu) variant (Yeap et al. 2011, Xekouki et al. 2012, Denes et al. 2015) was diagnosed with acromegaly (insulin-like growth factor-1 105.3 nmol/L; age-adjusted reference range 11.6–32.2 nmol/L; nadir growth hormone 9.3 ng/mL on oral glucose tolerance testing) and concomitant hyperprolactinaemia (3182 mIU/L; reference range 63–245 mIU/L). He had secondary hypogonadism. The other tests of anterior pituitary function were normal. Serum prolactin and insulin-like growth factor-1 levels were found in the AIP, MEN1 and CDKN1B genes. The patient’s father did not have SDHx variants. The patient underwent transsphenoidal surgery. Light microscopic features of the resected PitNET are shown in Fig. 6B.

Given the young age, tumour histotype and the presence of cytoplasmic vacuoles, the possibility that the SDHD variant resulted in the tumour was considered. In addition, the same SDHD exon 3 c.242C>T (p.Pro81Leu) missense variant resulting in maternally transmitted disease was also previously reported (Hensen et al. 2004, Denes et al. 2015, Xekouki et al. 2015). SDHB IHC showed normal expression and metabolomic profiling confirmed that the SDHD variant was not tumorigenic (Fig. 6B and C). This result has informed the future screening strategy for this patient and his family.

The performance of metabolomics appears far superior to SDHB IHC. The detection of succinate as an SDH-related tumour screening test has shown remarkable sensitivity and specificity (Imperiale et al. 2015). The succinate:fumarate ratio also has excellent performance with sensitivity and specificity of 93% and 97%, respectively (Richter et al. 2014). In a recent assessment of IHC vs novel metabolomics and machine learning techniques, IHC resulted in a specificity of 86.7–93.8% in PPGL vs 99.2% of metabolomics (Wallace et al. 2020). The sensitivity of both techniques was comparable (85.2% for SDHB IHC and 88.1% for metabolomics) (Wallace et al. 2020).

Metabolomics has predominantly been applied to tumour tissue. Its application to liquid biopsy and the possibility of obtaining rapid results on urine or blood to detect accumulated metabolites circulated from SDHx-related tumours could change future clinical practice (Martins et al. 2019). However, further data and understanding of the peripheral metabolomics signatures of heterozygous carriers vs affected patients must be developed before the technique can be routinely implemented in the clinical setting.

Management of SDHx-mutated PitNETs

Just over 50% of the PitNET tissue examined in the literature to date shows evidence of SDHx variants playing a role in tumour development. No comprehensive study performing whole exome or genome sequencing has been performed. Therefore, the causative role of an SDHx variant can only be confirmed in a small number of PitNETs. The evidence to suggest that SDHx-mutated PitNETs should be managed any differently to sporadic PitNETs is inconclusive. That said, it is evident that the incidence of PPGL in PitNET patients is significantly higher than expected (2 in 828 cases vs 0.33 expected) (Denes et al. 2015). Moreover, a mechanism has been established in an animal model suggesting that not all PitNET-PPGL cases are coincidental. Clinicians should be mindful of the potential for dual endocrine pathology and consider having a lower threshold to screen for PPGL in PitNET patients. In patients with PitNET in association with SDHx variant, it may be prudent to consider annual serum prolactin and insulin-like growth factor-1 levels during follow-up. In addition, screening MRIs should include imaging of the neck and may visualise some of the skull base so large PitNETs could be detected by standard SDHx variant radiological follow-up.

Sixty-seven percent of the reported SDHx-associated PitNETs and 100% of PitNETs with a causative SDHx variant were larger than 1 cm (macro) at diagnosis (Fig. 4), but tumour size and even invasion do not necessarily indicate
aggressiveness. The pituitary carcinoma reported by Tufton and colleagues might indicate a potential aggressive behaviour of SDHx-mutated PitNETs (Tufton et al. 2017). Notably, both the carcinoma and metastases responded to the alkylating agent temozolomide. Resistance to first-generation somatostatin receptor ligand has been reported in one case (Xekouki et al. 2012). The majority of reported prolactinomas have responded well to dopamine agonists. In one case of macroprolactinoma and dopamine-secreting PGL in the setting of SDHC germline variant,
the authors described the PGL responding to dopamine agonist therapy intended to treat the lactotroph PitNET. They highlighted the potential clinical pitfall of dopamine agonist therapy lowering 3-methoxytyramine levels and obscuring biochemical evidence of PGL metastases (Hussein et al. 2021).

The pseudohypoxia induced by accumulation of succinate is likely to cause changes in the tumour microenvironment. This may be a fruitful avenue for biomarkers and therapeutic targets in aggressive SDHx-mutated PitNETs. Metabolic profiling has documented increased levels of methionine, glutamine and myoinositol in SDHx-related PPGls (Imperiale et al. 2015), indicating that targeting of metabolic pathways could have future therapeutic potential in these rare PitNETs.

**Management of MAX-associated PitNETs**

There is not enough evidence on PitNETs in the setting of MAX germline variants to comment on management and on behaviour. MAX-associated lactotroph tumours seem to have a good biochemical response to dopamine agonists. The response of MAX-associated somatotroph tumours to somatostatin receptor ligands has been less convincing and multimodal therapies have been required (Roszko et al. 2018, Kobza et al. 2018, Seabrook et al. 2021).

**Conclusions**

PitNETs caused by SDHx and MAX variants are rare. Several studies reported co-existing PPGL and PitNET with SDHx variant, but many of them did not perform tumour tissue analysis.

The immunoreactions for SDHB and MAX and LOH analysis are useful tools to support or refute the contribution of SDHx and MAX variants to disease, but these techniques have limitations. For this reason, metabolic profiling of SDH-associated disease is likely to have an important role in the future.

A vast amount remains to be learned about PitNET pathogenesis in the setting of SDHx and MAX variants and particularly on the role of pseudohypoxic and kinase signalling pathways in pituitary disease, which may reveal novel biomarkers and medical therapies. Pituitary tumours thought to be caused by SDHx and MAX variants are indeed rare. While the data does not firmly establish that the presence of these variants predicts future tumour behaviour, close follow-up of these patients would seem prudent.
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