A review of the tumour spectrum of germline succinate dehydrogenase gene mutations: Beyond phaeochromocytoma and paraganglioma

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Abstract

The citric acid cycle, also known as the Kreb’s cycle, plays an integral role in cellular metabolism and aerobic respiration and mutations in genes encoding citric acid cycle enzymes including; succinate dehydrogenase, fumarate hydratase and malate dehydrogenase predispose to hereditary tumour syndromes. The succinate dehydrogenase enzyme complex (SDH) is a key enzyme coupling the oxidation of succinate to fumarate in the citric acid cycle and the reduction of ubiquinone to ubiquinol in the electron transport chain. A loss of function in the succinate dehydrogenase (SDH) enzyme complex is most commonly caused by an inherited mutation in one of the four SDHx genes (SDHA, SDHB, SDHC and SDHD) and was first implicated in familial phaeochromocytoma and paraganglioma. However, over the past two decades the spectrum of tumours associated with SDH deficiency has been extended to include; gastrointestinal stromal tumours (GIST), renal cell carcinoma (RCC) and pituitary adenomas. The aim of this review is to describe the extended tumour spectrum associated with SDHx gene mutations and to consider how functional tests may help to establish the role of SDHx mutations in new or unexpected tumour phenotypes.
**Introduction**

Disruption of the SDH complex results in accumulation of the ‘oncometabolite’ succinate, which is shuttled from the mitochondrial matrix to the cytoplasm and drives tumorigenesis by inhibiting 2-oxoglutarate-dependent dioxygenases, including DNA and histone demethylase enzymes and hypoxic gene response regulators. As a consequence, SDH-deficient tumours demonstrate epigenetic abnormalities, namely hypermethylation and an activated hypoxic gene response (1)(2).

Pathogenic germline variants in each of the four genes (*SDHx*) encoding the four sub-components of this complex (SDHA/B/C/D) have been implicated in hereditary tumourigenesis. *SDHx* mutations are heterozygous mutations inherited in an autosomal dominant fashion and tumorigenesis is initiated following bi-allelic loss as per the Knudson ‘two hit’ hypothesis. An additional inheritance mode has also been identified for *SDHD*, namely a parent of origin effect, where disease penetrance is almost exclusively dependent on paternal transmission (3).

Currently the accepted spectrum of hereditary tumours associated with germline *SDHx* mutations include; phaeochromocytoma (PC) and paraganglioma (PGL) (4), gastrointestinal stromal tumours (GIST) (5), renal cell carcinoma (RCC) (6) and pituitary adenomas (7). Surgery is the only curative treatment option for patients with SDH deficient tumors as effective treatment options are limited and palliation is often the only benefit of these patients (8). There is a need for further development of targeted therapies and current research studies are focusing on targets such as; demethylation, HIF2A inhibition and inhibition of glycolysis in SDH deficient tumours (9).

Surveillance for *SDHx* gene carriers is predominately focused on the early detection of PC and PGL, which is the most common *SDHx* associated phenotype. Initially the penetrance of
SDHB variants was estimated at 70-80% (10), but as genetic screening for asymptomatic relatives has become more common place, it is now clear that SDHx mutations are associated with an age dependant and incomplete penetrance. A recent study comparing allelic frequencies has estimated the lifetime penetrance for SDHB was estimated at 20%, whereas it was significantly lower for SDHA and SDHC (<1.8% and <8.3%, respectively) (11). Surveillance should therefore be tailored to the individual SDHx gene subunit and should include interval cross-sectional imaging from skull base to pelvis and plasma or urine metanephrine testing(12) (13). However, interval imaging increases the risk of incidental findings which may or may not be related to the patient’s genotype. Unexpected or incidental findings on imaging can also negatively impact on the psychological wellbeing of the affected individual and their family. Psychological and emotional distress are common amongst patients with hereditary cancer syndromes including those with hereditary PPGL syndromes (14). Patient reported triggers provided by patient advocacy groups include; annual screening, decisions about treatment and surgery and new tumour diagnoses (15). This impact on patient quality of life must be carefully considered when deciding on the most appropriate surveillance and clinical management strategies, and having a good understanding of the expected SDHx related phenotype is key to being able to effectively counsel patients on their life long tumour risks. Furthermore, determining whether an incidental tumour is related to a patient’s germline SDHx mutation may have future therapeutic implications as more targeted therapies for SDH deficient disease are developed and introduced into clinical practice.

The aims of this review are to; i) Describe the spectrum of SDHx related disease beyond phaeochromcoytoma and paraganglioma, and ii) provide a recomended approach to an incidental or unexpected tumour phenotype in patients with germline SDHx gene mutations.
**Methods:**

i) Literature review

A MEDLINE search was performed using PubMed and the following search terms i) ‘SDH’ or ‘succinate dehydrogenase’ or ‘succinate dehydrogenase gene’ and ii) ‘thyroid cancer’, iii) ‘breast cancer’, ‘breast tumour’, ‘cancer’, ‘tumour’, ‘neoplasia’ ‘pituitary adenoma’, ‘pituitary tumour’. Reference selection was based on cases with a proven germline $SDHx$ mutation and a tumour other than PPGL, GIST or RCC.

i) **SDH deficient pheochromocytoma and paraganglioma**

A phaeochromocytoma (PC) is a tumour arising from the adrenal medulla and a paraganglioma (PGL) refers to its extra adrenal counterpart, which can develop from sympathetic or parasympathetic tissue anywhere between the skull base to the pelvis. Pathogenic variants in one of the four $SDHx$ genes account for 30-40% of hereditary PC and PGL cases (16) and PC and PGL are the most common tumours arising in patients with germline $SDHx$ mutations and tumours can be multi-focal and synchronous. The lifetime risk of malignant PC and PGL with pathogenic $SDHx$ variants is approximately 10-20%, with the highest risk observed in patients with germline $SDHB$ mutations (17). $SDHB$ gene mutations are more commonly associated with abdominopelvic and head and neck PGL, and a lower risk of developing a PC or bilateral PC compared with $SDHD$ gene mutations. The mediastinum is an uncommon site for PGL development but is more commonly reported for $SDHB$ gene carriers than $SDHD$ (13) (Table 1).

$SDHD$ is the most common $SDHx$ gene implicated in head and neck paraganglioma (HNPGL) and tumours are frequently multifocal with a lower malignant potential compared
to SDHB mutant tumours (13) (Table 1). HNPGL can arise from the carotid bifurcation, near the jugular vein, vagus nerve or glomus tympanicum. Rare cases of SDH deficient PGL arising from the larynx, thyroid or oral cavity including the tongue have been reported (18). Importantly, malignant PPGL have been reported in association with all SDHx subunit genes and metastatic recurrence may present many years after initial presentation (19).

SDH deficient PC and PGL have reduced expression of a catecholamine conversion enzyme called Phenylethanolamine N-methyltransferase (PNMT) due to poor differentiation of paraganglia cells, resulting, biochemically, in a predominant noradrenergic secretory pattern (20). Expression of the enzyme tyrosine hydroxylase is also affected in SDH deficient PC and PGL so SDH deficient PC and PGL may be non-secretory and therefore biochemical testing alone is inadequate for the detection of SDH deficient PPGL (21).

ii) SDH deficient Gastrointestinal stromal tumours (GIST)

Gastrointestinal stromal tumors (GISTs) are the second most common tumour associated with SDHx mutations after PPGL (16). GISTs are the most common mesenchymal tumour of the gastrointestinal tract and the majority of GISTs occurring in adults are driven by somatic activating mutations in the receptor tyrosine kinase genes KIT (22) and platelet derived growth factor alpha (PDGFRA) (23). Wild type gastrointestinal stromal tumours, refer to those tumours that are negative for KIT and PDGFRA somatic gene variants (24)(25). These account for 15% of adult and 85% of paediatric GIST. The reported frequency of SDH deficient GIST ranges between 40% and 88% of wild type GIST (25)(26). With the exception of a single case of small bowel SDH deficient GIST reported to date, SDH deficient GIST occur in the stomach (27). Patients often present with multifocal tumours along the stomach wall and SDH deficient GIST have a characteristic histological epithelioid appearance.
SDH deficient GIST have a high tendency to metastatic spread both locally and to distant sites. Observational studies suggest that metastatic disease can be indolent, overall the clinical course of these rare tumours remains unpredictable (28).

The majority of SDH deficient GISTs are related to a germline $SDHx$ mutation. A strong genotype-phenotype correlation exists for $SDHA$ germline variants (Table 1), which account for 47% of reported SDH deficient GIST cases (16). The remaining SDH deficient GIST (without a germline $SDHx$ variant) are caused by CpG island hypermethylation in the promoter region of the $SDHC$ gene also referred to as an $SDHC$ epimutation. $SDHC$ epimutations predisposes to Carney’s triad defined by synchronous or metachronous GIST, PGL and pulmonary chondroma (25)(26)(29).

### iii) SDH deficient renal cell carcinoma

SDH deficient RCC account for approximately 0.2% of all cases of RCC (30) and a SDHB germline variant has been described in 83% of SDH deficient RCCs (16) (Table 1). Patients with germline $SDHB$ variants have an estimated 14% life time risk of developing an $SDH$ deficient RCC(31)(30)(32). Germline variants in the other three subunits; $SDHA$ (33), $SDHC$ (34), $SDHD$ (35), have also been associated with hereditary RCC and SDH deficient RCC cases can mimic sporadic RCC, with no family history of RCC or PPGL(36). With the exception of $VHL$, mutations in the $SDHB$ gene are the most common reported cause of familial pheochromocytoma and renal cell carcinoma (37). We have identified a higher than expected proportion of deletions in $SDHB$-associated renal tumours and PPGL in our cohort, highlighting the need to consider analysis for exonic deletions/duplications in patients presenting with SDH deficient RCC (37).
SDHB deficient renal carcinoma have a unique morphology consisting of solid architecture, intra-cytoplasmic inclusions and intra tumour mast cells (Figure 1). SDH deficient RCC was accepted as a separate sub-type of RCC in the 2013 International Society of Urological Pathology Vancouver Classification (38). SDH deficient RCC have been described to present bilaterally in up to 26% the cases and with metastatic disease in 33% of patients (39). The risk of metastatic disease is believed to be highest for those patients with a high histological grade, specific histological features such as coagulative necrosis and tumours with features of de-differentiation (30)(40).

iv) **SDH deficient pituitary adenomas**

Pituitary adenomas (PAs) are benign proliferations of the anterior pituitary gland. Clinically significant PAs have a prevalence of approximately 1:1000 (41). Unselected cohorts of PAs have relatively low rates of SDH deficiency and unpublished data from our centre has shown 1 case in 150 unselected surgical PA cases (0.7%). This is in-keeping with published data suggesting a prevalence of 0.3-1.8% in unselected PA cases (42)(43). A recent study reviewing the frequency of germline genetic mutations in a select cohort of patients with a history of PPGL and pituitary tumours, demonstrated that SDHx mutations were the most common germline mutation observed (19/82 cases (23%)) (43).

Vacuolated clear cytoplasm has been documented as a characteristic histological hallmark of SDH deficient PA (44) and it has been hypothesised these may be by-products of autophagy of abnormal mitochondria (43) (Figure 1). Definitive description of the SDH-deficient PA clinical phenotype is difficult given the low number of reported cases thus far, but the majority of the reported PAs are of PIT-1 lineage (lactotropinomas or somatotropinomas) although isolated reports of silent gonadotroph adenomas and corticotropinomas have been documented (43). There is no clear genotype-phenotype correlation for SDH deficient PA.
and mutations in all four $SDHx$ subunit genes have been reported to associate with the presence of these tumours (43). Reported cases of SDH deficient PA have frequently required more than one type of treatment modality suggesting a more aggressive phenotype and/or treatment resistance (43). A recent report of a recurrent and metastatic SDHB deficient pituitary carcinoma presenting a decade after initial diagnosis supports the general assumption that SDH deficiency may be associated with a more aggressive pituitary tumour phenotype (45).

v) SDH deficient thyroid neoplasia

Five cases of epithelial thyroid cancers with concurrent PPGL and underlying germline $SDHx$ mutations have been reported to date (Table 2). A further 62 cases of differentiated thyroid cancer alone have been reported in patients with germline $SDHx$ mutations, however none of these cases have demonstrated SDHB immunonegativity or loss of heterozygosity to support the diagnosis of SDH deficient thyroid neoplasia (ST1). Given the high prevalence rate of thyroid cancer, it is possible that incidental thyroid tumours are being identified in $SDHx$ carriers undergoing frequent radiological surveillance of the neck. Indeed, two population-based studies looking at $SDHx$ cohorts [295 $SDHB$ (31) and 417 $SDHB/D$ (46)] reported rates of thyroid neoplasia similar to that of the general population, although in one study, thyroid neoplasia was detected in two young $SDHx$ carriers aged 14 and 26 years (46). Papathomas et al performed SDHB immunohistochemistry on 60 papillary thyroid carcinoma (PTC) samples from patients with unknown $SDHx$ germline mutational status and one case of PTC from a patient with a pathogenic $SDHD$ variant was identified (47).
Another hypothesis supports a pathway signal interaction between the tumour suppressor gene phosphatase and tensin homolog (PTEN) and germline SDHx variants, inducing a ‘Cowden syndrome-like’ tumorigenesis (48)(49). This notion is based on the observation that the co-occurrence of germline SDHx mutations and germline PTEN mutations conferred a higher risk of breast and thyroid cancers over those with germline PTEN mutations alone (48); however, these studies did not include functional analysis aimed at demonstrating SDH deficiency in the thyroid tumours (48)(49). Furthermore, in the study by Ni et al, 4/10 cases identified with co-existing PTEN and SDHx germline mutations had a germline SDHD variant p.(Gly12Ser), which is now classified as a benign single nucleotide variant (48).

A large series examining 754 cases of sporadic differentiated thyroid cancer identified underlying germline SDHx variants (SDHB, SDHC or SDHD) in 48 (6%) cases however it is notable that 16 of these referred to the same benign germline SDHD variant p.(Gly12Ser) (50).

vi) ‘Other’ SDH deficient tumours

A range of malignancies have been reported in patients with germline SDHx mutations (ST2). We have identified eight cases in which putative ‘extended spectrum’ malignancies have occurred in patients with a germline SDHx mutation and concurrent PCC/PGL or RCC (ST2). These eight cases include three unpublished cases from our research group (one breast adenocarcinoma, one follicular lymphoma and one case of a rectal adenocarcinoma co-occurring with a GIST in the same individual) and six additional cases identified from the literature (ST2). Only two of these cases, demonstrated SDHB immunonegativity (a pancreatic neuroendocrine tumour (NET)(51) and a case of Hodgkin’s lymphoma(52) and additional analysis of the pancreatic NET also demonstrated loss of heterozygosity, thus supporting causality.
A further 28 cases of ‘other’ tumours in individuals with germline SDHx mutations but without concurrent PCC/PGL were identified (ST2). Only one of these cases, a neuroblastoma in a 3-year-old boy with a germline pathogenic SDHA variant, had reported SDHB immunonegativity and chromosomal copy-number loss in the tumor cells encompassing the SDHA gene locus, supporting the causative role of the germline SDHA mutation in this tumour(53).

vii) Confirming SDHx variant pathogenicity

a) Loss of heterozygosity

The SDHx genes are tumour suppressor genes and therefore follow the Knudson ‘two-hit hypothesis’, requiring a mutation in both alleles for initiation of tumorigenesis. Loss of heterozygosity (LOH) has been identified as one of the most common mechanisms accounting for the ‘second hit’ and is an accepted functional assessment tool for confirming variant pathogenicity for tumour suppressor genes (55). However, LOH appears to be a less common mechanism causing loss of activity of the wild-type allele in SDH deficient disease. A study evaluating the frequency of second allele inactivation in syndromic phaeochromocytoma identified an LOH related inactivation of the second allele in 80% of SDHB but only 50% of SDHD related PC and PGL (56). Similarly, in a study analysing SDHA mutated wild-type GIST, only 36% of tumours exhibited LOH (26). In those cases where inactivation of the wild type allele is not identified, further analysis to investigate the effect of methylation or inactivation of other genes on the activity of the wild type allele should be considered as well as functional assessment of SDH enzyme function or protein expression as an additional step to confirm SDH deficiency (Figure 2).
b) SDHB immunohistochemistry

Bi-allelic inactivation of any $SDHx$ gene can cause destabilisation of the SDH enzyme complex, which leads to proteolytic degradation of the anchor protein, SDHB. This can be visualised by loss of staining for the SDHB protein by immunohistochemistry (57). A loss SDHB staining is now a validated method for diagnosing pathogenic germline variants in the $SDHx$ genes and can provide a rapid assessment of pathogenicity for a novel $SDHx$ variant of uncertain significance (58). SDHA antibody testing directed against the SDHA protein is also available and predicts pathogenic variants in the $SDHA$ gene (59). A specific IHC for the $SDHD$ gene was reported in a retrospective study in 2015, and those tumours harbouring a variant in SDHD were immunonegative for SDHB but demonstrated preservation of the SDHD protein compared to those tumours that did not harbour a variant in $SDHD$, where expression of SDHD was absent (60). These data suggest that in equivocal cases of SDHB IHC, the addition of SDHD IHC may be useful for determining pathogenicity of SDHx gene variants in PPGL.

The clinical application of SDHB IHC has been demonstrated in a number of clinical studies and includes; i) the early diagnosis of SDH deficient tumours such as PPGL, wild type GIST and specific histological subtypes of RCC (Figure1), ii) as a biomarker for malignant risk in PPGL(61), iii) as a functional tool for the assessment of $SDHx$ variant pathogenicity and iv) to identify SDH deficient tumours caused by somatic $SDHx$ variants or those tumours harbouring an epimutation in the $SDHC$ gene (29)(58). Furthermore, SDHB IHC can be utilised to investigate the potential that a new tumour phenotype in a patient with a known germline $SDHx$ variant is SDH deficient. For example, Papathomas et al used SDHB IHC to identify non-PPGL SDH deficient tumours in 26 $SDHx$ mutation carriers and identified eight SDH deficient non-PPGL tumours including; one ganglioneuroma, one pituitary adenoma, two wtGIST and three SDH deficient RCC.
cases (51). A loss of heterozygosity supported the SDHB IHC results in 7/8 SDH deficient tumours in this study (51).

Similarly, our research group has applied SDHB IHC to investigate 20 non-PPGL tumours from 17 patients with germline SDHx mutations (unpublished data). Six of the 20 tumours analysed showed evidence of SDH protein loss on SDHB IHC and these included five wild type GIST and one RCC (Figure1). Five tumours not ordinarily associated with the SDHx spectrum of disease from patients with germline SDHB mutations were included in this analysis; such as a breast cancer, one papillary thyroid cancer, one follicular lymphoma and a colorectal adenocarcinoma and a rectal GIST in the same individual (Figure 1). All five tumours had preserved SDHB expression on SDHB IHC and no LOH was noted for 2/5 samples analysed (Figure1) (ST1+2). All four patients who presented with these atypical tumours had a SDH deficient PPGL as an index tumour, suggesting again that patients may present with synchronous or metachronous tumours over their lifetime which may not be associated with their germline SDHx variant and emphasises the need to assess each tumour individually.

Important limitations of SDHB IHC include inter-observer variation and the risk of false negative results particularly notable for SDHA and SDHD mutated tumours (57)(58). The specificity of SDHB IHC can also be affected by pathogenic somatic or germline VHL variants, which can lead to an equivocal SDHB staining pattern on immunohistochemistry (37). Finally, there is also a risk of false negatives with certain missense variants in the SDHx genes, which may affect enzyme activity without affecting the SDH complex stability.

c) Metabolomics

The accumulation of the ‘oncometabolite’ succinate as a result of SDH deficiency is believed to be the main driver in tumourigenesis and studies have demonstrated that succinate
concentrations are elevated 25-fold in $SDHx$ mutated tumours compared to non-$SDHx$ mutated tumours (62). Analysis of tissue metabolites including succinate requires 10-50mg of tissue and employing techniques such as nuclear magnetic resonance (NMR) spectroscopy and mass spectroscopy. Metabolites are assigned using standard metabolite chemical shift tables available in the literature (63) and succinate appears at 2.4ppm. Richter et al analysed a ratio of succinate : fumarate concentration in PPGL and found that a ratio cut off of 97.7 had a diagnostic sensitivity for SDH deficient PPGL of 93% and a specificity of 97% (62). Imperiale and colleagues used high resolution magic angle spinning (HRMAS) nuclear magnetic resonance (NMR) mass spectroscopy and also identified higher succinate concentrations in $SDHx$ mutated PC and PGL compared to sporadic PC and PGL(64). Our research group has also applied HRMAS NMR spectroscopy to investigate succinate accumulation in PC and PGL and wild-type GIST tumour samples and have demonstrated that succinate concentrations were 15 fold higher in $SDHx$ mutated tumours compared to non-SDH mutated tumours (65), highlighting that metabolite profiling can also be applied to non-PC/PGL tumours.

Limitations of *ex-vivo* metabolite analysis include the requirement for adequate tissue samples and the potential for the surgical procedure and storage conditions to introduce variance in the metabolomics profile. These limitations can be overcome by moving the analysis of tumours to the *in vivo* setting using high magnetic resonance spectroscopy $^1$H-MRS. A recent study by Lussey-Lepoutre and colleagues demonstrated that succinate accumulation *in vivo* can be detected using $^1$H-MRS and has a sensitivity of 87% and a specificity of 100% for the diagnosis of an SDH deficient PC and PGL (66). We have previously published our experience using $^1$H-MRS to detect succinate accumulation *in vivo* and have described the clinical utility of $^1$H-MRS in suspected SDH deficient disease (PC/PGL and non PC/PGL tumours) including the application of $^1$H-MRS to determine
whether a pituitary adenoma in a SDHB mutation carrier was SDH deficient (67). *In vivo* analysis of the latter did not identify a succinate accumulation which correlated well with a preserved SDHB protein expression using IHC and no evidence of LOH in the tumour (67). Our data highlights the potential application of $^1$H-MRS to investigate non-PC/PGL tumours for evidence of SDH deficiency in patients with SDHx gene mutations.

**viii) Recommended surveillance strategies for SDHx gene carriers**

The Endocrine Society guidelines recommended that asymptomatic carriers of pathogenic SDHx variants undergo regular surveillance in order to facilitate early detection of tumours, enable early intervention and reduce the morbidity and mortality associated with undiagnosed tumours or delayed presentation (12). It is worth noting that current surveillance programs are directed at the detection of PC and PGL, the most common SDHx related phenotype. In recent years, as information regarding disease penetrance of the pathogenic SDHx variants and further genotype-phenotype data has emerged, it has become apparent that the approach to clinical surveillance should be tailored to the SDHx subunit gene affected (13). Furthermore, surveillance programs will differ between probands (who will often require more frequent follow up) and asymptomatic gene carriers.

This group has previously recommended that surveillance begin at the age of 5 years for asymptomatic SDHB carriers and at age 10 years for SDHA, SDHC and SDHD carriers and continues life-long, if acceptable to the patient. The current recommendation is that annual clinical review is performed in addition to measurement of plasma metanephrines or urine metanephrines (12). We recommend that asymptomatic SDHx gene carriers are offered a full body MRI scan, which includes of the neck, thorax, abdomen and pelvis at diagnosis and at varying intervals thereafter depending on the SDHx subunit gene, annual biochemical testing
and patient symptoms (Table 2). MRI is the preferred imaging modality because it is radiation sparing and sensitive (68). In addition the above mentioned imaging protocol will provide a good survey for extra adrenal PGL and PC as well as renal tumours and wtGIST. Although the incidence of $SDHx$ mutations in sporadic PAs is low, the frequency of $SDHx$ mutations is significantly higher in cohorts of patients with familial PPGL (43) and akin to the incidence of pituitary adenomas in $MEN1$ gene mutation carriers (69). Therefore, screening with annual prolactin and IGF-1 measurements and an MRI pituitary at diagnosis in asymptomatic $SDHx$ gene carriers (and if normal every five years or sooner if symptomatic) similar to the guidance for $MEN1$ mutation carriers, should be considered (69) (Table 2). Taking into account the age of reported cases of PA in $SDHx$ carriers (43) and the penetrance of PPGL in pediatric $SDHx$ carriers, we advise that pituitary screening should be considered from the age of 15 years in asymptomatic $SDHx$ gene carriers or earlier depending on clinical symptoms and clinical judgment.

ix) Summary and future directions

Almost two decades ago, $SDHx$ mutations were first implicated in familial PC/PGL syndrome and since then several tumors, including GISTs, RCCs, and PAs, have expanded the spectrum of disease associated with $SDHx$ mutations. Some genotype-phenotype correlations have emerged such as the association between $SDHB$ mutations and malignant PPGL, $SDHD$ and HNPGL and $SDHA$ gene mutations and GIST. The causative role of $SDHx$ mutations in other tumour types remains unclear and reports with functional analysis are limited to isolated cases within studies and individual case reports as demonstrated in this
review. Radiological surveillance for the accepted tumour spectrum in SDHx carriers is now standard of care and the detection of incidental tumours in this patient cohort is likely to increase with the application of modern and sensitive imaging techniques. Identifying incidental tumours which may be SDH deficient can now be facilitated by techniques such as SDHB IHC and tissue metabolomics and may help to inform further expansion of the SDH tumour spectrum and may enable a more personalised approach to genetic counselling, clinical surveillance and treatment in the future.
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Table 1: Phenotype described in association with specific SDHx gene subunits

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<tr>
<th>SDHx subunit gene</th>
<th>PC</th>
<th>HNPGL PGL</th>
<th>Thoracic PGL</th>
<th>Malignant PGL</th>
<th>Abdominal/ pelvic PGL</th>
<th>wtGIST</th>
<th>RCC</th>
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<td>SDHA</td>
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<td>+</td>
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PC= phaeochromocytoma
HNPGL= Head and neck paraganglioma
PC= phaeochromocytoma
PGL= paraganglioma
wtGIST= wild type gastrointestinal stromal tumour
RCC= Renal cell carcinoma
PA= pituitary adenoma
Table 2: Guidance on clinical, biochemical and radiological surveillance in asymptomatic SDHx gene carriers

<table>
<thead>
<tr>
<th>SDHx subunit gene</th>
<th>Age to consider clinical surveillance</th>
<th>Age to consider radiological surveillance</th>
<th>Recommended surveillance</th>
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<tbody>
<tr>
<td>SDHB</td>
<td>• 5 years</td>
<td>• 10 years for PPGL</td>
<td>• Annual clinical review and plasma/urine metanephrine testing, prolactin, IGF-1</td>
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<td></td>
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<td>• 15 years for pituitary</td>
<td>• Abdominal and pelvic MRI at baseline and if normal every 12-24 months</td>
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<td>• MRI of neck and thorax at baseline and if normal every 3 years</td>
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<td>• MRI pituitary at baseline and if normal every 5 years</td>
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<td>SDHA</td>
<td>• 10 years</td>
<td>• 15 years for PPGL</td>
<td>• Annual clinical review and plasma/urine metanephrine testing, prolactin, IGF-1</td>
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<td>SDHD</td>
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<td>• 15 years for PPGL</td>
<td>• Screening should only be offered to patients who have a paternally inherited SDHD variant</td>
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<td>• 15 years for pituitary</td>
<td>• Annual clinical review and plasma/urine metanephrine testing, prolactin, IGF-1</td>
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1A shows a hematoxylin and eosin (H+E)-stained pituitary adenoma with prominent cytoplasmic vacuolation marked by the black arrows and tumour cells with non-granular eosinophilic cytoplasm. 1B shows the same pituitary tumour stained with an SDHB antibody (SDHB polyclonal rabbit antibody (Sigma Aldrich, United Kingdom) at a dilution of 1:300) showing evidence of a loss of SDHB staining in the tumour cells (regular SDHB staining can be observed in endothelial cells). 1C demonstrates the H+E-stained histological appearance of a SDHB-deficient RCC with evidence of intracytoplasmic vacuoles marked by the black arrows. 1D shows loss of SDHB protein expression on immunostaining of the same RCC. 1E shows the H+E-stained histological appearance of a SDH deficient wild type GIST with evidence of an epithelioid histology. 1F shows loss of SDHB protein expression on immunostaining of the same GIST. 1G shows a H+E stained histological appearance of an SDH deficient paraganglioma in a 63 year old man with a germline SDHB mutation (c.380T>G, p.(lle127ser)) and 1H shows SDHB immunonegativity in this tumour. 1I demonstrates the H+E stained appearance of a rectal adenocarcinoma and 1K shows an 8mm rectal GIST with a somatic KIT E11 deletion from the same 63-year-old gentleman detected two years after the paraganglioma. Figure 1J and 1L shows preservation of SDHB protein expression on immunohistochemistry of the rectal adenocarcinoma and rectal GIST respectively.
Figure 2: An algorithm for the approach to a non-PPGL tumour in an \textit{SDH}x mutation carrier


** = Specific histological feature such as cytoplasmic vacuolation in SDH deficient PA and intracytoplasmic vacuoles in SDH deficient RCC

*** = Reporting on ClinVar or case publication
Supplementary Data

**Supplementary Table 1A+1B:** Reports of thyroid tumours in *SDHx* mutation carriers with (1A) and without (1B) a history of PPGL

**Supplementary Table 2A+2B:** Reports of other rare tumours in *SDHx* mutation carriers with (2A) and without (2B) a history of PPGL