Hereditary phaeochromocytoma and paraganglioma in *SDHB* pathogenic variant carriers: clinical outcomes, surveillance strategies and economic evaluation

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Abstract

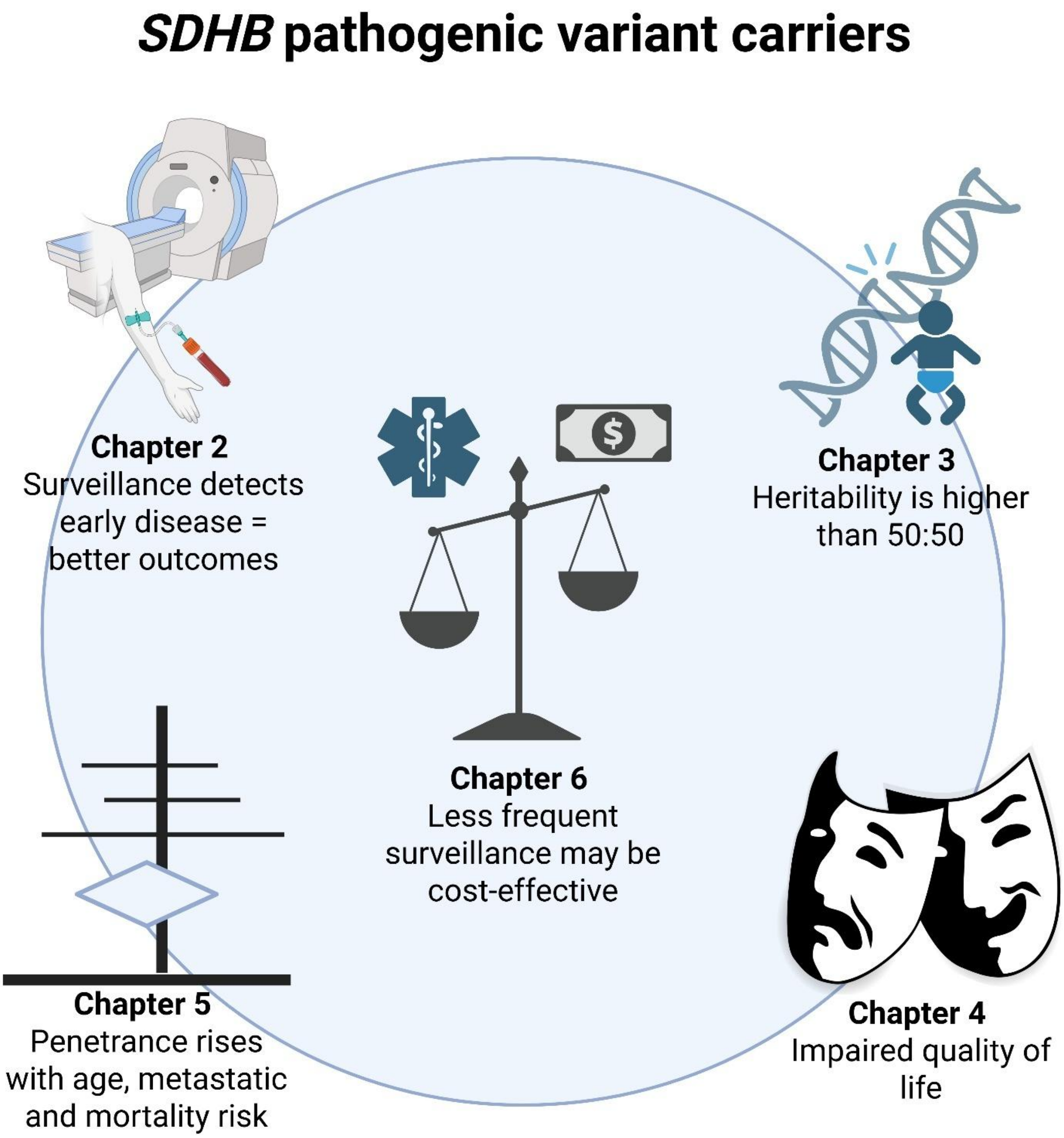
Phaeochromocytomas and paragangliomas (PPGL) are rare neuroendocrine tumours. Around 10-15% are caused by germline variants in the *succinate dehydrogenase* (*SDH*) gene family. Carriers of *SDHB* pathogenic variants (PVs) face high risk of recurrence, metastasis and death. Surveillance is recommended, but many uncertainties remain. These include true penetrance, predictors of progression, psychosocial burden and cost-effectiveness.

This thesis aimed to provide evidence to guide surveillance, genetic counselling and management of *SDHB* PV carriers. Chapter 2 presented a multicentre cohort study. Surveillance enabled earlier tumour detection. Carriers detected through surveillance had smaller tumours, higher surgical cure rates and better survival than those detected due to symptoms. Chapter 3 investigated inheritance patterns. Transmission ratio distortion was demonstrated, with PVs inherited more often than predicted by Mendelian genetics. This finding had implications for genetic counselling.

Chapter 4 assessed quality of life in carriers. Carriers had lower health utility scores than population norms. Anxiety and depression were common, even in carriers without tumours. This highlighted the psychosocial cost of lifelong surveillance. Chapter 5 was a systematic review and meta-analysis. It showed that penetrance rose steadily with age. Carriers remain at risk of second tumours after treatment. Lifetime risk of metastasis in non-probands was lower than earlier reports, but still significant. Chapter 6 developed a Markov model of different frequencies of surveillance. Annual surveillance achieved slightly higher QALYs but at greater cost compared to 3- and 5-yearly strategies. Results were highly sensitive to assumptions about anxiety from more frequent surveillance.

In conclusion, this thesis integrated clinical, genetic, psychosocial and economic perspectives. It provides an evidence base for surveillance and genetic counselling in *SDHB* PV carriers.

Graphical abstract



Statement of originality

This is to certify that the content of this thesis is my own work. This thesis has not been submitted for any other degree or purpose.

I certify that the intellectual content of this thesis is the product of my own work, and that all assistance received in preparing this thesis and all sources have been acknowledged.

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I assess my contribution to the results described in each chapter to be:

Chapter 1: 100%

Chapter 2: 100%

Chapter 3: 50%

Chapter 4: 100%

Chapter 5: 100%

Chapter 6:100%

Chapter 7: 100%

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Table of Contents

Abbreviations

3MT 3-methoxytyramine

ATP adenosine triphosphate

BRAF v-raf murine sarcoma viral oncogene homolog B1

CgA Chromogranin A

CSDE1 Cold shock domain containing E1

CT Computed tomography

DNMT3A DNA methyltransferase 3 alpha

EGLN1 Egl-9 family hypoxia-inducible factor 1

EORTC QLQ-C30 European Organisation for Research and Treatment Quality of Life Questionnaire

EPAS1 endothelial PAS domain protein 1

EQ5D5L EuroQol 5 Dimensions 5 Levels

EQ-VAS EQ visual analogue scale

ExAC Exome Aggregation Consortium

FACT-L Functional Assessment of Cancer Therapy Lung

$FADH_2$ Flavin adenine dinucleotide (reduced form)

FAP Familial adenomatous polyposis

^{18}F-FDG-PET/CT ^{18}F-fluorodeoxyglucose positron emission tomography/ computed tomography

FGFR1 Fibroblast growth factor receptor 1

FH Fumarate hydratase

FN False negative

FP False positive

^{68}Ga-DOTATATE PET/CT ^{68}Ga-DOTA-(Tyr3)-octreotate positron emission tomography/ computed tomography

GIST Gastrointestinal stromal tumors

GOT2 glutamate oxaloacetate transaminase 2

HADS Hospital Anxiety and Depression Scale

HIF Hypoxia-inducible factor

HN Head and neck

HNPGL Head and neck paragangliomas

HNPCC Hereditary non polyposis colorectal cancer

HRAS Harvey rat sarcoma viral oncogene homolog

HRQoL Health-related quality of life

ICERs Incremental cost-effectiveness ratios

IES-6 Impact of Event Scale 6

IRP Iron regulatory protein

IQR interquartile range

JmjC Jumonji-domain histone demethylases

KIF1B Kinesin family member 1B

Lutate ^{177}Lu-octreotate

MAML3 Mastermind like transcriptional coactivator 3

MDH2 Malate dehydrogenase 2

MFI-20 Multidimensional Fatigue Index 20

^{123}I-MIBG ^{123}I-metaiodobenzylguanidine

MAX MYC-associated factor X

MDH2 malate dehydrogenase 2

MEN1 multiple endocrine neoplasia 1

MEN2 multiple endocrine neoplasia 2

MERTK MER proto-oncogene tyrosine kinase

MET MET proto-oncogene

MRI Magnetic resonance imaging

mTOR Mechanistic target of rapamycin

NA not applicable

na not available

NF1 Neurofibromin 1

PC Phaeochromocytoma

PGL Paraganglioma

PHD Prolyl-4-hydroxylase

PHD1 Prolyl hydroxylase domain-containing protein 1

PPGL Phaeochromocytomas and paragangliomas

PoWH Prince of Wales Hospital

PROMIS-29 Patient-Reported Outcomes Measurement Information System 29-item profile measure

PV Pathogenic variants

QALY quality-adjusted life year

QoL Quality of life

RCC Renal cell carcinomas

REDCap Research Electronic Data Capture

RET Rearranged during transfection

RNSH Royal North Shore Hospital

RHH Royal Hobart Hospital

ROC Receiver operated curve

s-d Surveillance-detected

SDHA Succinate dehydrogenase subunit A

SDHAF2 Succinate dehydrogenase complex assembly factor 2

SDHB Succinate dehydrogenase subunit B

SDHC Succinate dehydrogenase subunit C

SDHD Succinate dehydrogenase subunit D

SF-36 Short Form 36

SLC25A11 Solute carrier family 25 member 11

TA Thoraco-abdominal

TAP Thorax, abdomen and pelvis

TCGA The Cancer Genome Atlas

TET Ten-eleven translocation methylcytosine dioxygenases

TMEM127 Transmembrane protein 127

TP True positive

TP53 Tumour protein

TRD Transmission ratio distortion

UK United Kingdom

VHL Von Hippel-Lindau

VUS Variants of uncertain significance

Chapter 1: Introduction

1.0 Phaeochromocytomas and paragangliomas

Phaeochromocytomas and paragangliomas (PPGL) are rare neuroendocrine tumours (1, 2). Phaeochromocytoma (PC) arise from chromaffin cells of the adrenal medulla, while paraganglioma (PGL) originate from extra-adrenal paraganglia (2). Sympathetic PGL arise from chromaffin cells and are capable of secreting catecholamines, leading to symptoms such as hypertension, palpitations, and sweating (2). In contrast, head and neck paragangliomas (HNPGLs) typically arise from chief cells associated with the parasympathetic nervous system and are usually non-secreting (2). PGL that are biochemically silent may be detected incidentally, symptomatically through mass effect or through surveillance imaging (3). Up to 40% of all patients presenting with PPGL will ultimately be found to carry germline pathogenic variants (PVs) in one of at least 20 susceptibility genes (4, 5). These genes are grouped into molecular clusters based on gene expression and tumour biology (Table 1.1).

Cluster 1 includes genes associated with pseudohypoxia (5-8). These genes are involved in oxygen sensing or the tricarboxylic acid (TCA) cycle, and includes *von Hippel-Lindau (VHL)*, *succinate dehydrogenase subunit A (SDHA)*, *subunit B (SDHB)*, *subunit C (SDHC)*, *subunit D (SDHD)*, *succinate dehydrogenase complex assembly factor 2 (SDHAF2)*, *fumarate hydratase (FH)*, *malate dehydrogenase 2 (MDH2)*, *solute carrier family 25 member 11 (SLC25A11)*, *glutamate oxaloacetate transaminase 2 (GOT2)*, *egl-9 family hypoxia-inducible factor 1 (EGLN1)*, *endothelial PAS domain protein 1 (EPAS1)*, *prolyl hydroxylase domain-containing protein 1 (PHD1)* and *iron regulatory protein (IRP)* (5, 7, 8).

Cluster 2 includes genes linked to kinase signalling and mechanistic target of rapamycin (mTOR) pathways such as *rearranged during transfection (RET)*, *neurofibromin 1 (NF1)*, *transmembrane protein 127 (TMEM127)*, *MYC-associated factor X (MAX)*, *MET proto-oncogene (MET)*, *MER proto-oncogene tyrosine kinase (MERTK)*, *DNA methyltransferase 3 alpha (DNMT3A)*, *kinesin family* *member 1B (KIF1B)*, *Harvey rat sarcoma viral oncogene homolog (HRAS)*, *fibroblast growth factor receptor 1 (FGFR1)* and *v-raf murine sarcoma viral oncogene homolog B1 (BRAF)* (5, 6).

Cluster 3 is a newer group involving Wnt signalling and chromatin remodelling and includes *mastermind like transcriptional coactivator 3 (MAML3)*, *cold shock domain containing E1 (CSDE1)* and *tumour protein p53 (TP53)* (5, 6).

The earliest PPGL susceptibility genes to be discovered were *RET*, *VHL* and *NF1* in the 1990s (5). These genes were associated with well described syndromes (multiple endocrine neoplasia 2 (*MEN2*), VHL disease and neurofibromatosis type 1 respectively) and were identified through linkage analysis and positional cloning (6, 9). Tumours with mutations in these genes often showed high penetrance and syndromic features (5).

The discovery of *SDHx* genes came in the 2000s and was a landmark in understanding non-syndromic hereditary PPGL. Bora Baysal and colleagues identified *SDHD* mutations in hereditary HNPGL (Baysal 2000) (10). They hypothesized a role for oxygen sensing due to the observation that HNPGL was more common at high altitude (10). This led to the identification of *SDHD* as a component of mitochondrial complex II and its role in oxygen sensing and tumourigenesis (10).

In 2005 Dahia *et al.* proposed clustering of PPGLs by gene expression phenotype (9). They showed tumours with *VHL*, *SDHB* and *SDHD* mutations shared a hypoxia gene expression profile known as Cluster 1 (9). Cluster 2 was defined by *RET* and *NF1* mutations and linked to kinase signaling pathways (9). In 2013 Letouzé *et al*. expanded Cluster 1 by identifying a hypermethylator phenotype in tumours with TCA cycle gene mutations (7). Subsequent phenotype-driven gene discovery revealed additional Cluster 1 genes linked to mitochondrial metabolism and pseudohypoxia: *FH*, *MDH2*, *IDH1/2*, *SLC25A11* and *GOT2* (11, 12). In 2017 Fishbein *et al*. conducted a comprehensive analysis using The Cancer Genome Atlas (TCGA) to characterize the genomic landscape of PPGL (6). They identified germline mutations in *SDHB*, *VHL*, *RET*, *NF1*, *SDHD*, *MAX*, *TMEM127* and *EGLN1*. Common somatic mutations involved *HRAS*, *NF1*, *EPAS1*,

RET, *CSDE1*, *SETD2*, *VHL*, *FGFR1*, *TP53*, *BRAF*, *ATRX*, *ARNT* and *IDH1*. Fusion genes included *MAML3*, *BRAF*, *NGFR* and *NF1* (6).

Germline PVs in the *SDH* gene family are the commonest predisposition drivers and occur in 10-15% of all PPGL cases (2, 13). *SDHB* PVs are of particular concern. They are typically associated with extra-adrenal tumours, a high risk of metastasis and increased mortality (2, 14, 15). Although previously considered to be rare, analysis of population genetic databases such as the Exome Aggregation Consortium (ExAC) dataset suggests approximately 1 in 6000 individuals carry pathogenic *SDHx* variants, equivalent to around 4000 individuals in Australia (16).

Because of these risks, lifelong surveillance for *SDHB* PV carriers is recommended. Guidance has been provided by international consensus groups such as the Endocrine Society and the European Society of Endocrinology (1, 14), as well as national bodies such as Cancer Institute NSW in Australia (17). Surveillance generally includes annual biochemical testing and periodic whole body imaging. However, these recommendations have mostly been based on expert opinion, single centre series or data from affected individuals (probands).

Many uncertainties remain regarding management and outcomes of *SDHB* PV carriers. The current literature provides limited evidence on the lifetime risk of tumour development in unaffected carriers. Data are also lacking on the likelihood of surgical cure once tumours are detected. The true risk of recurrence or metastatic progression is not well established. It remains unclear how frequently individuals develop multiple tumours over their lifetime. Predictors of metastatic progression have not been definitively identified. There is limited evidence on which imaging or biochemical tests offer the highest sensitivity for early detection of tumours. This thesis aimed to address these questions through clinical research, quality of life analysis, and health economic modelling in *SDHB* PV carriers.

Table 1.1 Clinical features of genes causing PPGL

Gene	Disease	Clinical features	Inheritance	Risk of PPGL	Risk of metastatic progression	References
Cluster 1: Pseudohypoxia						
VHL (3p25.3)	von Hippel-Lindau disease	Hemangioblastomas (CNS and retina), PC, pancreatic neuroendocrine tumors, RCC, endolymphatic sac tumors	AD	10-20%	~5%	Wachtel H *et al.* 2021 (18), Gimenez-Roqueplo AP *et al.* 2023 (5), Turchini J *et al.* 2018 (8)
SDHB (1p36.13)	Hereditary paraganglioma pheochromocytoma syndrome	PPGL, HNPGL, RCC, GIST, PA	AD	~22-35% by age 60 y	~28%	Niemeijer ND *et al.* 2017 (19), Eijkelenkamp K. *et al.* 2017 (20), Andrews KA *et al.* 2018 (21), Tufton N *et al.* 2019 (22)
SDHD (11q23.1)	Hereditary paraganglioma pheochromocytoma syndrome	HNPGL, PPGL, GIST, RCC	AD, paternal inheritance, maternal inheritance	~43% by age 60 y	Low	Andrews KA *et al.* 2018 (21)
SDHA (5p15.33)	Hereditary paraganglioma pheochromocytoma syndrome	PPGL, HNPGL, GIST, PA	AD	~10% by age 70 y	~10%	Van der Tuin K *et al.* 2018 (23)
SDHC (1q23.3)	Hereditary paraganglioma pheochromocytoma syndrome	HNPGL, PPGL, GIST, RCC	AD	~25% by age 60 y	Low	Andrews KA *et al.* 2018 (21)
SDHAF2 (11q12.2)	Hereditary paraganglioma pheochromocytoma syndrome	HNPGL	AD, paternal inheritance	Low	Low	Bausch B *et al.* 2017 (24)
FH (1q43)	Hereditary leiomyomatosis and renal cell carcinoma syndrome (HLRCC)	Leiomyomas, RCC, PPGL	AD	Low	~47% if RCC	Grubb RL *et al.* 2007 (25), Clark GR *et al.* 2014 (26)
EGLN1 (1q42.2)	Familial erythrocytosis type 3	Polycythemia vera, PGL	AD	Unknown	Unknown	Ladroue C *et al.* 2012 (27), Maule L *et al.* 2024 (28)
EPAS1 (2p21)	Pacak Zhuang syndrome	Polycythemia, PGL, somatostatinoma	AD, somatic	Unknown	Unknown	Mancini M *et al.* 2024 (29)
MDH2 (7q11.23)	-	PGL, uterine cancer, prostate cancer	AD	Unknown	~40%	Cascon A *et al.* 2015 (30), Buffet A et al. 2020 (31)
SLC25A11 (17p13.2)	-	PGL, GIST	AD	Unknown	~70%	Buffet A *et al.* 2018 (32), Freitas-Castro F et al. 2025 (33)
Cluster 2: Kinase signaling						
RET (10q11.21)	Multiple endocrine neoplasia type 2	PC; 2A: Medullary thyroid cancer, primary hyperparathyroidism, cutaneous lichen amyloidosis. 2B: as	AD, somatic	~20-50%	Low	Amodru V *et al.* 2020 (34)

		for 2A plus Marfanoid habitus, neuromas				
NF1 (17q11.2)	Neurofibromatosis type 1	PC, cafe au lait spots, Lisch nodules, optic gliomas, dysplastic bone lesions, neurofibromas, axillary and inguinal freckling	AD	~0.1-14.6%	~12%	Bausch B *et al*. 2006 (35), Zinnamosca L *et al*. 2011 (36)
TMEM127 (2q11.2)	Familial phaeochromocytoma	PC, RCC	AD	~33% by age 65 y	Low	Toledo S *et al*. 2015 (37)
MAX (14q23.3)	Familial phaeochromocytoma	PC, PA, other NETs	AD	Unknown	~18.8%	Lian B *et al*. 2024 (38)
Cluster 3: Wnt signalling						
TP53 (17p13.1)	Li-Fraumeni syndrome	PC, multiple cancer types	AD	Rare	Unknown	Loizidis S *et al*. 2025 (39)
MAML3 (4q31.1)	-	PC	Somatic	Unknown	Unknown	Fishbein L *et al*. 2017 (6)

Abbreviations: AD, autosomal dominant; EGLN1, egl-9 family hypoxia-inducible factor 1; EPAS1, endothelial PAS domain protein 1; FH, Fumarate hydratase; GIST, gastrointestinal stromal tumour, HNPGL, head and neck paraganglioma, MDH2, malate dehydrogenase 2; NET, neuroendocrine tumour; PA, pituitary adenoma; PC, phaeochromocytoma; PGL, paraganglioma; PPGL, pheochromocytoma and paraganglioma; RCC, renal cell carcinoma; SDHA, Succinate dehydrogenase subunit A; SDHAF2, Succinate dehydrogenase complex assembly factor 2; SDHB, Succinate dehydrogenase subunit B; SDHC, Succinate dehydrogenase subunit C; SDHD, Succinate dehydrogenase subunit D; VHL, von Hippel Lindau

1.1 SDHB in Mitochondrial Metabolism and Tumourigeneses

1.1.1 Pseudohypoxic Pathway and PPGL

The pseudohypoxic pathway is a key mechanism in the development of PPGL. It refers to the activation of hypoxia-related signalling in the absence of true hypoxia (8, 13). This occurs when mutations disrupt mitochondrial metabolism and cause accumulation of metabolites such as succinate and fumarate (40, 41). These metabolites inhibit prolyl hydroxylase enzymes that normally degrade hypoxia-inducible factors (HIF) (41). As a result, HIFs remain stable and active even in normal oxygen conditions (40). HIFs are transcription factors that promote angiogenesis, cell proliferation, and glycolysis (41). Their activation mimics the cellular response to low oxygen and contributes to tumour growth (40, 41).

Pseudohypoxic signalling is a hallmark of cluster 1 PPGLs whereby metabolic derangement can drive cancer development in PPGL (8, 13). It links mitochondrial dysfunction with oncogenic signalling and epigenetic dysregulation (40, 42). This pathway is especially relevant for *SDHB* PV carriers.

1.1.2 SDH

SDH is a mitochondrial enzyme found in all aerobic organisms (43). It catalyses the oxidation of succinate to fumarate in the tricarboxylic acid cycle (43). It also participates in the mitochondrial electron transport chain as complex II, transferring electrons to ubiquinone in the generation of adenosine triphosphate (ATP) (43).

SDH was first described in 1909 in frog muscle (44). It is located in the inner mitochondrial membrane (43). SDH consists of four subunits (45). SDHA is a flavoprotein that binds FAD (45). SDHB contains iron-sulphur clusters for electron transfer (45). SDHC and SDHD anchor the complex to the membrane and connect it to the quinone pool (45).

SDHB encodes the iron-sulphur protein subunit (45). This subunit is essential for transferring electrons from SDHA to ubiquinone (43). Loss of SDHB function leads to accumulation of succinate (2). Succinate is now considered an oncometabolite (2). It inhibits α-ketoglutarate (AKG)-dependent dioxygenases, including prolyl-4-hydroxylase domain (PHD), ten-eleven translocation methylcytosine dioxygenases (TET) and jumonji-domain histone demethylases (JmjC) (46). Inhibition of PHD stabilises HIF-1α subunits leading to pseudohypoxic signalling and angiogenesis (46). Inhibition of TET and JmjC leads to a hypermethylation of promoter regions and epigenetic dysregulation (7, 46). This disruption of cellular metabolism and chromatin architecture contributes to tumourigenesis and may influence tumour behaviour and aggressiveness (46). Succinate may also increase reactive oxygen species and further drive oncogenic signalling (1). The role of the SDH enzyme in converting succinate to fumarate in the tricarboxylic acid cycle is shown in Figure 1.1. The oncometabolic consequences of succinate is shown in Figure 1.2.

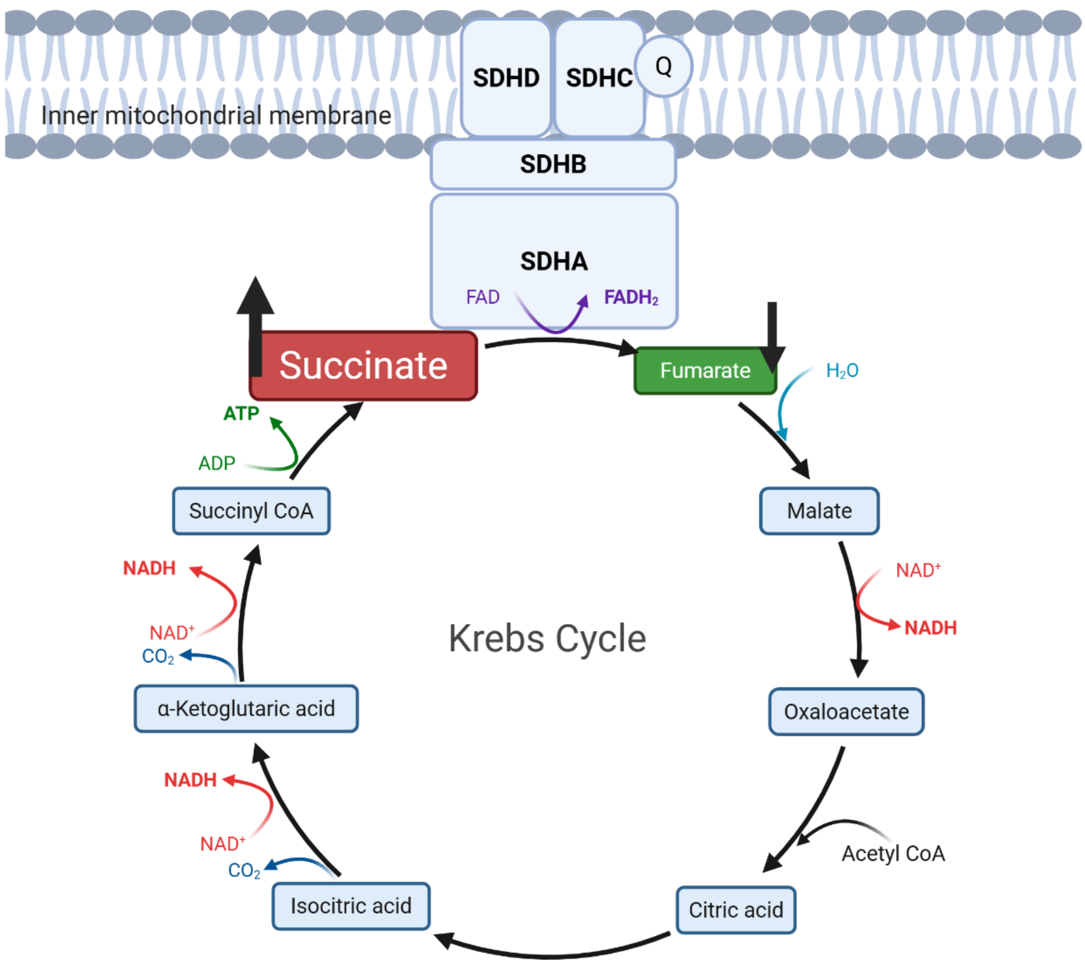

Figure 1.1 Succinate dehydrogenase converts succinate to fumarate in the tricarboxylic acid cycle

SDH also known as complex II, is part of both the TCA cycle and the mitochondrial electron transport chain (46). In the TCA cycle, SDH catalyses the oxidation of succinate to fumarate (46). This reaction is performed by the SDHA subunit, which produces $FADH_2$ (46). The SDHB subunit then transfers the electrons through iron-sulphur clusters to SDHC and SDHD (46). These membrane bound subunits pass electrons to ubiquinone (46). This links the TCA cycle to oxidative phosphorylation. Loss of SDH function interrupts the TCA cycle and leads to succinate accumulation (40, 46).

Abbreviations: $FADH_2$, flavin adenine dinucleotide (reduced form); SDH, succinate dehydrogenase; TCA, tricarboxylic acid; SDHA, succinate dehydrogenase subunit A; SDHB, succinate dehydrogenase subunit B; SDHC, succinate dehydrogenase subunit C; SDHD, succinate dehydrogenase subunit D.

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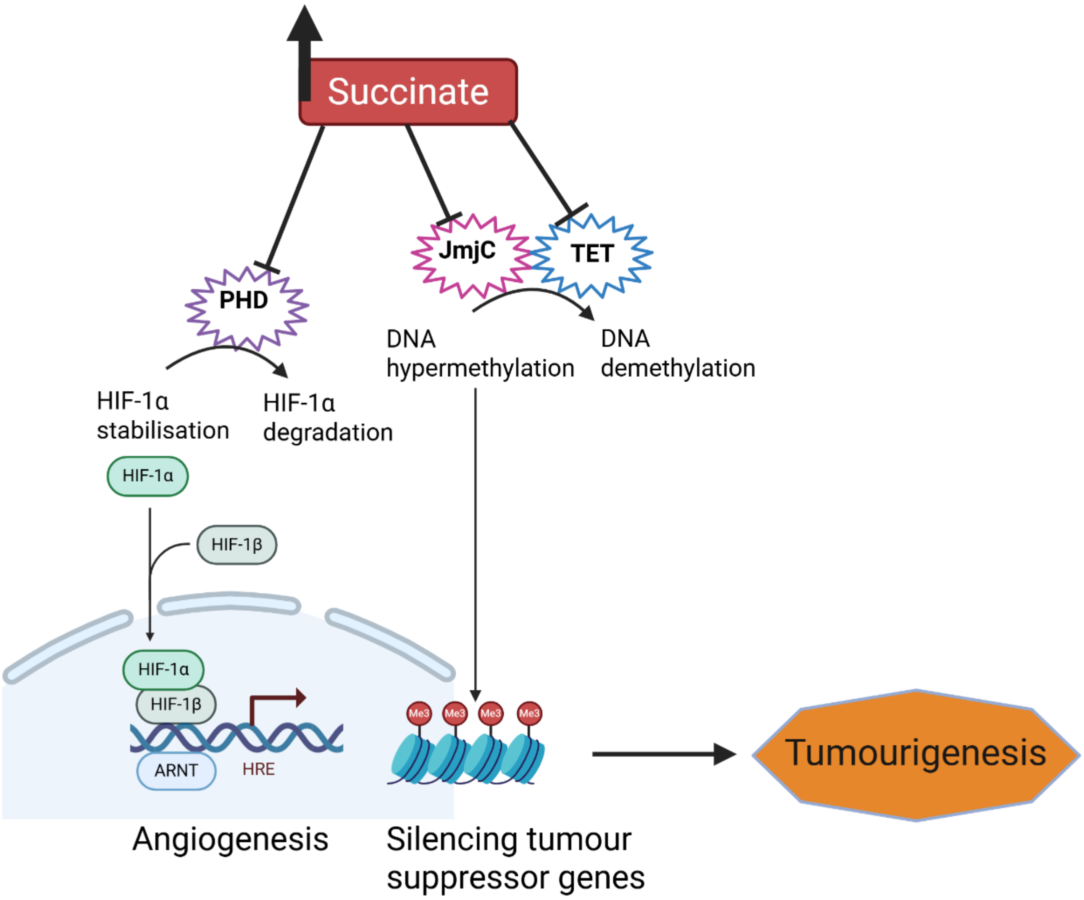

Figure 1.2 The oncometabolic consequences of succinate

Loss of SDH function leads to the accumulation of succinate (46). Succinate accumulation in cells leads to inhibition of AKG-dependent dioxygenases including PHD, JmjC and TET (46). PHD inhibition results in HIF-1α stabilisation while JmjC and TET inhibition lead to DNA hypermethylation (46). These effects lead to molecular events that promote tumourigenesis (46).

Abbreviations: HIF, Hypoxia-inducible factor; JmjC, jumonji-domain histone demethylases; PHD, prolyl-4-hydroxylase; TET, ten-eleven translocation methylcytosine dioxygenases.

Adapted from: Esteban-Amo *et al.* (2024) (46)

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1.2 Clinical Course and Inheritance in *SDHB* PV Carriers

1.2.1 Clinical Manifestations and Tumour Characteristics of *SDHB* PV Carriers

SDHB-related tumours in PV carriers are often extra-adrenal in the thorax, abdomen, pelvis and bladder but can be adrenal PC or HNPGL (20, 47-52). *SDHB*-related tumours have a high risk of metastatic progression (48, 52, 53). These features highlight the importance of routine surveillance to detect disease. Based on the author's appraisal of the literature, the key studies describing clinical presentations of *SDHB* PV carriers are shown in Table 1.2.

Table 1.2 Key studies describing the clinical presentations of *SDHB* PV carriers

Study	Country	Study design	Population studied	Number of *SDHB* PV carriers	Findings	Importance for understanding *SDHB* manifestations
Astuti *et al.* 2001 (47)	UK, USA, Sweden	Genetic association study	Familial and sporadic cases of PPGL	9	Tumours associated with *SDHB* PV carriers were frequently extra-adrenal sympathetic PGL rather than adrenal PCs. Metastatic disease was more common in *SDHB* PV carriers than in carriers of other *SDHx* mutations like *SDHD*	The first publication to report *SDHB* as a PPGL susceptibility gene
Amar *et al.* 2005 (48)	France	Retrospective multicentre	Patients with PPGL	21	*SDHB* mutations associated with extra-adrenal and large tumours. High metastatic risk.	The first large study to show the association between *SDHB* mutations and aggressive tumour features.
Benn *et al.* 2006 (49)	Australia, France, New Zealand, USA, UK, Germany and Canada	Retrospective multicentre	Germline *SDHB* or *SDHD* PV carriers	82	Compared *SDHB* and *SDHD* PV carriers. *SDHB* PV carriers more frequently had extra-adrenal PPGL in the thorax, abdomen and pelvis compared to *SDHD* PV carriers who mostly had HNPGL. *SDHB*-related tumours were diagnosed at a younger average age than *SDHD*-related tumours, with the youngest age at tumour diagnosis being age 7 y. Malignant tumours were more common in *SDHB* PV carriers. Two asymptomatic *SDHB* PV carriers were detected to have tumours following surveillance.	The first study to show that asymptomatic *SDHB* PV carriers can harbour tumours. *SDHB*-related tumours tend to occur in the abdomen and thorax rather than the head and neck.

Study	Country	Study design	Population studied	Number of *SDHB* PV carriers	Findings	Importance for understanding *SDHB* manifestations
Timmers *et al*. 2007 (50)	USA	Retrospective single centre	Patients with *SDHB*-related abdominal or thoracic PGL	29	*SDHB* PV carriers tended to have extra-adrenal PGL, younger age at tumour diagnosis and a higher incidence of metastatic disease compared to those with sporadic PPGL or other PPGL susceptibility genes such as *RET* and *VHL*. *SDHB*-associated tumours often produced norepinephrine or dopamine. Symptoms such as headaches, palpitations and sweats were more common in patients with functional tumours.	This study compared the biochemical and clinical profile of *SDHB*-associated PPGL to other known PPGL susceptibility genes such as *RET* and *VHL*. It described the catecholaminergic symptoms of functional *SDHB*-associated PPGL.
Ayala-Ramirez *et al*. 2013 (53)	USA	Retrospective single centre	Patients with malignant PPGL	9	In the group with bone metastases, half of the patients who underwent genetic testing were found to be *SDHB* PV carriers. Bone metastases caused pain and mobility issues.	The first study to describe bone metastases is a key clinical feature of malignant *SDHB*-related PPGL.
Eijkelenkamp *et al*. 2017 (20)	The Netherlands	Retrospective single centre	*SDHB* PV carriers	91	The overall penetrance of *SDHB*-related tumours was 35% by age 60 y, but once probands were excluded this dropped to 12% by age 60 y. HNPGL were frequently biochemically silent.	This study refined penetrance estimates by excluding index cases to reduce bias. This study described how *SDHB*-associated HNPGL were frequently biochemically silent.

Study	Country	Study design	Population studied	Number of *SDHB* PV carriers	Findings	Importance for understanding *SDHB* manifestations
Tufton *et al*. 2017 (51)	UK	Prospective single centre	*SDHB* PV carriers	92	Probands presented with tumour symptoms while non-probands frequently had tumours detected only through imaging surveillance (particularly MRI). *SDHB*-associated tumours included HNPGL, extra-adrenal PGL, PC, RCC and GIST. 12 of 14 patients with metastatic disease were probands.	This study compared clinical outcomes in probands and non-probands. Surveillance detected tumours in non-probands. Metastatic disease was more common in probands than non-probands. RCC and GIST were associated with *SDHB* PV carriers.
Andrews *et al*. 2018 (21)	UK	Retrospective multicentre	Patients referred for *SDHB*/*SDHC*/*SDHD* genetic testing due to a personal or family history of PPGL	584	The penetrance of PPGL/HNPGL in *SDHB* probands and non-probands by age 60 y was 60.2% but when excluding probands was 22.5%.	The largest study of penetrance for *SDHB* PV carriers. Performed an analysis of non-probands to exclude ascertainment bias.
Jochmanova *et al*. 2020 (52)	USA	Retrospective single centre	*SDHB* PV carriers with PPGL who presented with their primary tumour before age 20 y	64	In children and adolescent *SDHB* PV carriers, thoraco-abdominal PGL were the most common tumour type observed. Metastatic progression occured in one third of patients median 2 years following initial diagnosis. The mean age of tumour diagnosis was age 13 y but some cases presented as early as age 6 y.	This paper focused on presentations of *SDHB*-associated PPGL specifically in children and adolescents.

Abbreviations: GIST, gastrointestinal stromal tumours; IQR, interquartile range; na, not available; PC, phaeochromocytoma; PGL, paraganglioma; PPGL, pheochromocytoma and paraganglioma; PV, pathogenic variant; RCC, renal cell carcinoma; SDHB, Succinate dehydrogenase subunit

1.2.2 Penetrance of PPGL in *SDHB* PV Carriers

Penetrance refers to the proportion of individuals with a pathogenic variant who developed PPGL or HNPGL. For *SDHB* PV carriers, early studies based on probands suggested high penetrance (54, 55). Subsequent data including non-probands report lower risk.

Jafri *et al*. (2013) studied 187 non-proband *SDHB* PV carriers (56). Penetrance was estimated using the Kaplan-Meier method. They found 40% of non-probands developed HNPGL or other PPGL by age 60 y (56). Most non-probands remained asymptomatic (56). This contrasted with proband data and showed penetrance in non-probands was much lower than previously believed (56).

Jochmanova *et al*. (2017) studied a cohort of 241 non-probands (57). They found only 16.6% developed PPGL during follow up and estimated a penetrance of 49.8% by age 85 y (57). Males developed PPGL earlier than females in this study (57). Niemeijer *et al*. (2017) estimated penetrance in a Dutch cohort of *SDHB* PV carriers (19). They found 25% of non-probands developed PPGL by age 60 y. The penetrance of HNPGL specifically was 20% by age 60 y (19). Eijkelenkamp *et al*. (2017) reported a penetrance of 35% by age 60 y in a cohort that included both probands and non-probands (20).

Andrews *et al*. (2018) provided one of the most robust estimates of penetrance (21). In 371 non-proband *SDHB* PV carriers, penetrance of PPGL or HNPGL by age 60 y was 21.8% (21). The estimated risk of metastatic disease by age 60 y was 4.2% (21). In contrast, probands had significantly higher risks. When adjusted for ascertainment bias, the overall cumulative penetrance was 30.6% by age 80 y (21). They also found a sex difference, with male *SDHB* PV carriers having a higher penetrance of PPGL compared with females ($p = 0.0087$) (21).

These studies show penetrance is lower in non-probands than previously estimated and that risk varies by sex, highlighting the importance of personalised surveillance in *SDHB* PV carriers. However uncertainties remain regarding the true penetrance in *SDHB* PV carriers, particularly in non-probands, and how this risk varies by age. These gaps have important implications for surveillance recommendations and will be addressed in the multicentre cohort analysis (Chapter 2), systematically reviewed in Chapter 5 and incorporated into cost-effectiveness modelling of different surveillance frequencies (Chapter 6).

1.2.3 Risk of Second Tumour in *SDHB* PV Carriers

A second tumour refers to development of a new primary PPGL or HNPGL after initial diagnosis (51, 58). Tumours may be synchronous, defined as being detected at the same time as the first tumour, or metachronous, defined as being detected later (51, 59).

Srirangalingam *et al*. (2008) reported 3 of 16 (19%) patients with disease had multifocal tumours including synchronous or metachronous lesions over mean follow up of 5.8 years (59). Bausch *et al*. (2014) described a prospective paediatric cohort from a population-based registry of PPGL of whom 25 were *SDHB* PV carriers (58). Six (24%) of *SDHB* PV carriers developed a second PPGL at mean 30 years after initial diagnosis (58). These included ipsilateral, contralateral adrenal, and extra-adrenal tumours (58). Daniel *et al*. (2016) evaluated 47 *SDHx* PV carriers including 36 with *SDHB* variants, using surveillance imaging with MRI over a mean follow-up of 6.4 years (60). Two of nine *SDHB* index cases developed a second PPGL (60). This study supported the use of MRI imaging as effective for detecting new tumours during follow up (60).

Tufton *et al*. (2017) described outcomes from a UK surveillance cohort of 92 *SDHB* PV carriers (51). Based on Appendix 1 of the paper, 8 patients were found to have multifocal disease or a second tumour (51). All were index cases and no non-probands were reported to have developed a second tumour (51). These findings suggest second tumours may be more common in probands than in carriers identified through family screening (51).

These studies suggest affected *SDHB* PV carriers remain at ongoing risk of developing a second primary tumour and support the need for long term surveillance. The true risk of developing a second tumour remains uncertain and further data are needed to clarify risk and inform the optimal frequency and duration of surveillance. These gaps will be addressed in the multicentre cohort analysis (Chapter 2), systematically reviewed in Chapter 5 and incorporated into cost-effectiveness of different surveillance frequencies (Chapter 6).

1.2.4 Risk of Recurrence in *SDHB* PV Carriers

Recurrence refers to the reappearance of PPGL or HNPGL at a previously treated site or nearby region after an initial disease-free period (52, 61).

One of the first papers to report on recurrence risk for *SDHB*-related tumours was Gimenez-Roqueplo *et al.* (2003) (62). The authors described 8 patients with *SDHB* PVs. Case 1 initially had a PGL of the organ of Zuckerkandl which recurred during follow up (62). The patient later developed metastatic progression and died 87 months after diagnosis (62).

Lepoutre-Lussey *et al.* (2015) followed 27 *SDHB* PV carriers who underwent baseline and follow-up imaging (63). All patients with disease were alive without evidence of recurrence at a median follow up 40.4 months (63). Assadipour *et al.* (2017) studied a cohort of *SDHB* PV carriers who were probands (61). Local recurrence occurred in 20 of 42 patients (47.6%) (61). The median disease-free interval following surgery was 89.8 months (61). Tufton *et al.* (2017) in their UK surveillance cohort found that of the 27 index cases with multiple tumours and/or metastatic disease, 6 (22%) experienced recurrence at the original tumour site during follow up (61).

Jochmanova *et al.* (2020) investigated recurrence in a paediatric cohort who presented with their primary PPGL or HNPGL tumour before age 20 y (52). Recurrence occurred in 13 of 64 patients (20%) at a median age 16 y (52). The median time from first diagnosis to recurrence was 2 years (52). Most recurrent tumours were sympathetic extra-adrenal (52).

In summary, these studies show recurrence occurs in a substantial proportion of proband *SDHB* PV carriers and that more data is needed regarding recurrence risk in non-probands. It also reinforces the need for long term surveillance following initial treatment. More evidence is needed to inform which *SDHB* PV carriers are at greatest risk of recurrence and to tailor surveillance accordingly. These gaps will addressed in the multicentre cohort study presented in Chapter 2.

1.2.5 Metastatic Progression in *SDHB* PV Carriers

Tufton *et al.* (2019) conducted a pooled analysis of tumours in *SDHB* PV carriers across multiple studies (22). Across 344 PPGLs, 95 were metastatic giving an overall metastatic rate of 27.6% (22). The risk of metastatic progression varied by tumour location. HNPGL had the lowest risk at 12% (22). Thoracic PGLs had a risk of 17.2% (22). PC and abdominal PGLs had similar risks of 34.1% and 32.9% respectively (22). Pelvic PGLs had a risk of 30% (22). Bladder PGLs had the highest observed risk with 87.5% reported as malignant (22). These findings suggested PC and abdominal PGLs had a similar risk of malignant transformation in *SDHB* PV carriers (22). Tufton *et al.* raised concern about the notably high rate of malignancy in bladder PGLs, although numbers were small (22). In contrast, the risk of metastasis in HNPGL appeared lower.

Metastatic disease is a significant concern in *SDHB* PV carriers, particularly in those who present as probands. Martins *et al.* (2020) reported patients diagnosed based on clinical symptoms had a higher prevalence of metastases than those diagnosed through genetic screening (64). These patients also had larger tumours and worse clinical outcomes. Niemeijer *et al.* (2017) found all patients with metastatic disease in their nationwide Dutch cohort were probands (19). None of the non-probands developed metastatic disease during follow up (19). Similarly, Tufton *et al.* (2017) found 12 of 14 patients with metastatic disease were probands, while only two were non-probands with tumours detected through surveillance imaging (51). These findings suggest metastatic progression is primarily observed in probands, likely related to a longer "incubation" period before diagnosis (51). There is limited long term data on the risk of metastasis in non-probands and further research is needed to define this risk.

Several studies have used Kaplan Meier analysis to estimate the cumulative risk of metastatic progression in *SDHB* PV carriers. Andrews *et al.* (2018) found the risk of metastatic PPGL or

HNPGL was 4% at age 20 y, 8% at age 40 y, and 31% at age 60 y (21). Ma *et al.* (2020) reported a higher risk estimating nearly 60% of SDHB-related PPGL patients developed metastasis by age 60 y (65). Niemeijer *et al.* (2017) found the risk of malignancy in probands was 3% at age 20 y, 10% at age 40 y and 28% at age 60 y, rising to 53% by age 80 y (19). Ricketts *et al.* (2009) reported a cumulative risk of malignancy of 5% at age 20 y, 14% at age 40 y and 29% at age 60 y (66). These data highlight wide variability across studies but suggest metastatic risk increases with age in *SDHB* PV carriers.

Despite consistent evidence that metastatic risk increases with age and is more common in probands, long-term outcomes for non-probands remain poorly characterised. Existing studies vary widely in their estimates of cumulative risk, and few have examined risk factors that predict metastatic progression. More robust and generalisable data are needed to inform surveillance timing. This gap will be addressed through the multicentre cohort analysis in Chapter 2, the systematic review and meta-analysis in Chapter 5 and incorporated into the cost-effectiveness modelling of different surveillance frequencies in Chapter 6.

1.2.6 Tumour Size and Metastatic Risk

Tumour size is a known predictor of metastatic progression in *SDHB* PV carriers (61, 67). Larger tumours are more likely to metastasize (61, 67). Schovanek *et al.* found patients with tumours ≥4.5 cm had a median time to metastasis of 2 years compared to 8 years for those with smaller tumours (67). Jochmanova *et al.* reported children with tumours ≥5 cm were more likely to develop metastases within 5 years (52). In a multicentre cohort study, Hescot *et al.* reported that 76% of patients with metastatic PPGL had a primary tumour size ≥5cm, supporting the association between larger tumour size and metastatic behaviour (68). Michalowska *et al.* reported similar findings with median tumour size 6.2 cm in metastatic cases and 3.6 cm in non-metastatic cases (69). Alzahrani *et al.* studied *SDHB* p.R90X carriers (70). Those with metastatic disease had a median tumour size of 12.6 cm compared to 7.0 cm in non-metastatic *SDHB* PV carriers (70). Assadipour *et al.* found tumours ≥5 cm were associated with both recurrence and distant metastases (61). Dhir *et al.* performed multivariable analysis in 157 PPGL patients (71). They found larger tumour size, extra-adrenal location and *SDHB* mutation were independently associated with malignancy (71). Further investigation is needed to determine whether tumour size thresholds can reliably inform tumour risk stratification and imaging frequency. This question will be addressed in Chapter 2 and incorporated into the cost-effectiveness model evaluating different surveillance frequencies in Chapter 6.

1.2.7 Mortality in *SDHB* PV Carriers

Understanding mortality outcomes in *SDHB* PV carriers is essential to guide prognosis and long-term management. Hamidi *et al.* (2017) conducted a systematic review and meta-analysis of metastatic PPGL (72). In a subgroup analysis of two studies that reported on *SDHB* PV carriers, mortality ranged between 35-55% at median 28 and 42 months follow up following the diagnosis of metastatic disease (72). Choi *et al.* (2021) studied patients with aggressive PPGL and found *SDHB* PV carriers had significantly worse survival compared to non-carriers (73). The hazard ratio for death in patients with SDHB mutations was 4.66 (95% CI 1.10-19.75, $p = 0.04$) compared to wild-type at median survival 15.6 years (73). Overall mortality, while variable, has been reported as elevated for *SDHB* PV carriers.

Several studies have also reported on 5-year survival outcomes. Amar *et al.* (2007) found the 5-year probability of survival after the diagnosis of the first metastasis was 36% (95% CI 15-57%), with most tumours being extra-adrenal (74). King *et al.* (2011) reported a 5-year survival rate of 95.8% in a cohort of *SDHB* PV carriers diagnosed in childhood or adolescence including both adrenal and retroperitoneal tumours (75). Turkova *et al.* (2016) reported a 5-year survival rate of 91.8% in a mixed adult and paediatric cohort with predominantly extra-adrenal tumours (76). Jochmanova *et al.* (2020) studied a paediatric cohort and found a 5-year survival rate of 100%, although the confidence interval was undefined (52). Buffet *et al.* (2019) compared outcomes in a genetic group, defined as patients diagnosed after identification of a germline mutation and enrolled in surveillance, to a historic group diagnosed before genetic testing was available (11). Five-year overall survival was significantly higher in the genetic group (1.0) compared to the historic group (0.50), suggesting a benefit of early genetic diagnosis and surveillance (11).

Although several studies have reported mortality rates in *SDHB* PV carriers, existing data are heterogeneous and lack long term follow up. The effect of early diagnosis through surveillance on mortality remains uncertain, and time-specific mortality data in *SDHB* PV carriers is limited. Mortality outcomes will be further explored in Chapters 2, 5 and 6.

1.2.8 Transmission Ratio Distortion and Inheritance of the *SDHB* PV

Genetic counselling for individuals with *SDHB* PVs requires accurate information on inheritance to inform family members about their risks. This is crucial for reproductive counselling, including decisions around prenatal screening, preimplantation genetic testing and cascade testing. The *SDHB* PV is generally assumed to follow autosomal dominant inheritance where each child of a carrier has a 50% chance of inheriting the variant. However, this has not been rigorously tested in large familial cohorts and deviation from Mendelian transmission could significantly affect the accuracy of genetic risk communication.

One possible source of such deviation is called Transmission Ratio Distortion (TRD). TRD refers to any statistically significant departure from the expected 1:1 segregation ratio of alleles from heterozygous parents to offspring (77, 78). This can happen during different stages of reproduction and development such as egg or sperm formation, fertilisation or early embryo development (77). TRD may arise due to unequal survival of gametes, preferential segregation in meiosis or reduced embryo viability depending on which allele is inherited (77, 79). This means that some embryos carrying the PV might be more or less likely to implant or survive than others, skewing the observed inheritance ratios in live births.

Evidence for TRD has been reported in other hereditary cancer syndromes. In *RB1*-associated retinoblastoma, fewer maternally inherited mutations were seen in some families (80). In *RET*-associated MEN2, studies in Japanese families suggested more frequent paternal transmission of *RET* mutations (81). These examples show that TRD is not just a theoretical concept. It may alter the actual risk of transmission and can influence family planning. For *SDHB* PV carriers, if TRD is present then the real inheritance risk might be higher or lower than 50%. This matters not only for families but also for healthcare professionals who treat this high-risk group. The possibility of TRD has important implications for genetic counselling and risk communication. This phenomenon and its relevance to *SDHB* PV carriers will be examined in more detail in Chapter 3.

1.3 Surveillance for *SDHB* PV Carriers

1.3.1 Imaging Modalities for Surveillance

1.3.1.1 Computed tomography (CT)

CT is a widely used anatomical imaging modality for localizing PPGLs in *SDHB* PV carriers. Timmers *et al.* (2007) reported CT identified the primary tumour in 28 of 29 patients with *SDHB*-associated thoraco-abdominal (TA) PGLs, indicating high sensitivity for lesion detection (50). CT is most effective in detecting PC and sympathetic PGL, which typically appear as dense and hypervascular masses (50). In their review of imaging modalities for PPGL detection, Castinetti *et al.* (2015) noted CT is a first-line modality for local staging of sympathetic PPGLs and HNPGL (82). CT offers some advantages over MRI for detection of HNPGL including better spatial resolution, fewer motion artifacts, but may be less effective for detecting small lesions or differentiating scar tissue from residual tumour (82). Saie *et al*. assessed asymptomatic *SDHx* PV carriers with at least one imaging work-up (83). CT was found to have a sensitivity of 92% (95% CI 51-99) and specificity of 98% (95% CI 93-99) for detection of TA PPGLs, but the authors did not investigate CT for detection of HNPGLs (83).

1.3.1.2 Magnetic resonance imaging (MRI)

MRI is widely used in the surveillance of *SDHB* PV carriers due to its lack of ionising radiation. It is recommended for long-term follow up starting from a young age (84). Whole body MRI allows assessment of the head, neck, thorax, abdomen, and pelvis in a single study (84). Saie *et al.* (2021) reported excellent diagnostic performance for MRI (83). For HNPGL MRI had a sensitivity of 100% (95% CI 80-100) and specificity of 99% (95% CI 96-100) (83). For TA and pelvic PPGL, sensitivity was 100% (95% CI 32-100) and specificity was 100% (95% CI 93-100) (83). Castinetti *et al.* (2015) noted MRI is the preferred modality for evaluating HNPGL and sympathetic PPGLs (82). MRI is a sensitive and radiation free tool for tumour surveillance in *SDHB* PV carriers (82).

1.3.1.3 ^{123}I-metaiodobenzylguanidine (^{123}I-MIBG)

Historically ^{123}I-MIBG scintigraphy had been a widely used functional imaging technique for PPGL (82) and now is largely replaced by PET imaging (discussed below). However, its sensitivity is limited in *SDHB* PV carriers (85). Timmers *et al.* (2012) found ^{123}I-MIBG had a lower sensitivity for detecting metastases compared to ^{18}F-FDG PET/CT (85). For metastatic lesions ^{123}I-MIBG had a sensitivity of only 50%, while ^{18}F-FDG PET/CT was 82.5% (85). Fonte *et al.* studied 21 patients with false-negative ^{123}I-MIBG scans (86). Most of these had *SDHB* PVs and later developed metastatic disease (86). The authors concluded false-negative ^{123}I-MIBG scans were unfortunately common in *SDHB*-related tumours and associated with poor outcomes (86). Michalowska *et al.* confirmed low sensitivity of ^{123}I-MIBG in *SDHB* carriers where the sensitivity of ^{123}I-MIBG for PPGL was 22% for HNPGL, PC or TA PPGL (87). Saie *et al.* also reported limited utility of ^{123}I-MIBG (83). They found overall lesion-based sensitivity of 63% and specificity of 95% for ^{123}I-MIBG in SDHB carriers (83). Together these studies suggest ^{123}I-MIBG is not a reliable imaging modality for detecting PPGL in *SDHB* PV carriers.

1.3.1.4 ^{18}F-fluorodeoxyglucose PET/CT (^{18}F-FDG-PET/CT)

^{18}F-FDG-PET/CT is a functional imaging modality that detects tumours with high glucose metabolism (15, 85). It is especially useful in *SDHB* PV carriers who often develop aggressive tumours (15). Taieb *et al.* in 2012 recommended ^{18}F-FDG-PET/CT for detection of *SDHB*-related PPGL or HNPGL due to high uptake in metastatic and extra-adrenal disease (15). Timmers *et al.* found ^{18}F-FDG-PET/CT was more sensitive than ^{123}I-MIBG for detecting metastatic PPGL (85). In 2016 Kornaczewski *et al.* reported ^{18}F-FDG-PET/CT had 100% sensitivity and 95.5% specificity for *SDHB*-related HNPGL, PC or TA PPGL (88). Saie *et al.* (2021) found ^{18}F-FDG-PET/CT detected tumours in 13% of asymptomatic *SDHB* PV carriers and reported a lesion-based sensitivity of 87% (95% CI 51-98) and specificity of 99% (95% CI 93-100) (83). These findings support the use of ^{18}F-FDG-PET/CT for detecting both primary tumours and metastatic progression in *SDHB* PV carriers.

1.3.1.5 ^{68}Ga-DOTA-(Tyr3)-octreotate PET/CT (^{68}Ga-DOTATATE PET/CT)

^{68}Ga-DOTATATE PET/CT is a highly sensitive functional imaging modality for detecting PPGL in *SDHB* PV carriers (89, 90). It binds to somatostatin receptor type 2, which is frequently expressed in these tumours (91). Janssen *et al.* compared ^{68}Ga-DOTATATE PET/CT to ^{18}F-FDG-PET/CT and ^{123}I-MIBG (89). They found ^{68}Ga-DOTATATE PET/CT had superior lesion detection particularly for HNPGL (89). In a follow-up study the authors confirmed it was especially effective for localizing HNPGL (92). Jha *et al.* (2018) studied a paediatric *SDHx* cohort and reported a per-lesion detection rate of 93.5%, significantly higher than ^{18}F-FDG-PET/CT and anatomic imaging (91). The 2019 European Association of Nuclear Medicine guidelines endorsed ^{68}Ga-DOTATATE PET/CT as a first-line modality for *SDHx*-related PPGL due to its high sensitivity and ability to guide treatment planning (90). This modality is now considered a preferred functional imaging option for surveillance and staging in *SDHB* PV carriers.

1.3.1.6 Summary of imaging modalities

In summary, multiple imaging modalities have been used for tumour surveillance in *SDHB* PV carriers. CT and MRI offer high anatomical resolution, with MRI preferred due to the absence of radiation (82-84). Among functional imaging techniques, ^{68}Ga-DOTATATE PET/CT provides the highest sensitivity and is now considered the preferred option (90). ^{18}F-FDG-PET/CT is also accurate, particularly for detecting aggressive or metastatic disease (15, 88). In contrast ^{123}I-MIBG has limited sensitivity in *SDHB* PV carriers and is no longer recommended as a first-line modality. Together, these data support the use of a combination of anatomical and functional imaging for routine surveillance. The sensitivity and specificity of imaging modalities for *SDHB* PV carriers is shown in Figure 1.3. Further analysis of imaging performance in *SDHB* PV carriers will be presented in the multicentre cohort (Chapter 2) and incorporated into the cost-effectiveness modelling of different surveillance frequencies (Chapter 6).

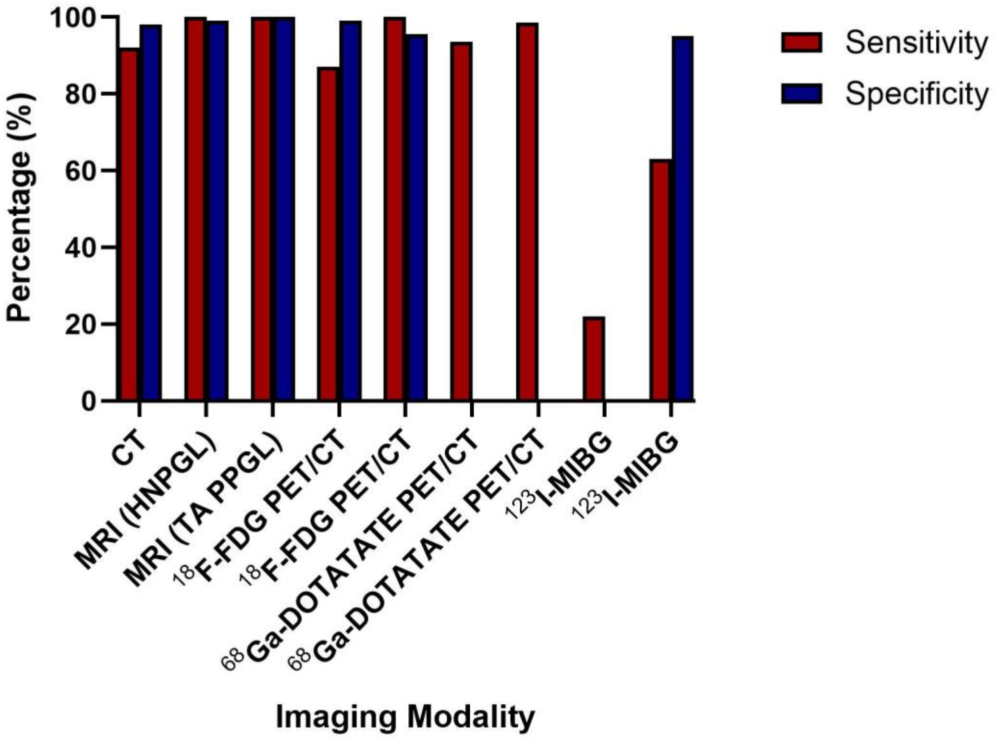

Figure 1.3 The sensitivity and specificity of imaging modalities for detection of *SDHB*-associated tumours

CT for TA PPGL sensitivity 92%, specificity 98% (83). MRI for HNPGL sensitivity 100%, specificity 99% (83). MRI for TA PPGL sensitivity 100%, specificity 100% (83). ^{18}F-FDG-PET/CT lesion-based sensitivity 87%, specificity 99% (83). ^{18}F-FDG-PET/CT for HNPGL, PC or TA PPGL sensitivity 100%, specificity 95.5% (88). ^{68}Ga-DOTATATE PET/CT sensitivity 93.5% specificity not stated (91). ^{68}Ga-DOTATATE PET/CT sensitivity 93.5% specificity not stated (89). ^{123}I-MIBG for HNPGL, PC or TA PPGL sensitivity 22% specificity not stated (87). ^{123}I-MIBG lesion-based sensitivity 63%, specificity 95% (83). Abbreviations: CT, Computed tomography; MRI, Magnetic resonance imaging; HNPGL, Head and neck paragangliomas; TA, Thoraco-abdominal; PPGL, Phaeochromocytomas and paragangliomas; ^{18}F-FDG-PET/CT, ^{18}F-fluorodeoxyglucose PET/CT; ^{68}Ga-DOTATATE PET/CT, ^{68}Ga-DOTA-(Tyr3)-octreotate PET/CT; ^{123}I-MIBG, ^{123}I-metaiodobenzylguanidine.

1.3.2 Biochemical Detection of PPGL in *SDHB* PV Carriers

Biochemical testing is a first-line tool to detect PPGL in *SDHB* PV carriers (3, 93). Given that PPGL can produce and metabolize catecholamines, surveillance usually includes an annual assessment of catecholamine metabolites (22). Production of catecholamines normetanephrine, epinephrine and dopamine in chromaffin cells, are best assessed by measuring their O-methylated metabolites, namely free plasma normetanephrine, metanephrine and methoxytyramine respectively (93). These metabolites have the advantage of being more sensitive and specific for the detection of functional PPGL as they are produced within tumour cells continuously and independently of catecholamine secretion (94). Plasma normetanephrine and methoxytyramine are commonly elevated in *SDHB*-associated PPGL. Methoxytyramine is especially useful in *SDHB* PV carriers and may indicate aggressive disease (93). Eisenhofer *et al.* reported this plasma panel had a sensitivity of 98% and specificity of 94% for PPGL detection (95). Diagnostic performance of plasma normetanephrine was superior to that of urinary free or deconjugated normetanephrine especially in high risk patients (95). Greenberg *et al.* confirmed both plasma and urine normetanephrine have high diagnostic yield in *SDHB* PV carriers (96). However biochemically silent tumours remain challenging to recognise without imaging (3, 59). Biochemical variation in tumours may be explained by tumour enzymology. *SDHB*-associated PPGLs typically show low expression of phenylethanolamine N-methyltransferase (PNMT), the enzyme that converts norepinephrine to epinephrine. As a result metanephrine levels are often normal, while normetanephrine or methoxytyramine may be elevated (97). This explains the frequent absence of elevated metanephrine and reinforces the need for imaging in surveillance protocols. The catecholamine pathway and formation of free metabolites in plasma is shown in Figure 1.4.

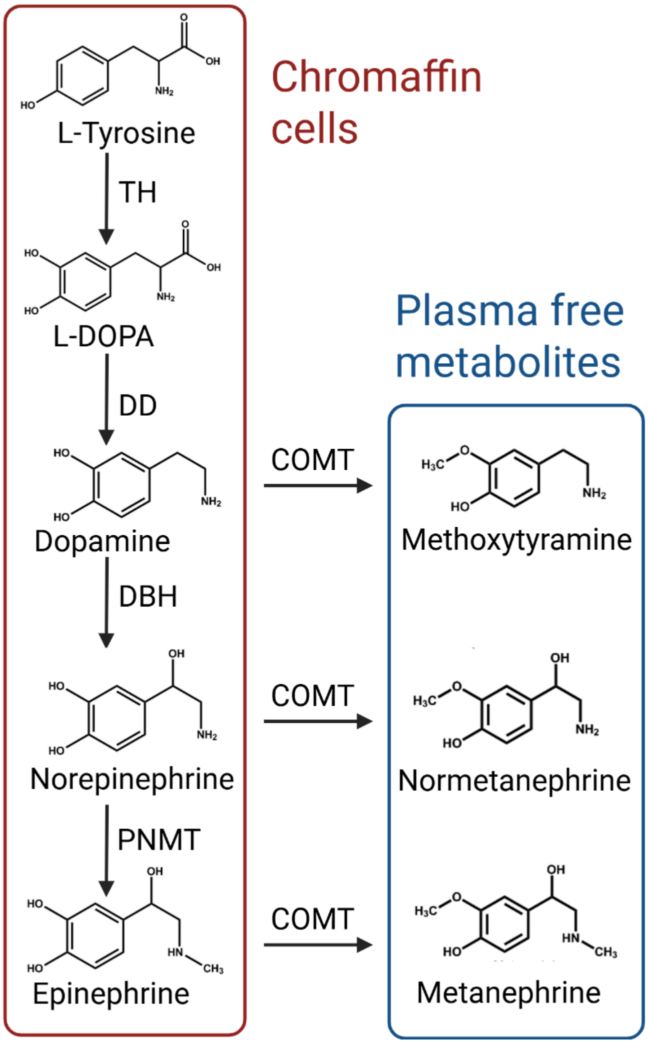

Figure 1.4 The catecholamine pathway and formation of free metabolites in plasma.

Plasma metanephrines are formed in chromaffin cells (93). Plasma metanephrines normetanephrine, metanephrine and methoxytyramine are the respective O-methylated metabolites of normetanephrine, metanephrine and dopamine (93). In plasma the three O-methylated metabolites are commonly measured as free metabolites (93).

Abbreviations: TH, tyrosine hydroxylase; L-DOPA, L-Dihydroxyphenylalanine; DD, Dihydroxyphenylalanine Decarboxylase; DBH, Dopamine β-Hydroxylase; PNMT, Phenylethanolamine N-methyltransferase; COMT, Catecholamine-O-methyltransferase.

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Chromogranin A (CgA) is a neuroendocrine secretory protein that can be elevated in PPGL (98). It is secreted alongside catecholamines and has been evaluated as a potential biochemical surveillance test in *SDHB* PV carriers (52). However the utility of CgA is limited. Thompson *et al*. found in a cohort of *SDHB* PV carriers, CgA had a sensitivity of 67% and specificity of 79% (98). CgA also produced frequent false positives, and plasma normetanephrine and metanephrine had higher specificity. Jochmanova *et al*. also measured CgA in children with *SDHB*-related PPGL and found elevations in only 75% of tested cases (52). These findings suggest CgA may have some use in surveillance but it lacks sufficient sensitivity and specificity to be used as a primary biochemical surveillance test.

While plasma normetanephrines and methoxytyramine show high diagnostic accuracy in *SDHB*-associated PPGL, the detection of biochemically silent tumours remains a challenge. Further evaluation of sensitivity and specificity of biochemical testing for *SDHB*-associated tumours is warranted, These issues will be further evaluated in the multicentre cohort study (Chapter 2) and incorporated into the cost-effectiveness analysis of different surveillance frequencies (Chapter 6).

1.3.3 Surveillance Recommendations for *SDHB* PV Carriers

Surveillance is recommended for all *SDHB* PV carriers due to the high risk of developing PPGL and risk of recurrence or metastasis years after initial treatment (1, 14). Several original studies and clinical guidelines have proposed structured surveillance protocols for *SDHB* PV carriers, summarised in Table 1.3. One of the earliest studies to recommend a surveillance strategy was put forward by Benn *et al*. They studied 82 individuals with *SDHB* or *SDHD* PV, including 38 asymptomatic carriers (49). Their surveillance protocol involved annual plasma or urinary metanephrines and biennial CT and/ or MRI neck and TAP (49). The authors concluded early detection through structured surveillance might improve clinical outcomes and recommended lifelong surveillance for all *SDHx* PV carriers beginning in childhood or early adulthood (49).

Several international and local clinical practice guidelines for surveillance of *SDHB* PV carriers were published prior to the commencement of my PhD. The Endocrine Society USA Guidelines in 2014 also advised biochemical testing and periodic MRI, with imaging tailored to tumour location and biochemical activity (1). The European Society of Endocrinology in 2016 recommended lifelong annual follow up for high-risk patients (14). High-risk groups included young patients, those with *SDHB* PV, large tumours or extra-adrenal location. Recommended follow included yearly plasma or urinary metanephrines and imaging every 1-2 years, particularly for biochemically silent tumours (14). The Dutch national protocol for adult *SDHB* PV carriers offered annual biochemical screening and regular imaging with head and neck MRI every 3 years and TA MRI or CT every 2 years (99). Functional imaging such as ^{123}I-MIBG and ^{18}F-FDG-PET/CT was recommended to be used selectively when biochemistry was positive or further localization was required (99). These clinical practice guidelines were based on expert consensus and supported by clinical experience. However, they were not based on prospective data and many questions remain about optimal frequency and modality of testing.

Original research studies have proposed updated surveillance recommendations for *SDHB* PV carriers since the publication of international guidelines (2, 22, 52). Neumann *et al*. (2019) recommended annual biochemical testing and biennial whole-body MRI for all *SDHB* PV carriers starting from age 10 y (2). Jochmanova *et al*. (2020) supported imaging from age 5-6 y with biochemical screening annually and anatomical imaging every 2 years with MRI to avoid ionising radiation (52). Tufton *et al*. (2019) summarised surveillance protocols across European and Australian centres (22). The authors found most centres recommended annual or biennial biochemical screening, and MRI of the head, neck, chest, abdomen and pelvis every 1-3 years (22). The optimal frequency and modality for surveillance of *SDHB* PV carriers is not well established and deserves further evaluation.

Since the commencement of this PhD in 2019, national and international expert consensus statements have been published proposing updated approaches to the surveillance of *SDHB* PV carriers (100-103). These guidelines reflect growing recognition of the unique risks associated with *SDHB*-related tumours including young onset, multifocal and often biochemically silent tumours with a high risk of metastasis (100-103). There is now broader agreement on the need for early and lifelong surveillance, the use of whole-body MRI to minimise radiation exposure and structured protocols tailored to age, genotype and tumour behaviour. These developments highlight the importance and evolving nature of surveillance in hereditary PPGL syndromes. Further detail on these recommendations and their relevance to this thesis will be explored in the Discussion and Conclusion chapters.

Table 1.3 Summary of surveillance recommendations for *SDHB* PV carriers

Source/ guideline	Biochemical surveillance	Imaging surveillance frequency and modality	Age to start
Benn *et al.* 2006 (49)	Annual urinary catecholamines or plasma metanephrines	Biennial CT and/or MRI neck and TAP	Age 10 y
Lenders *et al.* 2014 (1)	Urinary metanephrines or plasma metanephrines	Periodic MRI; CT/ nuclear medicine used only to evaluate detected lesions	NA
Plouin *et al.* 2016 (14)	Annual urinary catecholamines or plasma metanephrines with 3MT	Every 1-2 years imaging tests	NA
Rijken *et al.* 2018 (99)	Annual urinary catecholamines or plasma metanephrines with 3MT	Every 3 years HN MRI and every 2 years TAP CT or MRI. Following PPGL detection, functional imaging such as ^{123}I-MIBG and ^{18}F-FDG-PET/CT	NA
Neumann *et al.* 2019 (2)	Annual urinary catecholamines or plasma metanephrines	Initial imaging with MRI skull base, neck, thorax, retroperitoneum and pelvis or ^{68}Ga-DOTATATE PET/CT then MRI every 3 years	NA
Tufton *et al.* 2019 (22)	Annual urinary catecholamines or plasma metanephrines	Every 1-3 years MRI HN and TAP	Age 5 or 10 y
Jochmanova *et al.* 2020 (52)	Annual urinary catecholamines or plasma metanephrines	If biochemistry comes back positive, it should be followed by anatomical imaging (CT/MRI)	Age 5-6 y
Amar *et al.* 2021 (100)	Annual urinary catecholamines or plasma metanephrines in childhood, annual plasma metanephrines in adulthood	Initial imaging MRI HN and TAP in childhood and combination of MRI HN and TAP and PET-CT in adulthood, then every 2–3 years MRI HN and TAP	Age 6-10 y
Casey *et al.* 2024 (101)	Biannual urinary catecholamines or plasma metanephrines with 3MT	Every 2-5 years MRI HN and TAP	Age 5 y
Taïeb *et al.* 2024 (102)	Urinary catecholamines or plasma metanephrines with 3MT	Initial MRI HN and TAP and ^{68}Ga-DOTATATE PET/CT. Following PPGL detection, active surveillance for patients without symptoms who have a low tumour burden or otherwise indolent tumour behaviour. If surgical removal performed, ^{68}Ga-DOTATATE PET/CT within 6 months then MRI HN and TAP every 1-2 years.	NA
Cancer Institute of NSW 2024 (103)	Annual urinary catecholamines or plasma metanephrines and if available 3MT	Biennial MRI base of skull to coccyx, ^{68}Ga-DOTATATE PET/CT once with initial imaging	Age 5 y for biochemical testing, age 10 y for MRI and age 18 y for ^{68}Ga-DOTATATE PET/CT

Abbreviations: 3MT, 3-methoxytyramine; CT, computed tomography; HN, head and neck; MRI, magnetic resonance imaging; NA, not applicable; PET/CT, positron emission tomography/computed tomography; PPGL, pheochromocytoma and paraganglioma; TAP, thoracic abdominal and pelvis; ^{18}F-FDG-PET/CT, ^{18}F-fluorodeoxyglucose PET/CT; ^{68}Ga-DOTATATE PET/CT, ^{68}Ga-DOTA-(Tyr3)-octreotate PET/CT; ^{123}I-MIBG, ^{123}I-metaiodobenzylguanidine.

1.4 Impact of Surveillance on Patients and Health systems

1.4.1 Quality of Life in *SDHB* PV Carriers

Quality of life (QoL) is an important outcome in genetic cancer syndromes. In *SDHB* PV carriers, the lifelong risk of developing cancer can lead to ongoing psychological distress, uncertainty about the future and a sense of vigilance that affects daily life. Carriers must undergo repeated imaging, biochemical tests and clinical appointments. This may cause significant psychological burden even in the absence of cancer.

A Danish meta-ethnographic synthesis found that women with hereditary cancer risk often lived what they called a "cancer surveillance life" (104). Their everyday routines were shaped by medical vigilance. They described emotional tension, worry about future disease and difficulties with planning family life. Decisions about risk-reducing surgery, reproductive planning and disclosing genetic status to family were experienced as overwhelming (104).

A systematic review in *BRCA1/2* PV carriers showed that cancer worry was common, especially shortly after disclosure of their genetic test result (105). While distress often lessened over time, some studies still reported elevated distress in carriers compared to non-carriers in long term follow up. Younger women appeared more vulnerable to disruptions in role functioning, particularly those with a family history of early or aggressive cancers (105).

A cross-sectional study of PGL patients confirmed reduced QoL (106). In a Dutch study, *SDHB* and *SDHD* mutation carriers reported worse fatigue, lower physical functioning, and higher anxiety than matched controls as assessed by 3 measures: Hospital Anxiety and Depression Scale (HADS), Multidimensional Fatigue Index 20 (MFI-20) and Short Form 36 (SF-36) (106). Notably even patients without active disease described reduced motivation and increased general fatigue. Asymptomatic *SDHB* and *SDHD* mutation carriers without manifest disease had

QoL scores comparable to the general population, suggesting that tumour diagnosis or treatment may contribute to the reduced QoL.

These findings highlight that QoL may be adversely affected in *SDHB* PV carriers, particularly after tumour diagnosis or treatment. Given the lifelong burden of surveillance and potential psychological impact, quality of life deserves dedicated assessment in this population. This issue will be explored in depth in Chapter 4, which focuses on QoL of *SDHB* PV carriers

1.4.2 Health Economic Evaluation of Surveillance Strategies in *SDHB* PV Carriers

Health economic evaluation helps guide decisions about the best use of healthcare resources (107). It compares the costs and outcomes of different strategies. In genetic cancer syndromes this is especially important. Patients often require lifelong follow up, with expensive imaging and clinical care. Health economic models are well-suited to evaluate the value of different surveillance approaches. They can incorporate test sensitivity, tumour detection rates, health outcomes, costs and quality of life (107). A cost-effectiveness analysis is a method used to compare the costs and health outcomes of different interventions and is expressed as cost per quality-adjusted life year (QALY) gained (107).

Cost-effectiveness analyses have been done in other hereditary cancer syndromes. These include *BRCA1/2* (108), familial adenomatous polyposis (FAP) (109) and Lynch syndrome (HNPCC) (110). In *BRCA1/2* PV carriers, Hallsson *et al.* modelled 16 prevention strategies and found that early risk-reducing surgery was highly cost-effective, with prophylactic bilateral mastectomy and salpingo-oophorectomy at age 30 y being cost effective compared to intense surveillance (108). In FAP, Greenblatt *et al.* modelled prophylactic surgery for duodenal cancer and found that pancreaticoduodenectomy performed at Spigelman stage IV duodenal polyposis was the most cost-effective strategy compared with surgery at cancer diagnosis (109). For Lynch syndrome, Alblas *et al.* modelled the cost-effectiveness of prophylactic hysterectomy and found it reduced endometrial cancer incidence by 98%, with cost-effectiveness maintained when surgery was offered between ages 40 and 80 y (110).

No cost-effectiveness assessments have been published to assess surveillance protocols for *SDHB* PV carriers. Despite the substantial burden of lifelong imaging and cancer risk in this population, the cost-effectiveness of varying surveillance intervals remains unknown. This represents a critical gap in the literature that warrants evaluation to support future guideline development and resource allocation.

1.5 Aims and hypotheses

This thesis investigates how best to monitor individuals with a germline *SDHB* PV. The overarching aim is to characterise the risks, clinical features, outcomes and patient experience of *SDHB* PV carriers, and to evaluate the cost-effectiveness of alternate surveillance strategies in this high-risk population. The overarching hypothesis is that, for *SDHB* PV carriers, extending the interval between surveillance episodes (from annual to less frequent) may maintain clinical benefit while reducing harms and costs, and may therefore represent a more cost-effective approach than annual surveillance.

Each of the five aims is addressed in a corresponding thesis chapter. **Aim 1** is addressed in **Chapter 2**, which uses a multicentre retrospective cohort to evaluate tumour characteristics and outcomes associated with surveillance-detected versus to tumours detected at symptomatic presentation (probands). **Aim 2** is addressed in Chapter 3, which explores the heritability of *SDHB* PVs across families. **Aim 3** is addressed in **Chapter 4**, which investigates health-related quality of life (HRQoL) in affected and unaffected *SDHB* PV carriers undergoing surveillance. **Aim 4** is addressed in **Chapter 5**, a systematic review and meta-analysis quantifying age-specific tumour risks, risks of second primaries, metastasis and mortality in *SDHB* PV carriers. **Aim 5** is addressed in **Chapter 6**, which presents a Markov model comparing the cost-effectiveness of different surveillance frequencies, using data from earlier chapters to inform clinical inputs and model assumptions.

Aim 1. To evaluate the characteristics and outcomes of *SDHB*-associated tumours detected during surveillance

Hypothesis: Surveillance-detected tumours are diagnosed at earlier stages and lead to better clinical outcomes compared to tumours detected at symptomatic presentation (probands).

Aim 1.1 To describe the nature of SDHB-related tumours detected during surveillance in a multicentre cohort.

Aim 1.2 To compare detection methods and timing of diagnosis between surveillance-detected and proband tumours.

Aim 1.3 To assess the relationship between surveillance and clinical outcomes including metastatic rate and mortality.

Aim 2. To examine the risk of inheriting an *SDHB* PV

Hypothesis: The observed inheritance of *SDHB* PVs deviates from Mendelian expectations of 50:50 risk of inheritance.

Aim 2.1 To quantify the observed vs expected inheritance of *SDHB* PVs across multiple families.

Aim 2.2 To assess whether TRD is present in *SDHB* PV families.

Aim 3. To evaluate HRQoL in *SDHB* PV carriers enrolled in long term surveillance

Hypothesis: Carriers of *SDHB* PVs experience reduced HRQoL due to psychological and physical impacts of lifelong cancer surveillance and tumour development.

Aim 3.1 To assess HRQoL in both affected and unaffected *SDHB* PV carriers.

Aim 3.2 To explore relationships between HRQoL and demographic and clinical variables.

Aim 3.3 To identify opportunities to enhance patient-centred care in surveillance programs.

Aim 4. To quantify the clinical risks associated with SDHB PVs in a systematic review and meta-analysis

Hypothesis: *SDHB* PV carriers have substantial lifetime risks of tumour development, metastatic disease, second primaries and mortality.

Aim 4.1 To synthesise age-specific tumour risks in asymptomatic *SDHB* PV carriers.

Aim 4.2 To estimate the rates of metastatic progression, recurrence and tumour-related death.

Aim 5. To model the cost-effectiveness of surveillance strategies for asymptomatic *SDHB* PV carriers

Hypothesis: Less frequent surveillance (every 3 or 5 years) is cost-effective compared to annual surveillance with acceptable trade-offs in clinical outcomes.

Aim 5.1 To develop a Markov model comparing annual, 3 and 5 yearly surveillance strategies.

Aim 5.2 To estimate cost per QALY gained and identify the most cost-effective strategy from a health system perspective.

Aim 5.3 To evaluate the impact of key variables and assumptions via sensitivity analyses.

These chapters are interrelated. For example, **Chapters 2** and **5** inform transition probabilities, costs and model assumptions in the health-economic model (**Chapter 6**), while the HRQoL findings from **Chapter 4** are incorporated as utility values in **Chapter 6**. **Chapter 3** examines inheritance patterns with direct implications for genetic counselling and family planning. Taken together, this thesis provides an integrated evidence base to guide healthcare professionals and genetic counsellors in supporting and managing the care of *SDHB* PV carriers.

Chapter 2: Surveillance for SDHB PV carriers: a multicentre cohort study

2.0 Preface

The following manuscript contains a multicentre cohort study conducted with local ethics approval (Northern Sydney Local Health District Ethics Committee Ref: 2019/ETH09870; Tasmania Health and Medical Human Research Ethics Committee Ref: H0018520). It provided detailed clinical, biochemical and radiologic profiling of *SDHB* PV carriers and demonstrated that surveillance improved outcomes by enabling earlier tumour detection and treatment. By establishing the clinical value of surveillance, this study informed guideline development and underpinned later analyses in this thesis including the systematic review of penetrance and outcomes (Chapter 5) and the cost-effectiveness modelling of surveillance strategies (Chapter 6). The manuscript was published in the *Journal of Clinical Endocrinology and Metabolism* in 2022 with the text in the thesis identical to the published paper. The full pdf version is available in the appendix.

Davidoff DF, Benn DE, Field M, Crook A, Robinson BG, Tucker K, De Abreu Lourenco R, Burgess JR, Clifton-Bligh RJ (2022). Surveillance Improves Outcomes for Carriers of *SDHB* Pathogenic Variants: A Multicenter Study. *The Journal of Clinical Endocrinology & Metabolism*. Volume 107, Issue 5, May 2022, Pages e1907–e1916, https://doi.org/10.1210/clinem/dgac019

Title Page

Full title:

Surveillance improves outcomes for carriers of *SDHB* pathogenic variants: a multicentre study

Short running title:

Surveillance improves outcomes for *SDHB* carriers

Authors:

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Structured abstract:

Context

Carriers of succinate dehydrogenase type B (SDHB) pathogenic variants (PV) are at risk of phaeochromocytoma and paraganglioma (PPGL) from a young age. It is widely recommended carriers enter a surveillance program to detect tumours but there are limited studies addressing outcomes of surveillance protocols for *SDHB* PV carriers.

Objective

The purpose of this study was to describe surveillance-detected (s-d) tumours in *SDHB* PV carriers enrolled in a surveillance program and to compare their outcomes to probands.

Methods

This was a multicentre study of *SDHB* PV carriers with at least one surveillance episode (clinical, biochemical, imaging) in Australian genetics clinics. Data were collected by both retrospective and ongoing prospective follow-up. Median duration of follow-up was 6.0 years.

Results

181 *SDHB* PV carriers (33 probands and 148 non-probands) were assessed. Tumours were detected in 20% of non-probands undergoing surveillance (age range 9-76 years). Estimated 10-year metastasis-free survival was 66% for probands and 84% for non-probands with s-d tumours (p=0.027). S-d tumours were smaller than those in probands (median 27 mm *versus* 45 mm respectively, p=0.001). Tumour size ≥40 mm was associated with progression to metastatic disease (OR 16.9, 95% CI 2.3-187.9, p=0.001). Patients with s-d tumours had lower mortality compared to probands: 10-year overall survival was 79% for probands and 100% for non-probands (p=0.029).

Conclusion

SDHB carriers with s-d tumours had smaller tumours, reduced risk of metastatic disease and lower mortality compared to probands. Our results suggest that *SDHB* PV carriers should undertake surveillance to improve clinical outcomes.

Disclosures:

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Disclosures: The authors have nothing to declare.

2.1 Introduction

Carriers of *succinate dehydrogenase type B* (SDHB) pathogenic variants (PV) are at risk of phaeochromocytoma and paraganglioma (PPGL), renal cell carcinomas (RCC) and gastrointestinal stromal tumours (GIST) from a young age (2). It is widely recommended carriers enter a surveillance program to detect tumours, as metastatic disease is associated with high mortality (51, 74). However, there are limited studies addressing outcomes of surveillance protocols for *SDHB* PV carriers.

A recent international Delphi consensus study recommended asymptomatic *SDHB* PV carriers commence surveillance for tumours from as young as age 6 to 10 years; baseline assessment should include clinical history for catecholaminergic symptoms, blood pressure, either plasma or urinary metanephrine and normetanephrine, and magnetic resonance imaging (MRI) of head and neck, thorax, abdomen and pelvis and the option of functional imaging in adulthood (100). These guidelines recommended ongoing assessment, after a first negative initial surveillance, with annual clinical examination, biennial biochemical testing and MRI every 2-3 years and/or functional imaging in adulthood (100).

The goal of surveillance is to detect *SDHB*-associated tumours early enough for curative surgical resection, since large tumour size is associated with metastatic progression (52, 61, 67, 68, 71). A multicentre European study of patients with metastatic PPGL reported primary tumour size was ≥ 5 cm in 76% of cases (68). Single-centre studies from the USA have reported PPGL cut-offs of 4 cm (71), 4.5 cm (67), 5 cm (52) and 6.1 cm (61) are associated with metastatic disease in *SDHB* PV carriers. Tumour location also impacts on risk of metastatic disease. HNPGL tend to have a lower risk of metastatic progression than *SDHB*-associated abdominal or pelvic PPGL (12% vs 33%) (22), and when metastases occur the disease course is often indolent (111).

The purpose of this study was to describe surveillance-detected (s-d) tumours in *SDHB* PV carriers undergoing surveillance compared to probands by: (a) describing the nature of *SDHB*-

associated tumours detected during surveillance; and, (b) investigating case-detection strategies that are helpful in detecting *SDHB*-related tumours and whether these translate into improved outcomes.

2.2 Materials and Methods

This was a multicentre observational cohort study of *SDHB* PV carriers with at least one surveillance episode (clinical, biochemical, imaging) in genetics clinics at Royal North Shore Hospital (RNSH), Prince of Wales Hospital (PoWH) and Royal Hobart Hospital (RHH) in Australia. Clinical data from medical records were collected by both retrospective and ongoing prospective follow-up using a bespoke data extraction form developed in Research Electronic Data Capture (REDCap) (112) software. Retrospective data was collected from July 2000 at RNSH, September 1994 at PoWH and February 2002 at RHH, up to 29 September 2019. Ongoing prospective data collection has been performed since 30 September 2019 with most recent data collection performed on 10 August 2021.

The data extraction form was tailored to the surveillance protocol at each site, but also had capacity to record types and frequencies of biochemical or imaging surveillance that differed to the standard protocol if relevant to that participant. Annual clinical assessment of symptoms, blood pressure and biochemical measurements of plasma metanephrines or urinary catecholamines were included in surveillance protocols of all three centres. At RHH biochemical assessment also included annual chromogranin A measurement, and imaging surveillance consisted of biennial MRI from base of skull to coccyx and after age 18 of four yearly ^{18}F-FDG-PET/CT alternating with four yearly neck and abdominal ultrasounds (98). RNSH and PoWH followed the Cancer Institute NSW guidelines for biennial MRI from base of skull to coccyx (103). After age 18 five yearly functional imaging with either ^{68}Ga-DOTATATE PET/CT or ^{18}F-FDG-PET/CT was included as clinically appropriate.

Probands were defined as the first individual in a family to be diagnosed with a *SDHB* PV after presenting with a tumour, and for this cohort were always the index case. Surveillance-detected (s-d) tumours were defined as tumours detected in non-probands during surveillance. These were classified either as 'clinical' s-d tumours if detected as part of familial surveillance prior to formal genetic diagnosis of *SDHB* PV i.e. before the advent of routine *SDHB* genetic testing, or ‘genetic’ s-d tumours if diagnosed following predictive genetic diagnosis. Incidence density of s-d tumours was defined as the total number of new tumours over the person-time at risk contributed by each participant during the period of surveillance. Incidence was reported as number of tumours per 100 person-years. Genotypes were classified as loss of function (nonsense, splicing, deletion or frameshift) or missense variants. Tumour diagnosis and size was obtained from histopathology reports, or from radiological appearance in combination with biochemistry if the tumour was not resected. ‘Classic’ symptoms (i.e. those typically associated with catecholamine excess) were defined as headaches, palpitations or excessive sweating and were recorded systematically in the clinical records at each site: the majority of clinic visits recorded presence or absence of headaches, palpitations or sweats and where absent were classified as not available. Poor adherence was defined as either attending only one follow-up visit or two or more years between surveillance episodes. Loss to follow-up was defined as two or more years since the last follow-up episode. Ethics approval was obtained from the Northern Sydney Local Health District Ethics Committee for RNSH and PoWH (Ref: 2019/ETH09870) and from Tasmania Health and Medical Human Research Ethics Committee for RHH (Ref: H0018520).

Statistical analysis was performed using GraphPad Prism version 7.03 and IBM SPSS version 26. Descriptive statistics were performed with numerical data presented as median and range. Groups were compared using the Mann-Whitney test for non-parametric data. Cumulative frequency analysis was conducted on s-d tumours to represent the timing of tumour diagnosis during surveillance. Sensitivity, specificity, positive predictive value and negative predictive value of clinical, biochemical and imaging methods of tumour detection were calculated using the Wilson-Brown method to compute 95% confidence intervals and Fisher’s exact test to determine statistical significance. Predictors of disease diagnosis, metastatic disease and mortality were assessed using a generalized linear model with binary logistic regression to perform a multivariate analysis. Explanatory variables included in this model were sex, genotype, history of smoking, classic symptoms, hypertension, age at first surveillance episode, tumour size, Ki-67, tumour location, tumour functional status (defined as production of catecholamines noradrenaline, adrenaline or dopamine producing or non-functioning), multifocality and synchronous metastases. Significant predictors in the main effects model were then assessed for interaction. We estimated metastasis-free survival and overall survival of probands and non-probands by Kaplan-Meier analysis in patients with any *SDHB*-associated tumours and in a subgroup of patients with thoraco-abdominal PPGL. Receiver operated curve (ROC) analysis of tumour size for prediction of metastatic disease was presented as area under the curve with an area of 0.7 considered acceptable and a Youden's index determined based on the optimal value that maximised the sum of sensitivity and specificity. Cox proportional hazards regression was also performed to examine the impact of tumour size and risk of probands compared to non-probands for metastatic progression and risk of a second primary tumour. A p-value ≤ 0.05 was considered as statistically significant.

2.3 Results

Baseline characteristics

This cohort includes 181 *SDHB* PV carriers from 59 families undergoing routine clinical surveillance (Table 2.1): 92 (51%) from RHH, 50 (27%) from RNSH and 39 (22%) from PoWH. There were 33 (18%) probands and 148 (82%) non-probands. Median age at first surveillance for non-probands was 33 years (range 1 to 81 years) and 84 (46%) were male. One proband and three non-probands had poor adherence to surveillance. Median duration of follow-up was 6.0 ± 0.4 years and was not different between probands and non-probands. Non-probands had 1059 person-years follow-up. Of the total cohort, 110 (61%) had at least 5 years follow-up, 54 (30%) had at least 10 years of follow-up, 24 (13%) had 15 years and 5 (3%) had 20 years follow-up. Twenty (11%) carriers were lost to follow-up.

Table 2.1 Baseline characteristics

	Complete cohort (n=181)	Probands (n=33)	Non-probands (n=148)	P value
Age at first surveillance, years; median (range)	33 (1-81)	35 (9-70)	33 (1-81)	0.68
Male; n (%)	84 (46)	17 (52)	67 (45)	0.50
Probands; n (%)	33 (18)	-	-	-
Genotype; n (%)				
Missense	57 (31)	9 (27)	48 (32)	0.52
Nonsense	43 (24)	10 (30)	33 (22)	
Splicing	35 (19)	3 (9)	32 (22)	
Deletion	43 (24)	10 (30)	33 (22)	
Duplication	2 (1)	1 (0.5)	1 (0.5)	
Not available	1 (0.5)	0	1 (0.5)	
Country of birth; n (%)				
Australia	155 (86)	26 (79)	129 (87)	0.86
UK	5 (3)	1 (3)	4 (3)	
Malaysia	1 (0.5)	1 (3)	0	
Philippines	2 (1)	2 (6)	0	
Brazil	2 (1)	0	2 (1)	
The Netherlands	2 (1)	1 (3)	1 (0.5)	
South Africa	1 (0.5)	0	1 (0.5)	
Not available	13 (7)	2 (6)	11 (8)	
Smoking status				
Non-smoker; n (%)	95 (52)	19 (58)	76 (51)	0.76
Current smoker; n (%)	29 (16)	4 (12)	25 (17)	
Past smoker	27 (15)	7 (21)	20 (14)	
Not available; n (%)	30 (17)	3 (9)	27 (18)	
Occupation; n (%)				
Student	49 (27)	3 (9)	46 (31)	0.22
Professional	33 (18)	9 (27)	24 (16)	
Clerical, sales and service worker	12 (7)	2 (6)	10 (7)	
Labourer	11 (6)	3 (9)	8 (5)	
Manager	10 (5.5)	3 (9)	7 (5)	
Tradesperson	10 (5.5)	3 (9)	7 (5)	
Retired	7 (4)	0	7 (5)	
Full time parent	4 (2)	0	4 (3)	
Production and transport worker	2 (1)	0	2 (1)	
Disability support pension	1 (0.5)	1 (3)	0	
Not available	42 (23)	9 (27)	33 (22)	
Duration of ongoing follow-up, years; median (range)	6.0 (1 month-25.6 years)	8.1 (14 month-25.6 years)	5.9 (1 month-23.9 years)	0.08

The nature of *SDHB*-associated tumours detected during surveillance

Surveillance-detected tumours

Tumours were detected in 29 (20%) of 148 non-probands during surveillance (Table 2.2 and Figure 2.1). The incidence of s-d tumours was 1.3 cases per 100 person-years (95% CI 0.7-2.3 per 100 person-years). Twenty two (15%) of 148 non-probands had their first surveillance aged ≤ 10 years. The youngest age of first surveillance was at age one year. The youngest age of a s-d tumour was a bladder paraganglioma diagnosed at age 9 years. Seventeen (11%) of the 148 non-probands had a final age of follow-up after 70 years of age, and 3 (2%) non-probands had follow-up after age 80 years. The oldest age of a surveillance detected tumour was an asymptomatic gastric GIST detected at age 76. The age of case detection of the 29 non-probands with s-d tumours is represented in Figure 2.2.

Table 2.2 *SDHB* PV carriers with disease diagnoses: tumour types

	Probands (n=33)	Non-probands with s-d tumours (n=29)	P value
HNPGL (n)	9	14	0.16
PC (n)	7	2	0.11
Thoracic PGL (n)	1	3	0.25
Abdominal PGL (n)	27	11	0.002*
RCC (n)	3	0	0.21
GIST (n)	1	3	0.25
Pituitary adenoma (n)	0	1	0.29
Total tumours (n)	48	34	0.03**

13 probands and 5 non-probands had multifocal tumours. For probands, one patient had a synchronous APGL and GIST, one patient had synchronous APGL and PC followed by a metachronous APGL, one patient had synchronous bilateral RCC, one patient had synchronous bilateral APGL. 10 probands had metachronous tumours (31%). For non-probands, one patient had synchronous APGL and GIST and one patient with synchronous HNPGL and APGL; three patients developed a metachronous tumour (two HNPGL, one PC). * P value < 0.01; ** P value < 0.05. GIST gastrointestinal stromal tumour; HNPGL head and neck paraganglioma; PC phaeochromocytoma; PGL paraganglioma; RCC renal cell carcinoma; s-d surveillance-detected

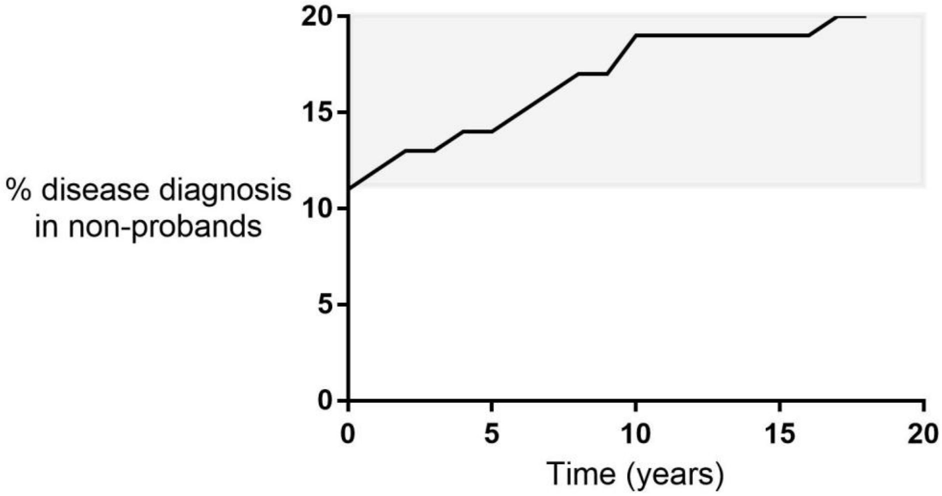

Figure 2.1 Cumulative frequency of disease diagnosis in non-probands

The prevalence of disease at the initial surveillance episode was 11% (n=17) and 12 new cases were detected during surveillance. In total, 29 of 148 non-probands were diagnosed with disease. The risk of disease in non-probands was 20%.

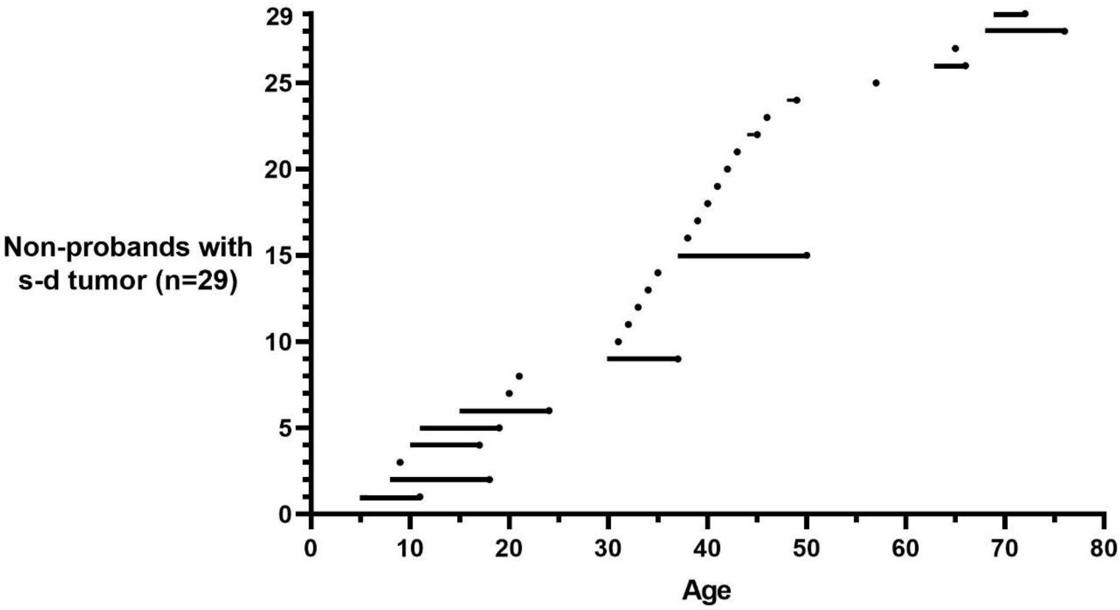

Figure 2.2 Non-probands diagnosed with s-d tumours

The age of case detection of each participant is represented by the black circle (n=29). Non-probands who were not diagnosed with disease at the initial screen have a line to represent the age from the start of surveillance until case detection.

<u>Clinical surveillance-detected tumours</u>

Twelve (41%) of 29 non-probands with disease had ‘clinical’ s-d tumours, defined as tumour detected as part of familial surveillance but prior to formal predictive genetic diagnosis of an *SDHB* PV (Table 2.2). Four (33%) patients with clinical s-d tumours had tumours smaller than 40 mm and one (8%) had a tumour under 20 mm. Of the eight non-probands with clinical s-d tumours whose records on symptoms were available, five (63%) had classic symptoms consistent with elevated catecholamines, two had hearing loss in the context of HNPGL and only one patient was asymptomatic.

Six (50%) of 12 patients with clinical s-d tumours had tumours resected and are in remission at most recent follow-up. One (8%) patient has a persistent localised HNPGL despite surgical excision. Four (33%) patients had metachronous tumours: one (8%) had a metachronous APGL resected and is in remission, two (17%) patients had an abdominal PGL resected but have persistent localised HNPGLs, and one (8%) patient has persistent localised HNPGLs. One (8%) patient developed recurrence of HNPGL and subsequently metastatic disease. One (8%) patient with clinical s-d tumour died from disease.

<u>Genetic surveillance-detected tumours</u>

Seventeen (57%) of 30 non-probands with disease had ‘genetic’ s-d tumours, defined as tumours diagnosed following predictive genetic diagnosis (Table 2.2 and Figure 2.3). Six patients had tumours diagnosed at the initial surveillance (35% of all genetic s-d tumours; Table 2.2). The remaining eleven (65%) patients had genetic s-d tumours detected at 3 to 152 months following the initial surveillance episode, of which one was a synchronous GIST and APGL. Notably 11 patients (65%) with genetic s-d tumours had tumours smaller than 40 mm and 7 (41%) patients had tumours smaller than 20 mm. Ten (59%) patients with genetic s-d tumours were asymptomatic. One patient had a 14 mm prolactinoma without suprasellar or cavernous sinus extension. Eleven patients (65%) with genetic s-d tumours had tumours resected and are in remission at most recent follow-up. Five (29%) have persistent localised disease (one TPGL, one pituitary adenoma, two HNPGL, one APGL) and one patient has metastatic disease. None of the patients with genetic s-d tumours experienced recurrence, a metachronous tumour or death from disease at most recent follow-up.

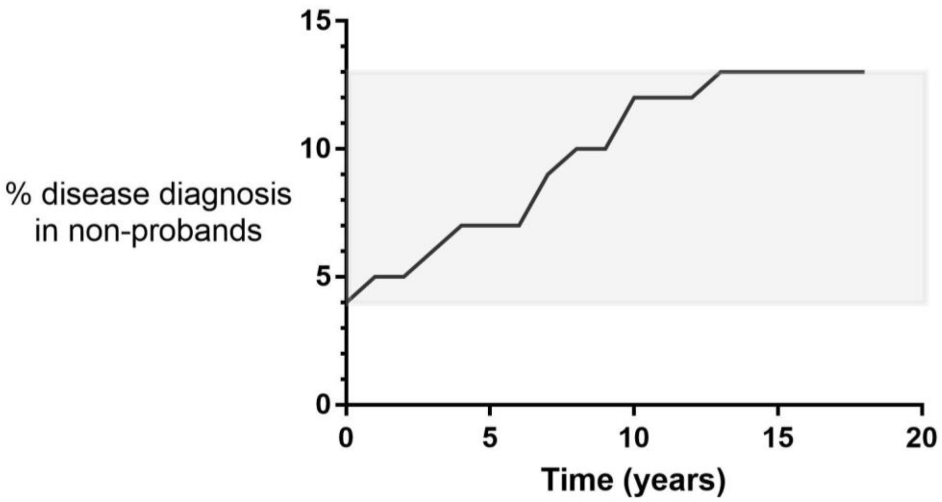

Figure 2.3 Cumulative frequency of disease diagnosis made following predictive genetic diagnosis of *SDHB* PV

The prevalence of disease at the initial surveillance episode was 4% (n=6) and 11 new cases were detected during surveillance. In total, 17 of 136 non-probands were diagnosed with disease following the genetic diagnosis. The risk of disease in non-probands following the genetic diagnosis was 13%.

Case-detection strategies helpful in detecting *SDHB*-related tumours and translating into improved clinical outcomes

Method of detection of tumours and tumour size

To describe the methods by which tumours were detected, we then reviewed data on all 82 tumours detected in these 62 patients (33 probands and 29 non-probands, Table 2.3). Forty (65%) patients had 49 diagnoses of thoraco-abdominal PPGL (9 PC, 4 TPGL and 38 APGL). Data on tumour functional status were available for 38 thoraco-abdominal PPGL. Twenty nine (76%) secreted noradrenaline of whom 21 (72%) had classic symptoms. Three (8%) secreted adrenaline of whom 2 had classic symptoms and 6 (16%) were apparently non-functional of whom 1 reported classic symptoms. Of those whose tumour functional status were unavailable, 9 (18%) had unavailable data from the 1990's and two (4%) patients were probands who entered surveillance following surgery at another centre where PPGL had not been suspected (of whom one survived an intra-operative hypertensive crisis). Median maximal tumour diameter was 40 mm (range 25-90 mm), 43 mm (range 35-75 mm) and 41 mm (range 8-190 mm) for PC, TPGL and APGL respectively. Tumours were detected by functional imaging followed by directed anatomical imaging in 3 HNPGL, 2 PC, 1 TPGL and 8 APGL (Table 2.3).

Table 2.3 *SDHB* PV carriers with disease diagnoses: method of detection of tumours

	Modality of detection	Probands (n=33)	Non-probands with s-d tumors (n=29)
HNPGL	MRI	MRI (n=1)	MRI (n=1)
	MRI and CT	MRI-detected followed by CT (n=1)	CT and MRI-detected (n=1) MRI-detected followed by CT (n=1)
	18F-FDG-PET/CT and MRI	18F-FDG-PET/CT followed by MRI (n=1)	None detected
	68-Ga-DOTATATE PET/CT, ultrasound and MRI	68-Ga-DOTATATE PET/CT-detected followed by ultrasound and MRI (n=2)	None detected
	MRI and 68-Ga-DOTATATE PET/CT	None detected	MRI followed by 68-Ga-DOTATATE PET/CT (n=2)
	Ultrasound and CT	None detected	Ultrasound-detected followed by CT (n=1)
	MRI, ultrasound and CT	MRI followed by ultrasound and CT (n=1)	None detected
	Ultrasound	None detected	Ultrasound (n=1)
	Chromogranin A, 68-Ga-DOTATATE PET/CT and MRI	None detected	Elevated chromogranin A and 68-Ga-DOTATATE PET/CT-detected followed by MRI (n=1)
	Unknown	Unknown (n=3)	Unknown (n=6)
PC	Urinary normetanephrine, plasma normetanephrine, 68-Ga-DOTATATE PET/CT and CT	Elevated urinary normetanephrine and plasma normetanephrine and 68-Ga-DOTATATE PET/CT followed by CT (n=1)	None detected
	Urinary normetanephrine and dopamine	Elevated urinary normetanephrine and dopamine, imaging unknown (n=1)	None detected
	Urinary norepinephrine and normetanephrine, plasma normetanephrine, ultrasound, CT, MRI, 68-Ga-DOTATATE PET/CT, 123I-MIBG scintigraphy, 18F-FDG-PET/CT	Urinary norepinephrine, normetanephrine, plasma normetanephrine, ultrasound-detected followed by CT, MRI, 68-Ga-DOTATATE PET/CT, 123I-MIBG scintigraphy and 18F-FDG-PET/CT (n=1)	None detected
	18F-FDG-PET/CT and 68-Ga-DOTATATE PET/CT	18F-FDG-PET/CT, 68-Ga-DOTATATE PET/CT (n=1)	None detected
	Urinary catecholamines, plasma catecholamines, CT, MRI, 68-Ga-DOTATATE PET/CT, 123I-MIBG	Urinary catecholamines, plasma catecholamines , CT, MRI, 68-Ga-DOTATATE PET/CT, 123I-MIBG (n=1)	None detected
	MRI	MRI (n=1)	None detected
	Unknown	Unknown (n=1)	Unknown (n=2)
Thoracic PGL	CT, urinary norepinephrine, normetanephrine and plasma normetanephrine	CT, urine norepinephrine, normetanephrine, plasma normetanephrine (n=1)	None detected
	MRI, CT, 68-Ga-DOTATATE PET/CT	None detected	MRI followed by CT and 68-Ga-DOTATATE PET/CT (n=1)
	Plasma normetanephrine, 3-MT, chromogranin A, 18F-FDG-PET/CT, MRI and 68-Ga-DOTATATE PET/CT	None detected	Elevated plasma normetanephrine, 3-MT and chromogranin A, 18F-FDG-PET/CT followed by MRI and 68-Ga-DOTATATE PET/CT (n=1)
	Unknown	None	Unknown (n=1)
Abdominal PGL	Urinary norepinephrine, plasma normetanephrine, MRI and 18F-FDG-PET/CT	Elevated urinary norepinephrine, plasma normetanephrine, MRI, 18F-FDG-PET/CT (n=1)	None detected

	Urinary norepinephrine, plasma normetanephrine, chromogranin A, ultrasound, CT, MRI, octreoscan, 68-Ga-DOTATATE PET/CT and 18F-FDG-PET/CT	None detected	Urinary norepinephrine, plasma normetanephrine, chromogranin A, ultrasound, CT, MRI, octreoscan, 68-Ga-DOTATATE PET/CT, 18F-FDG-PET/CT (n=1)
	Urinary normetanephrine, metanephrine	Elevated urinary normetanephrine, metanephrine, imaging unknown (n=1)	None detected
	Urinary normetanephrine, plasma normetanephrine, CT and MRI	Elevated urinary normetanephrine, plasma normetanephrine, CT and MRI (n=1)	None detected
	Urinary norepinephrine, normetanephrine, plasma normetanephrine, CT, ultrasound, MRI, 68-Ga-DOTATATE PET/CT, 123I-MIBG scintigraphy and 18F-FDG-PET/CT	Elevated urinary norepinephrine, normetanephrine, plasma normetanephrine, CT followed by ultrasound, MRI, 68-Ga-DOTATATE PET/CT, 123I-MIBG, 18F-FDG-PET/CT (n=2)	None detected
	Urinary epinephrine, chromogranin A and CT	None detected	Elevated urinary epinephrine, chromogranin A CT (n=1)
	Urinary normetanephrine, plasma normetanephrine, 68-Ga-DOTATATE PET/CT and CT	Elevated urinary normetanephrine, plasma normetanephrine and 68-Ga-DOTATATE PET/CT-detected followed by CT (n=1)	None detected
	Urinary catecholamines, plasma catecholamines, CT, MRI, 68-Ga-DOTATATE PET/CT and 123I-MIBG scintigraphy	Elevated urinary catecholamines, plasma catecholamines, CT, MRI followed by 68-Ga-DOTATATE PET/CT, 123I-MIBG (n=1)	None detected
	Urinary norepinephrine, plasma normetanephrine, 18F-FDG-PET/CT and 68-Ga-DOTATATE PET/CT	None detected	Elevated urinary norepinephrine and plasma normetanephrine and 18F-FDG-PET/CT-detected followed by 68-Ga-DOTATATE PET/CT (n=1)
	Urinary norepinephrine and dopamine, CT and MRI	Urinary norepinephrine, dopamine, CT, MRI (n=2)	None detected
	Urinary norepinephrine, plasma normetanephrine, chromogranin A, CT and 18F-FDG-PET/CT	Elevated urinary norepinephrine, plasma normetanephrine, chromogranin A and CT-detected followed by 18F-FDG-PET/CT (n=1)	None detected
	Urinary norepinephrine, dopamine, plasma normetanephrine, CT and 123I-MIBG scintigraphy	None detected	Elevated urinary norepinephrine, dopamine and plasma normetanephrine and CT-detected followed by 123I-MIBG scintigraphy (n=1)
	Urinary norepinephrine, normetanephrine, MRI, CT and 123I-MIBG scintigraphy	Elevated urinary norepinephrine and normetanephrine and MRI-detected followed by CT and 123I-MIBG scintigraphy (n=1)	None detected
	Urinary norepinephrine, chromogranin A, ultrasound, CT, 123I-MIBG and 18F-FDG-PET/CT	Urinary norepinephrine, chromogranin A, ultrasound, CT, 123I-MIBG, 18F-FDG-PET/CT (n=1)	None detected
	Urinary norepinephrine, plasma normetanephrine, epinephrine, norepinephrine, MRI, 123I-MIBG scintigraphy and 18F-FDG-PET/CT	Elevated urinary norepinephrine, plasma normetanephrine, epinephrine and norepinephrine, MRI followed by 123I-MIBG scintigraphy and 18F-FDG-PET/CT (n=2)	None detected
	Urinary norepinephrine, plasma norepinephrine, 18F-FDG-PET/CT, CT and 123I-MIBG scintigraphy	Elevated urinary norepinephrine, plasma norepinephrine, 18F-FDG-PET/CT followed by CT and 123I-MIBG scintigraphy (n=1)	None detected

	Urinary normetanephrine, plasma normetanephrine, MRI, 68-Ga-DOTATATE PET/CT	Urinary normetanephrine, plasma normetanephrine, MRI, 68-Ga-DOTATATE (n=1)	
	18F-FDG-PET/CT	None detected	18F-FDG-PET/CT (n=1)
	Ultrasound, CT and 68-Ga-DOTATATE PET/CT	Ultrasound, CT, 68-Ga-DOTATATE PET/CT (n=1)	None detected
	68-Ga-DOTATATE PET/CT and MRI	None detected	68-Ga-DOTATATE PET/CT followed by MRI (n=1)
	Plasma normetanephrine, chromogranin A and 18F-FDG-PET/CT	None detected	Elevated plasma normetanephrine and chromogranin A and 18F-FDG-PET/CT-detected (n=1)
	Plasma normetanephrine, 18F-FDG-PET/CT	None detected	Elevated plasma normetanephrine and 18F-FDG-PET/CT-detected (n=1)
	18F-FDG-PET/CT and 68-Ga-DOTATATE PET/CT	18F-FDG-PET/CT-detected followed by 68-Ga-DOTATATE PET/CT (n=1)	None detected
	Plasma normetanephrine	None detected	Plasma normetanephrine, imaging unknown (n=1)
	MRI and 18F-FDG-PET/CT	None detected	MRI-detected followed by 18F-FDG-PET/CT (n=1)
	18F-FDG-PET/CT, ultrasound, CT, MRI	18F-FDG-PET/CT, ultrasound, CT, MRI (n=1)	None detected
	Plasma normetanephrine, chromogranin A, ultrasound, CT, 68-Ga-DOTATATE PET/CT and 18F-FDG-PET/CT	Elevated plasma normetanephrine and chromogranin A, ultrasound, CT, 68-Ga-DOTATATE PET/CT and 18F-FDG-PET/CT (n=1)	None detected
	MRI	MRI (n=2)	None detected
	Plasma normetanephrine, MRI	Elevated plasma normetanephrine, MRI (n=1)	None detected
	Unknown	Unknown (n=4)	Unknown (n=1)
RCC	CT	CT-detected (n=3)	NA
GIST	CT, MRI, gastroscopy	None detected	CT and MRI followed by gastroscopy (n=1)
	Ultrasound, CT, 18F-FDG-PET/CT, gastroscopy	None detected	Ultrasound, CT, 18F-FDG-PET/CT, gastroscopy (n=1)
	MRI, 18F-FDG-PET/CT and gastroscopy	None detected	MRI, 18F-FDG-PET/CT and gastroscopy (n=1)
Pituitary adenoma	MRI	NA	MRI (n=1)

Table represents the positive biochemical and imaging modalities leading to tumour detection and is not inclusive of all the biochemical and imaging surveillance which did not detect the tumour. 3-MT 3-methoxytyramine; 18F-FDG-PET/CT fluorodeoxyglucose (18F) positron emission tomography/computed tomography; 68-Ga-DOTATATE PET/CT Gallium-68 DOTATATE PET CT; 123I-MIBG scintigraphy Iodine-123 meta-iodobenzylguanidine scintigraphy; CT computed tomography; GIST gastrointestinal stromal tumour; HNPGL head and neck paraganglioma; MRI magnetic resonance imaging; NA not applicable; PC phaeochromocytoma; PGL paraganglioma; RCC renal cell carcinoma; s-d surveillance-detected

When we compared the size of tumours detected before and after the introduction of functional PET imaging, the difference was not significant albeit possibly limited by power (median 34 versus 23 mm, p = 0.15; Table 2.4). The sensitivity and specificity of diagnostic modalities for detection of sympathetic PPGL are shown in Table 2.5.

Table 2.4 Non-proband s-d tumour characteristics and outcomes in those who commenced surveillance before and after 2013

	Surveillance began prior to 2013 (n=21)	Surveillance began after 2013 (n=8)	P value
Male; n (%)	11 (52)	4 (50)	0.91
Classic symptoms (headaches, palpitations or sweats); n (%)	3 (14)	3 (38)	0.18
Age at disease diagnosis; median (range)	37 (9-76)	46 (19-72)	0.08
Tumour size; median (range)	34 (8-85)	23 (10-41)	0.15
Metastases; n (%)	3 (14)	0	0.28
Mortality; n (%)	1 (5)	0	0.55
Duration of follow-up, years; median (range)	11.4 (8.3-23.9)	3.0 (0.8-5.3)	0.00

Table 2.5 Sympathetic PPGL method of detection

	Number of assessments	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	P value
Classic symptoms (headaches, palpitations or sweats)	607	76.9% (95% CI 61.7-87.3)	92.3% (95% CI 89.8-94.2)	40.5% (95% CI 30.1-51.9)	98.3% (95% CI 6.8-99.1)	<0.0001
Hypertension (BP >= 140/90)	439	47.4% (95% CI 27.3-68.3)	77.1% (95% CI 72.9-80.9)	8.6% (95% CI 4.6-15.5)	97.0% (95% CI 94.6-98.4)	0.0242
Urinary catecholamines	227	76.7% (95% CI 59.1-88.2)	85.8% (95% CI 80.2-90.0)	45.1% (95% CI 32.3-58.6)	96.0% (95% CI 92.0-98.1)	<0.0001
Plasma catecholamine metabolites	564	72.7% (95% CI 55.8-84.9)	96.4% (95% CI 94.5-97.7)	55.8% (95% CI 41.1-69.6)	98.3% (95% CI 96.8-99.1)	<0.0001
Plasma catecholamines	14	60.0% (95% CI 23.1-92.9)	88.9% (95% CI 56.5-99.4)	75.0% (95% CI 30.0-98.7)	80.0% (95% CI 49.0-96.5)	0.0949
Chromogranin A	210	61.5% (95% CI 35.5-82.3)	85.8% (95% CI 80.2-90.0)	22.2% (95% CI 11.7-38.1)	97.1% (95% CI 93.5-98.8)	0.0002
Ultrasound	226	45.5% (95% CI 21.3-72.0)	100% (95% CI 98.2-100)	100% (95% CI 56.6-100)	97.3% (95% CI 94.2-98.8)	<0.0001
CT	52	85.2% (95% CI 67.5-94.1)	100% (95% CI 86.7-100)	100% (95% CI 85.7-100)	86.2% (95% CI 69.4-94.5)	<0.0001
MRI	199	82.6% (95% CI 62.9-93.0)	98.3% (95% CI 95.1-99.5)	86.4% (95% CI 66.7-95.3)	97.7% (95% CI 94.3-99.1)	<0.0001
^{68}Ga-DOTATATE PET/CT	33	93.3% (95% CI 70.2-99.7)	100% (95% CI 82.4-100)	100% (95% CI 78.5-100)	94.7% (95% CI 75.4-99.7)	<0.0001
^{123}I-MIBG	19	41.2% (95% CI 21.6-64.0)	100% (95% CI 17.8-100)	100% (95% CI 64.6-100)	16.7% (95% CI 3.0-44.8)	0.5088
^{18}F-FDG-PET/CT	99	100% (95% CI 85.7-100)	100% (95% CI 95.2-100)	100% (95% CI 85.7-100)	100% (95% CI 95.2-100)	<0.0001

^{18}F-FDG-PET/CT fluorodeoxyglucose (^{18}F) positron emission tomography/computed tomography; BP blood pressure;^{68}Ga-DOTATATE PET CT Gallium-^{68} DOTATATE PET CT; CT computed tomography; MRI magnetic resonance imaging; PPGL phaeochromocytoma or paraganglioma

Twenty (32%) patients had 23 diagnoses of HNPGL of whom 5 (25%) reported classic symptoms typically associated with catecholamine excess. 2 (10%) patients with classic symptoms had concomitant APGL but 3 (15%) curiously had classic symptoms of headaches, palpitations or sweats despite absence of biochemical catecholamine excess. Median HNPGL tumour diameter was 23 mm (range 3-65 mm). Seven (35%) HNPGL were detected smaller than 20 mm and imaging modalities of detection for these small tumours were one US, five MRI and four ^{68}Ga-DOTATATE PET/CT scans. The sensitivity and specificity of diagnostic modalities in this cohort for detection of HNPGL are shown in Table 2.6.

Table 2.6 HNPGL method of detection

	Number of assessments	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	P value
Classic symptoms (headaches, palpitations or sweats)	582	28.6% (95% CI 11.7-54.7)	92.2% (95% CI 89.8-94.2)	8.3% (95% CI 3.3-19.6)	98.1% (95% CI 96.6-99.0)	0.0221
Hypertension (BP >= 140/90)	429	33.3% (95% CI 12.1-64.6)	77.1% (95% CI 72.9-80.9)	3.0% (95% CI 0.8-8.5)	98.2% (95% CI 96.1-99.2)	0.4370
Urinary catecholamines	202	0% (95% CI 0-4.3)	85.8% (95% CI 80.2-90.0)	0% (95% CI 0-12.1)	97.1% (95% CI 93.4-98.8)	>0.9999
Plasma catecholamine metabolites	540	11.1% (95% CI 0.6-4.4)	96.4% (95% CI 94.5-97.7)	5% (95% CI 0.3-23.6)	98.5% (95% CI 97.0-99.2)	0.2898
Plasma catecholamines	9	No value (95% CI 0-1)	88.9% (95% CI 56.5-99.4)	0% (95% CI 0-94.9)	100% (95% CI 67.6-100)	>0.9999
Chromogranin A	203	50% (95% CI 18.8-81.2)	85.8% (95% CI 80.2-90.0)	9.7% (95% CI 3.3-24.9)	98.3% (95% CI 95.0-99.5)	0.0470
Ultrasound	223	75.0% (95% CI 40.9-95.6)	100% (95% CI 98.2-100)	100% (95% CI 61.0-100)	99.1% (95% CI 96.7-99.8)	<0.00001
CT	33	75.0% (95% CI 40.9-95.6)	100% (95% CI 86.7-100)	100% (95% CI 61.0-100)	92.6% (95% CI 76.6-98.7)	<0.0001
MRI	189	100% (95% CI 77.2-100)	98.3% (95% CI 95.1-99.5)	81.3% (95% CI 57.0-93.4)	100% (95% CI 97.8-100)	<0.0001
^{68}Ga-DOTATATE PET/CT	24	100% (95% CI 61.0-100)	100% (95% CI 82.4-100)	100% (95% CI 61.0-100)	100% (95% CI 82.4-100)	<0.0001
^{123}I-MIBG	3	0% (95% CI 0-94.9)	100% (95% CI 17.8-100)	No value (95% CI 0-100)	66.7%	>0.9999

					(95% CI 11.9-98.3)	
^{18}F-FDG-PET/CT	79	66.7% (95% CI 11.9-98.3)	100% (95% CI 95.2-100)	100% (95% CI 17.8-100)	98.7% (95% CI 93.0-99.9)	0.0010

^{18}F-FDG-PET CT fluorodeoxyglucose (^{18}F) positron emission tomography/computed tomography; BP blood pressure;^{68}Ga-DOTATATE PET CT Gallium-^{68} DOTATATE PET CT; CT computed tomography; HNPGL head and neck paraganglioma; MRI magnetic resonance imaging.

Other *SDHB*-associated tumours are shown in Table 2.2. GIST tumour size was median 55 mm (range 36-60 mm). The imaging modalities for detection of GIST were one US, two CT, two MRI and two ^{18}F-FDG-PET/CT scans. RCC tumour size was median 41 mm (range 25-167 mm) and CT detected RCC in these cases. Finally, one pituitary adenoma was detected on MRI measuring 14 mm diameter.

Predictors of disease diagnosis

Male gender, classic symptoms (headaches, palpitations or sweats), absence of hypertension and young age were associated with tumours on a generalized linear model with binary logistic regression (Table 2.7). However, there were multiple interactions in the model. Male gender was associated with absent symptoms (p=0.023) and normal blood pressure (p=0.010) while female gender was associated with younger age at first presentation (p=0.007). There was no interaction between classic symptoms and hypertension (p=0.810). Younger age was associated with normal blood pressure (p=0.003), suggesting that young age rather than normal blood pressure was a predictor of disease. The age of disease diagnosis for females was median 34 years (range 9-72) and for males was median 41 years (range 9-76). Overall median age of diagnosis was 37 years (range 9-76). A generalized linear model with binary logistic regression for predictors of thoraco-abdominal PPGL was unable to be obtained due to sample size and therefore quasi-complete separation in the data. Probands had higher numbers of APGL and overall *SDHB*-associated tumours compared to non-probands with disease (p=0.002 and p=0.03 respectively, Table 2.2). Probands were more likely to develop multifocal disease over time compared to non-probands (OR 3.28, 95% CI 1.02-9.74, p=0.046) but were not more likely to have multifocal disease at baseline (p=0.68). Cox proportional hazard assessment found the risk of a second tumour was not determined by primary tumour size 40mm or larger (HR 2.61, 95% 0.69-9.87, p=0.157). No other factors were able to predict recurrence or multifocal disease including gender, loss of function genotype, history of smoking, typical symptoms, hypertension, age, tumour size and Ki-67.

Table 2.7 Generalized linear model with binary logistic regression of predictors of disease diagnosis, metastatic disease and death from disease for *SDHB* PV carriers

Predictors of disease diagnosis	**P value**	**OR (95% CI)**
Male	0.007*	3.05 (1.36-6.89)
Loss of function PV	0.43	0.72 (0.32-1.62)
History of smoking	0.33	1.75 (0.56-5.44)
Classic symptoms (headaches, palpitations or sweats)	0.000*	4.90 (2.03-11.84)
Hypertension (BP >= 140/90)	0.000*	0.10 (0.03-0.29)
Age at first surveillance episode	0.003*	0.97 (0.94-0.99)
Test	P value	χ^2
Overall model likelihood ratio test (Omnibus Test)	0.000*	38.79
Predictors of metastatic disease	**P value**	**OR (95% CI)**
Tumour size	0.004*	1.08 (1.03-1.14)
Male	0.36	0.29 (0.02-4.19)
Loss of function PV	0.39	0.29 (0.18-4.81)
History of smoking	0.81	1.21 (0.25-5.91)
Classic symptoms (headaches, palpitations or sweats)	0.48	3.35 (0.11-102.89)
Hypertension (BP >= 140/90)	0.63	2.17 (0.09-50.49)
Age at first surveillance episode	0.14	1.07 (0.98-1.16)
Multifocal tumours	0.13	8.05 (0.55-117.43)
Tumour location	0.09	5.99 (0.78-45.90)
Tumour functioning status	0.47	2.09 (0.28-15.68)
Ki-67	0.84	0.72 (0.02-8.44)
Test	P value	χ^2
Overall model likelihood ratio test (Omnibus Test)	0.009**	29.41
Predictor of death from disease	**P value**	**OR (95% CI)**
Tumour size	0.09	0.97 (0.94-1.01)
Synchronous metastases	0.99	No value[#]
Male	0.38	0.28 (0.02-4.93)
Loss of function PV	0.77	1.71 (0.05-59.16)
History of smoking	0.20	6.58 (0.67-62.06)
Typical symptoms (headaches, palpitations or sweats)	0.47	3.93 (0.10-157.93)
Hypertension (BP >= 140/90)	0.63	1.97 (0.13-29.97)
Age at first surveillance episode	0.74	0.98 (0.89-1.09)

Multifocal tumours	0.33	3.79 (0.25-56.57)
Tumour location	0.99	0.00 (no value)
Tumour functioning status	0.99	0.00 (no value)
Ki-67	0.99	0.00 (no value)
Test	P value	χ^2
Overall model likelihood ratio test (Omnibus Test)	0.19	18.47

* P value < 0.01; ** P value < 0.05 # No value due to a quasi-complete separation in the data; CI confidence interval; Loss of function defined as nonsense, splicing, deletion or frameshift pathogenic variant (PV)

Metastatic disease

Patients with s-d tumours were less likely to be associated with metastatic disease compared to probands. Three (10%) of 29 patients with s-d tumours developed metastatic disease at most recent follow-up compared to 10 (31%) of 32 probands (p=0.04). Overall, 13 (21%) of 62 patients with disease showed evidence of metastatic progression at a median of 66 months (range 0 to 127 months) with no difference in synchronous metastases between probands and non-probands (p=0.061). Two probands had synchronous metastases. Probands had increased risk of metastatic progression compared to non-probands with s-d tumours (HR 4.13, 95% CI 1.15-14.9, p = 0.03). Estimated 5- and 10-year metastasis-free survival was 82% and 66% for probands and 100% and 84% for non-probands (p=0.027, Figure 2.4). Median time between initial diagnosis of any *SDHB*-associated tumour and diagnosis of metastatic disease was 68 months (range 0-193 months).

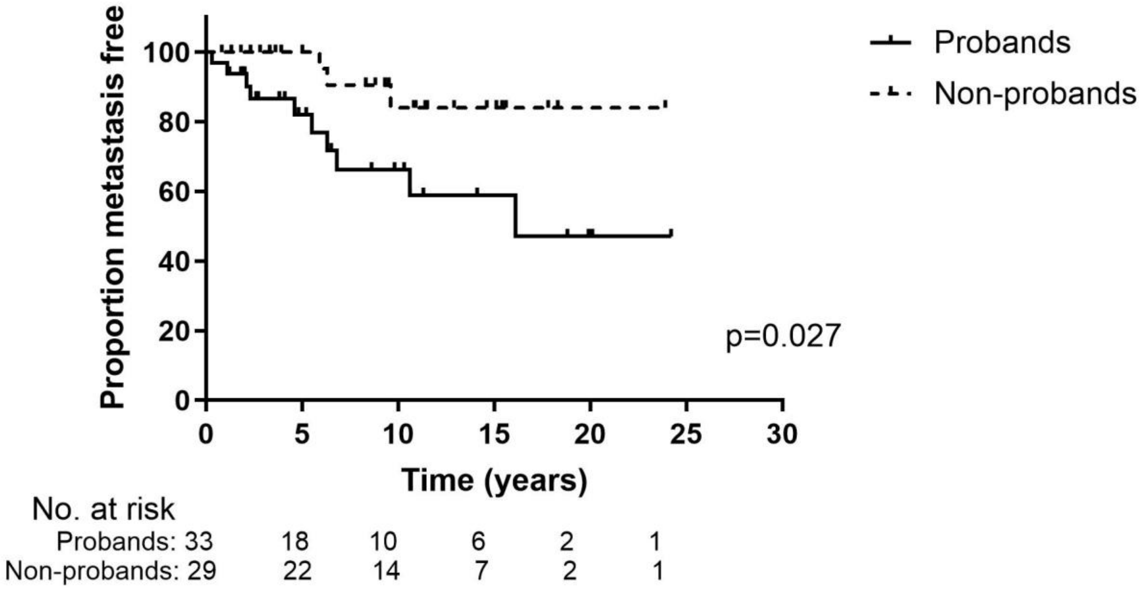

Figure 2.4 Kaplan-Meier curve of metastasis-free survival for all patients with *SDHB*-associated tumours

The risk of metastatic disease in HNPGL was 10% (2/20). In comparison, 10 (25%) of the 40 patients with thoraco-abdominal PPGL had evidence of metastatic progression at median 61 months (range 3 to 127 months) with higher risk of metastatic progression in probands compared to non-probands (OR 11.35, 95% CI 1.51-128.2, p=0.013). One proband with thoraco-abdominal PPGL had synchronous metastases. Estimated 5- and 10-year metastasis-free survival for patients with thoraco-abdominal PPGL was 77% and 57% for probands and 100% and 91% for non-probands (p=0.023, Figure 2.5). Median time between initial diagnosis of thoraco-abdominal PPGL and diagnosis of metastatic disease was 60 months (range 3-127 months).

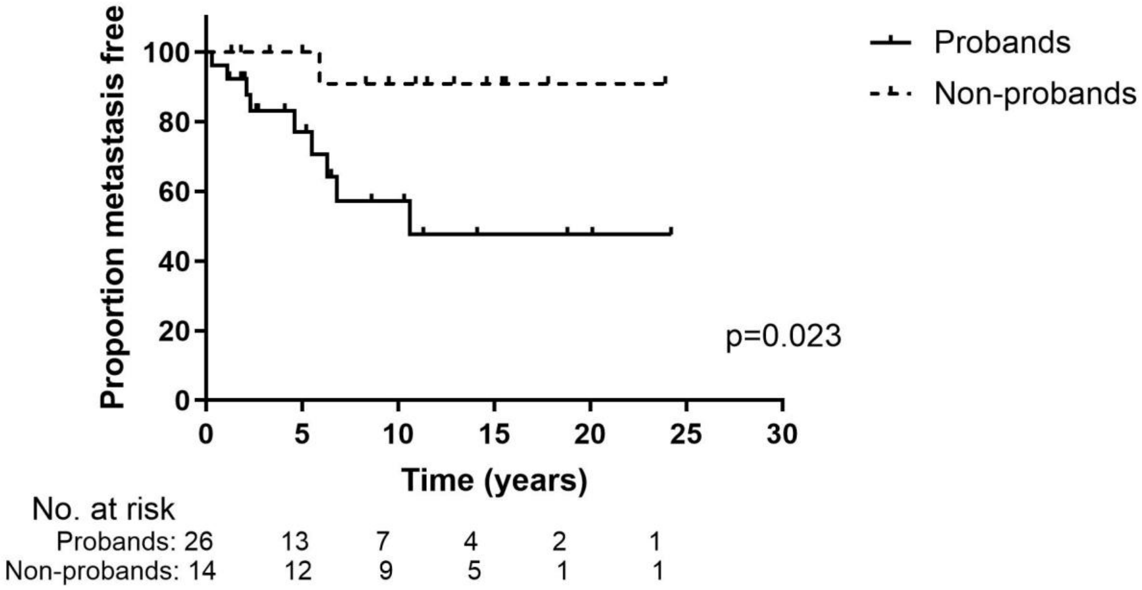

Figure 2.5 Kaplan-Meier curve of metastasis-free survival for patients with thoraco-abdominal PPGL

Tumour size was the only statistically significant predictor of metastatic disease on a multivariate generalized linear model with binary logistic regression (OR 1.08, 95% CI 1.03-1.14, p=0.004, Table 2.7). S-d tumours were smaller than those in probands (median 27 mm (range 8-85 mm) *versus* 45 mm (range 13-190mm) respectively, p=0.001). Tumour size of 40 mm or higher was associated with progression to metastatic disease for all *SDHB*-associated tumours (OR 16.9, 95% CI 2.3-187.9, p=0.001) and for patients with thoraco-abdominal PPGL (OR 11.5, 95% CI 1.57-133.1, p=0.02). ROC curve analysis determined that size of the primary tumour > 39 mm was the optimal cut-off value associated with increased risk of metastatic disease for all *SDHB*-associated tumours (Area Under the Curve = 0.86, sensitivity = 58.5% [95% CI 42.1-73.7], specificity = 100% [95% CI 75.3-100], Youden's index = 0.59, p=0.0001, Figure 2.6) and for patients with thoraco-abdominal PPGL (Area Under the Curve = 0.87, sensitivity = 56.0% [95% CI 34.9-75.6], specificity = 100% [95% CI 69.2-100], Youden's index = 0.56, p=0.0007, Figure 2.7). Cox proportional hazards regression could not determine the hazard ratio of metastatic disease associated with tumours 40mm or larger due to a quasi separation in the data, whereby there were zero cases of metastatic progression with tumour size below 40mm. Similarly, a multivariate generalized linear model with binary logistic regression for predictors of metastatic disease in patients with thoraco-abdominal PPGL was unable to be obtained due to a quasi-complete separation in the data.

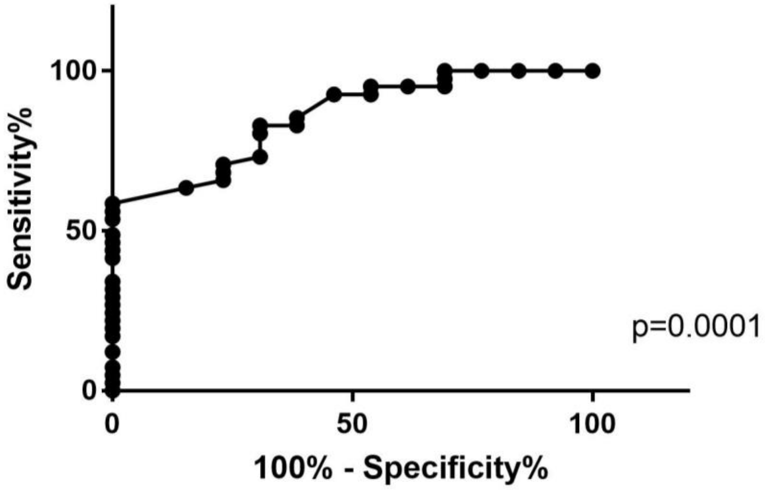

Figure 2.6 ROC curve analysis of tumour size for prediction of metastatic disease in all patients with *SDHB*-associated tumours

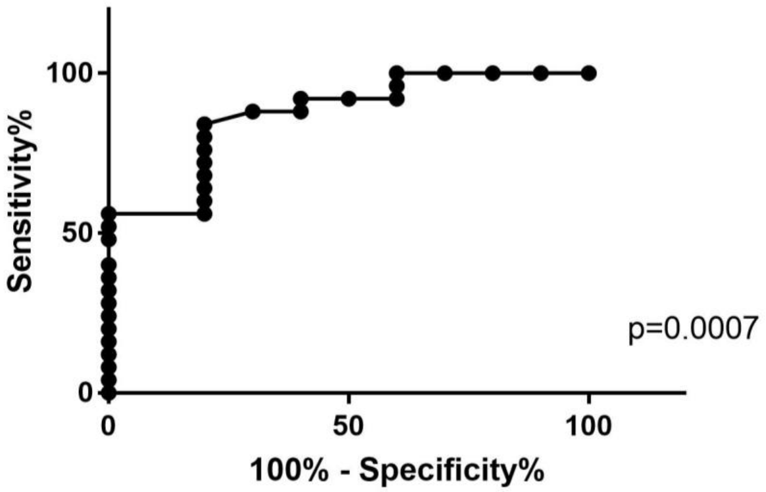

Figure 2.7 ROC curve analysis of tumour size for prediction of metastatic disease in patients with thoraco-abdominal PPGL

Mortality

Nine patients (14.5% of patients with disease) died in this cohort, one from a non-*SDHB* related cause (metastatic colorectal cancer on a background of ulcerative colitis). One non-proband and seven probands died from disease: one from bilateral RCC, three from APGLs, one from metachronous APGL (including one bladder PGL), one from metachronous APGL/PC and two from metachronous APGL/HNPGL. Of patients who died of disease, primary tumour size was median 68 mm (range 40-105 mm), age at initial disease diagnosis was median 38 years (range 27-60 years) and two patients had synchronous metastases at initial diagnosis. For the remaining patients who did not have synchronous metastases, time to metastatic disease following initial diagnosis was median 71 months (range 4-193 months). Duration of survival following the diagnosis of metastatic disease was median 61 months (range 27-311 months). The age at death was median 47 years (range 29-84 years).

Patients with s-d tumours had lower mortality compared to probands. Risk of death from disease was 21.9% for probands and 3.4% for non-probands (OR 7.8, 95% CI 1.2-90.1, p=0.054). Estimated 5- and 10-year overall survival was 89% and 79% for probands and 100% for non-probands (p=0.029, Figure 2.8). There was no difference in overall survival for patients with thoraco-abdominal PPGL between probands and non-probands (p=0.179, Figure 2.9).

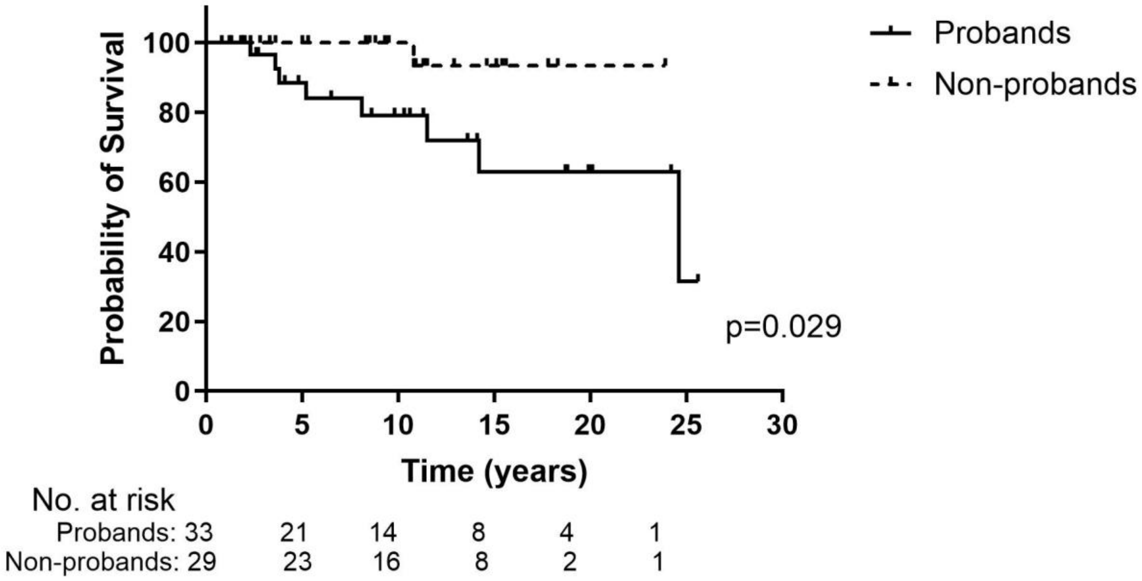

Figure 2.8 Kaplan-Meier curve of overall survival for all patients with *SDHB*-associated tumours

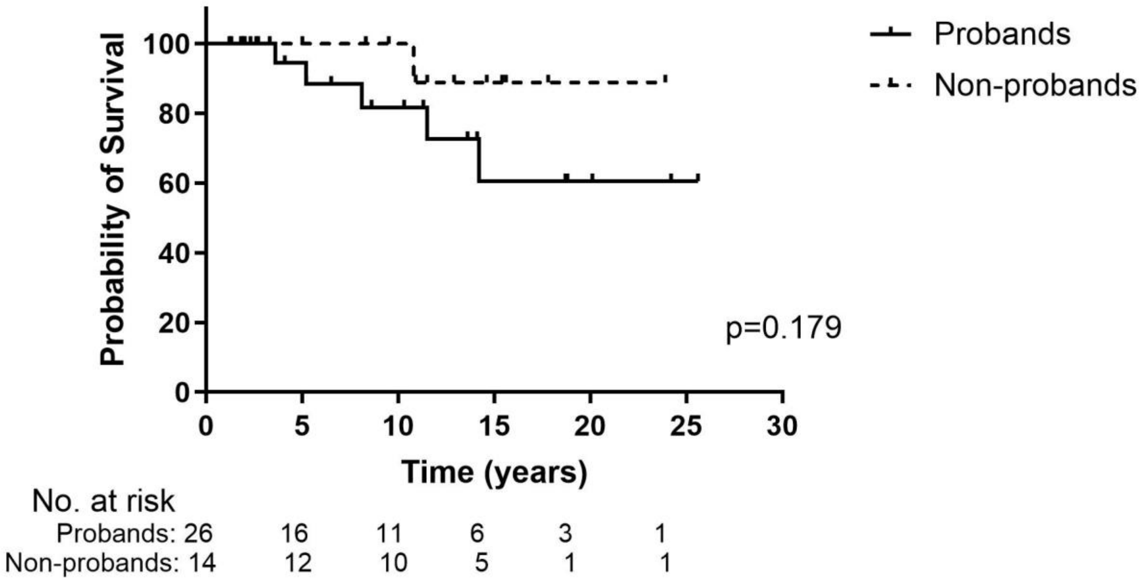

Figure 2.9 Kaplan-Meier curve of overall survival for patients with thoraco-abdominal PPGL

In non-probands diagnosed with disease, the risk of death was 8.3% and 0% for those with clinical and genetic s-d tumours respectively (P=0.433). Results from the multivariate generalized linear model with binary logistic regression showed that no factors were able to predict death from disease (Table 2.7).

2.4 Discussion

Our study is one of the largest to report surveillance practice, describe s-d tumours and disease-specific outcomes for *SDHB* PV carriers in a surveillance program. We found surveillance successfully detected *SDHB*-associated tumours prior to development of metastatic disease. We found that a tumour size of 40 mm or higher was associated with increased risk of metastatic progression. Ours is the first study to show surveillance is associated with lower mortality in an *SDHB*-specific population.

Median age of disease diagnosis in our cohort was 37 years in the 62 patients with disease diagnoses. Several studies have noted *SDHB* PV carriers are more likely to be diagnosed with disease at a young age (22, 56, 57, 113, 114). Median age at diagnosis was younger for females than males (34 *versus* 41 years respectively) although male gender was associated with a higher risk of disease. Jochmanova *et al*. found that the median age at diagnosis was younger for male *SDHB* PV carriers, but also observed a higher likelihood of disease in male compared to female carriers (57). Andrews *et al*. performed a retrospective multicentre study of 584 *SDHB* PV carriers with statistical adjustment for ascertainment bias, and similarly found a higher age-specific penetrance of HNPGL and PPGL in males (21).

The median age of first surveillance in non-probands was 33 years (range 1 to 81 years). The youngest age of a s-d tumour (n=29) was a bladder paraganglioma diagnosed at age 9 years; in the literature APGL has been described in a proband as young as 6 years (115). The oldest age of a s-d tumour was an asymptomatic gastric GIST detected at age 76 years.

Blood pressure measurement is routinely recommended for asymptomatic *SDHB* carriers (100), but paradoxically in our cohort pre-treatment hypertension was more prevalent in those without disease than those with disease. Our study may have found normotension was associated with disease due to interaction between young age and normotension, whereby young age was a risk factor for disease detection and essential hypertension is more common with advanced age (116).

Our study is the first to show the presence of 'classic' symptoms of headaches, palpitations or sweats is associated with disease detection (OR 4.9) and highlights the importance of clinical history during surveillance of these patients. While association between classic symptoms and disease may have been due to ascertainment bias, we felt this was unlikely in this cohort where patients were routinely asked about symptoms at surveillance visits. Our observation that a subset of patients with apparently non-functioning tumours had symptoms has been noted by others (117). In a case series of four patients with biochemically silent *SDHB*-associated tumours, one patient reportedly presented with palpitations, headaches and sweats (117). Absence of classic symptoms did not exclude disease, and indeed 10 of 29 (34%) s-d tumours were asymptomatic.

Regarding surveillance imaging modalities, MRI base of skull to coccyx formed the basis of surveillance in keeping with international guidelines (100). CT was used for anatomical imaging surveillance at all centres but less commonly than MRI, to minimize radiation exposure per international recommendations (100). For example as shown in the supplemental tables, 33 CT head and neck/ base of skull scans were performed on the entire cohort in comparison to 189 MRI head and neck/ base of skull scans. International guidelines suggest functional imaging could be considered for surveillance in adults (100). In our cohort, adults at RNSH and PoWH undertook ^{68}Ga-DOTATATE PET/CT or ^{18}F-FDG-PET/CT 5-yearly and at RHH adults had ^{18}F-FDG-PET/CT 4-yearly. At RHH, functional imaging with ^{18}F-FDG-PET/CT was undertaken as a key component of imaging surveillance and was noted to have high sensitivity and specificity for the detection of sympathetic PPGL with the advantage of providing both anatomical and functional detail. Functional imaging with ^{68}Ga-DOTATATE PET/CT had high sensitivity and specificity for sympathetic PPGL and HNPGL. PET imaging was 4-5 yearly rather than biennially to reduce radiation exposure, despite being sensitive and specific. Ultrasound surveillance 4-yearly at RHH was also noted to have high specificity for the detection of sympathetic PPGL and HNPGL. ^{123}I-MIBG imaging was rarely utilised after the advent of PET imaging. Future research could assess cost-effectiveness of different imaging modalities for surveillance of *SDHB* PV carriers.

We observed that probands had higher numbers of APGL and were more likely to have multifocal disease over time (OR 3.3). Given probands did not have a higher risk of multifocal disease at baseline, we speculate this finding is due to delayed diagnosis and therefore a longer period of 'tumour incubation' in probands compared to non-probands. Tufton *et al*. reported that ten (11%) of *SDHB* PV carriers in their cohort had multifocal disease and all were index cases (51). Bausch *et al*. found that of 6 of 25 (24%) probands with *SDHB* PV developed a second PPGL (58) and Daniel *et al*. reported 2 of 9 (22%) probands developed a second tumour (60). More research is needed with longer follow up to determine whether proband/index cases with *SDHB* PV have intrinsically increased risk of multiple tumours.

We found the risk of metastatic disease in *SDHB* carriers with s-d tumours was 10% and in the overall cohort (including probands) was 21%. A systematic review and meta-analysis found the risk of metastatic disease for *SDHB* PV carriers was 17% for a cohort including probands and asymptomatic carriers (118). A more recent review found overall risk of metastatic disease was 27.6% and like our study found the risk of metastatic disease was higher in thoraco-abdominal PPGL than HNPGL (22). Metastases were synchronous in two of 13 (15%) patients, which is slightly lower than reported rates in the literature ranging from 19-44% of *SDHB* PV carriers with metastatic disease (51, 64, 67, 119-121). Schovanek *et al*. noted synchronous metastases were associated with a median tumour size of 75 mm in *SDHB* PV carriers (67), whereas our patients with synchronous metastases had primary tumour sizes of 40 mm. In patients with disease (n=63), median time to development of metastases was 68 months (5.7 years), which was comparable to the literature where median time to metastases was 4-5 years (52, 59, 76).

To our knowledge, ours is the first study to compare metastasis and overall 5- and 10- year survival of *SDHB* carriers between patients with s-d tumours and probands. 14.5% of patients with disease died in this study. In other studies mortality of *SDHB* PV carriers with disease was between 2.1 and 13.0% (19, 49, 51, 52, 55, 61, 64, 122). In studies that only examined patients with metastatic PPGL, mortality rates were understandably higher from 18.5% to 60.9% (4, 74-76).

Our study has implications for surveillance recommendations for *SDHB* PV carriers. Patients with s-d tumours of 40mm or larger and those with abdominal or pelvic PPGL should be followed more frequently to detect metastatic progression. Probands/index cases may also be at higher risk of metastatic progression and/or multiple tumours and surveillance should be increased accordingly. Given that the age of tumour detection is broad as shown in Figure 2.2, we concur lifelong surveillance should start at a young age in keeping with the current consensus guidelines (100). The value of surveillance programs has also been reported by others (11, 51, 83, 96). Buffet *et al*. examined patients with PPGL who carried a germline PVs of whom 95 had a confirmed *SDHB* PV; they compared overall survival between those who received germline testing within 12 months after diagnosis of their first PPGL (the genetic group) and within 7 years of the PPGL (the historic group) (11). Overall survival was 100% in the genetic group and 50% in the historic group, and the authors postulated that patients with a knowledge of their genetic risk may have experienced a higher quality of surveillance (11). Greenberg *et al*. described surveillance of 188 *SDHB* PV carriers and observed 15% developed tumours over mean follow-up duration 1.81 ± 2.75 years (96). A multicentre study of asymptomatic *SDHx* mutation carriers (n=171 *SDHB* carriers) noted 22 of 171 (13%) asymptomatic *SDHB* carriers had a tumour detected during surveillance and 14 of 22 (64%) tumours were detected at the initial surveillance episode. Tufton *et al*. reported that ten of 15 (67%) tumours detected during surveillance were identified at the first surveillance episode (51) and we also noted that 59% (17/29) of all s-d tumours were detected at the initial surveillance episode. The optimal frequency of surveillance following an initial negative surveillance episode deserves further research.

There were several limitations to our study. Being a medical chart review some historic data on primary tumour characteristics and initial presentation were not available. Similarly, we were only able to observe the data available and the form in which it was entered, which varied across the centres. Extraction of data by one researcher ensured consistency with respect to the interpretation of chart notation where required. Although the study was multicentre it was not a nationwide Australian cohort. The association of classic symptoms with disease may have been due to ascertainment bias. More studies are needed to further evaluate the sensitivity and specificity of diagnostic modalities for *SDHB*-associated tumours. However, strengths of this study were the long duration of follow-up, small rate of loss to follow-up (123), large patient numbers, and good adherence to standardised surveillance for *SDHB* PV carriers in our genetics clinics.

2.5 Conclusion

This is one of the largest studies to describe surveillance-detected tumours and outcomes for *SDHB* PV carriers in a surveillance program. Patients with surveillance-detected tumours had smaller tumours, reduced risk of metastatic disease and lower mortality compared to probands. Tumour size ≥40 mm was associated with increased risk of metastatic progression. Our results suggest that *SDHB* PV carriers should undertake surveillance to improve clinical outcomes.

Chapter 3: Heritability of SDHB and SDHD PVs: a multicentre cohort study

3.0 Preface

The following manuscript contains a multicentre cohort study conducted with local ethics approval (Northern Sydney Local Health District Ethics Committee Ref: 2022/ETH01880; Cambridge East Medical Research Ethics Committee Ref: 06/Q0104/133). It assessed transmission of *SDHB* and *SDHD* PVs across multiple generations and demonstrated transmission ratio distortion, with alleles inherited more frequently than expected under Mendelian genetics. The findings had direct implications for genetic counselling by showing that inheritance risk was higher than the standard 50% quoted in autosomal dominant conditions. This work also contributed to the broader understanding of mechanisms underlying *SDHx*-associated disease and provided context for later chapters on the psychosocial impact of living with increased familial risk (Chapter 4). The manuscript was published in *Endocrine-Related Cancer* in 2023 with the text in the thesis identical to the published paper. The full pdf version is available in the appendix.

Davidoff DF, Lim ES, Benn DE, Subramaniam Y, Dorman E, Burgess JR, Akker SA, Clifton-Bligh RJ (2023). Distortion in transmission of pathogenic *SDHB*- and *SDHD*-mutated alleles from parent to offspring. *Endocrine Related Cancer*. Volume 30, Issue 5, April 2023, e220233, https://doi.org/10.1530/ERC-22-0233

Title Page

Full title:

Distortion in transmission of pathogenic *SDHB*- and *SDHD*-mutated alleles from parent to offspring

Short running title:

Transmission Ratio Distortion in *SDH-B* and *-D*

Authors:

Dahlia F. Davidoff,[1,2,3,*] Eugénie S. Lim,[4,5,*] Diana E. Benn, [1,2] Yuvanaa Subramaniam,[5] Eleanor Dorman,[5] John R. Burgess,[6,7] Scott A. Akker,[4,5,**] Roderick J. Clifton-Bligh[1,2,3,**]

Structured abstract:

Phaeochromocytoma and paraganglioma are highly heritable tumours; half of those associated with a germline mutation are caused by mutations in genes for Krebs's cycle enzymes, including *succinate dehydrogenase* (*SDH*). Inheritance of *SDH* alleles is assumed to be Mendelian (probability of 50% from each parent). The departure from transmission of parental alleles in a ratio of 1:1 is termed transmission ratio distortion (TRD). We sought to assess whether TRD occurs in the transmission of *SDHB* pathogenic variants (PVs). This study was conducted with 41 families of a discovery cohort from Royal North Shore Hospital, Australia, and 41 families from a validation cohort from St. Bartholomew's Hospital, United Kingdom (UK). Inclusion criteria were a clinically diagnosed *SDHB* PV and a pedigree available for at least two generations. TRD was assessed in 575 participants with the exact binomial test. The transmission ratio for *SDHB* PV was 0.59 ($p=0.005$) in the discovery cohort, 0.67 ($p<0.001$) in the validation cohort and 0.63 ($p<0.001$) in the combined cohort. No parent-of-origin effect was observed. TRD remained significant after

adjusting for potential confounders: 0.67 ($p<0.001$) excluding families with incomplete family size data; 0.58 ($p<0.001$) when probands were excluded. TRD was also evident for *SDHD* PVs in a cohort of 81 patients from 13 families from the UK. The reason for TRD of *SDHB* and *SDHD* PVs is unknown but we hypothesize a survival advantage selected during early embryogenesis. The existence of TRD for *SDHB* and *SDHD* has implications for reproductive counselling, and further research into the heterozygote state.

Disclosures:

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3.1 Introduction

The phaeochromocytoma and paraganglioma (PPGL) tumour group is the most heritable of tumours, with at least 40% of cases arising from a pathogenic germline mutation (9). Of these, around half are caused by pathogenic variants (PVs) in genes encoding critical enzymes of the tricarboxylic acid cycle, including *succinate dehydrogenase (SDH)*. PVs in *SDH* subunits result in loss of function of the SDH protein complex; *SDH*-deficient tumours are in the cluster of PPGL with a pseudo-hypoxic cellular response and the greatest potential for metastatic disease (124), and the metastatic tendency is particularly apparent with PVs in the *SDHB* subunit. Inheritance of *SDH* alleles is assumed to follow Mendelian laws of segregation, with a probability of 50% from each parent, but confirmation of this in clinical practice is made difficult by the highly variable penetrance across the subunits *SDH-A* to *-D* (22) and the rarity of *SDH*-deficient tumours in general.

There exist monogenic familial diseases which are not necessarily transmitted according to Mendelian laws of inheritance, including Factor V Leiden deficiency (79), Long QT syndrome (125), and some of the spinocerebellar ataxias (126, 127) (Table 3.1).

Table 3.1 Genes known to demonstrate transmission ratio distortion

Gene	**Function**
	Relates to tumourigenesis
CDKN1C	Tumour suppressor
HRAS1	Oncogene
IGF2	Intestinal adenoma
RB-1	Retinoblastoma tumour suppressor
SIRT3	Node-positive breast cancer
TNFa and *TNFb*	Tumour necrosis
	Relates to neurological development
ARX	Non-syndromic intellectual disability and brain malformations
CTDP1	Congenital cataract, facial dysmorphism, peripheral neuropathy
DMPK	Muscular dystrophy
HASH2 (ASCL2)	Neuronal precursor for central and peripheral nervous systems
SCA1, SCA3 (ATXN3)	Spinocerebellar ataxia types 1 and 3 (respectively)
SMN1	Spinal muscular atrophy
TH	Neuropathology
	Overlap of roles in tumourigenesis and early neurological development
DBC1, CDK5RAP2, MEGF9	Neuronal differentiation; bladder cancer
MTHFR	Acute leukaemia; colon cancer; neural tube defects
NBPF8 and *HFE2*	Neuroblastoma tumour suppressor, cognitive development; iron metabolism
	Other
ATG16L1, DLG5	Inflammatory bowel disease
BHLHA9	Split-hand/foot malformation +/- Long bone deficiency
CLC1, IGFR2 (FCGR2B)	Autoimmunity
F2 (Factor II / thrombin)	Thrombosis
F5 (Factor V Leiden)	Thrombophilia
HSP70.1	Graft versus host disease
INS	Hyperinsulinism

KCNQ1, KCNH2	Long QT syndrome
STX16-GNAS	Autosomal dominant pseudohypoparathyroidism type 1b
SUPT3H-MIRN586-RUNX2	Cleft palate; skeletal morphogenesis; haematological neoplasia
TGFB1	Cystic fibrosis severity and endophenotype

Adapted from Huang *et al*. (2013) (77)

The departure from a transmission of parental alleles in a ratio of 1:1 is termed transmission ratio distortion (TRD) (78). There are five key timepoints at which TRD can occur (77): (1) germline selection (e.g. mutation, recombination, non-allelic gene conversion) during mitosis; (2) mechanisms that occur in meiosis and prior to fertilization known as meiotic drive, where the structural characteristics of a certain chromatid result in increased transmission during oogenesis (maternal germline) or spermatogenesis (paternal germline); (3) gametic competition (by sperm) prior to fertilization, resulting in gamete selection; (4) imprint resetting at the post-implantation stage, when parental imprints are erased and re-established; and (5) post-fertilization mechanisms of embryonic or neonatal lethality from the inherited allele, resulting in differential survival of offspring. We suggest that at this time point, advantageous selection may also occur (Figure 3.1).

Our interest in whether *SDH* PVs are inherited according to Mendelian laws of segregation or in an imbalanced, distorted way arose anecdotally: an *SDHB* PV carrier underwent pre-implantation genetic testing and reported that high numbers of embryos harboured the affected allele. We sought to assess whether TRD occurs in the transmission of *SDHB* PVs, and posit that a post-fertilization survival advantage is the cause.

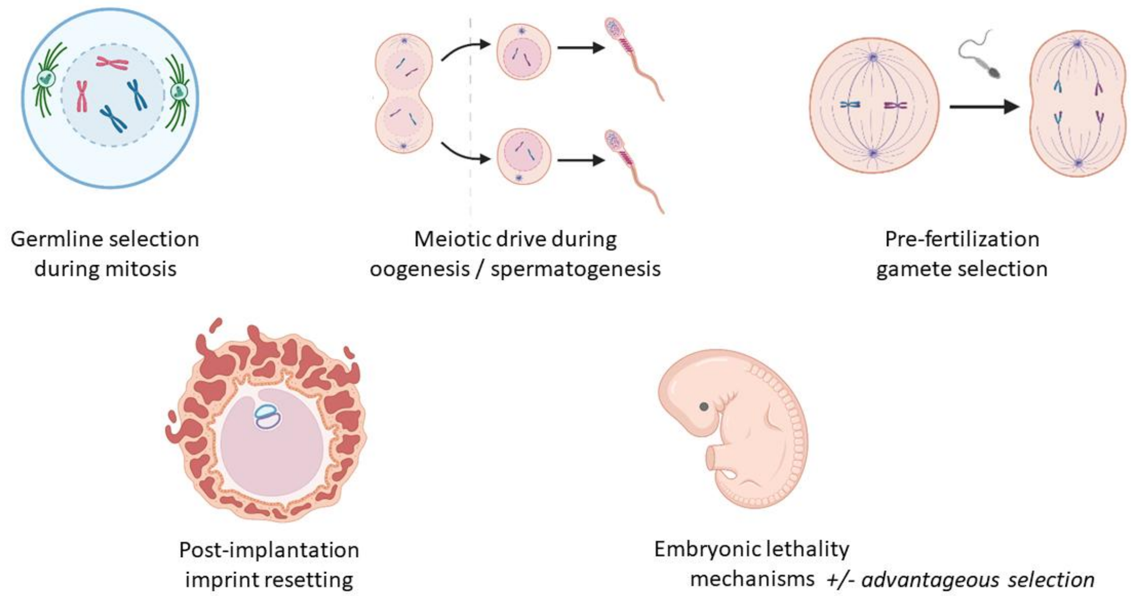

Figure 3.1 Five key timepoints at which transmission ratio distortion may occur

Graphics produced in Biorender.com

3.2 Materials and Methods

This study has been conducted with 41 families in each of a discovery cohort in Australia, from Royal North Shore Hospital (RNSH), and a validation cohort in the United Kingdom, from St. Bartholomew's Hospital (SBH), together representing a range of different pathogenic variants in the *SDHB* gene. Inclusion criteria were confirmed *SDHB* PV and a pedigree available for at least 2 generations. Probands were defined as the first individual in a family to be diagnosed with a *SDHB* PV after presenting with a PPGL. PVs were classified as loss of function (nonsense, splicing, deletion, or frameshift) or missense. PVs were defined as being in the proximal region of the *SDHB* gene if they occurred in exons 1-3 or intronic regions up to IVS3. Ethics approval was obtained from the Northern Sydney Local Health District Ethics Committee for the discovery cohort (Ref: 2022/ETH01880), including waiver of consent; and Cambridge East Medical Research Ethics Committee for the validation cohort (Ref: 06/Q0104/133.) Patients provided consent after full explanation of the purpose of the study.

Statistical analysis was performed using IBM SPSS version 28. The forest plot figure was produced using GraphPad Prism version 9. Categorical data were tested with the binomial test to obtain a true estimate, with 95% confidence intervals using the Clopper-Pearson method. Continuous data were assessed with the exact Mann-Whitney test for non-parametric data. A p-value ≤ 0.05 (two-tailed) was considered statistically significant. Results that were not significant were assessed for heterogeneity with Levene's test. Several sensitivity analyses were undertaken in this study by: (a) excluding probands; (b) excluding families with incomplete family pedigree data; and (c) excluding untested participants younger than age 20 years. Potential predictors of TRD were assessed in the cohort that underwent genetic testing and the cohort with complete family data, using a generalized linear model with binary logistic regression to perform a multivariate analysis. Explanatory variables included in this model were sex, genotype, parent of origin, birth order, and family size.

3.3 Results

575 participants from 82 families from RNSH and SBH were assessed. There was a difference between centres in the proportion that underwent genetic testing and in the number of generations assessed in each family (Table 3.2). 503 of the 575 participants assessed underwent genetic testing, with 316 found to harbour an *SDHB* PV. Thirty-five different *SDHB* PVs were represented in the combined cohort: 12 missense PVs were present in 29 families, and a further 23 PVs were loss of function mutations (Table 3.3). Disease had manifested in approximately 19% of *SDHB* participants at any timepoint, which is similar to reported disease penetrance in the literature (16, 99). In the validation cohort, most families (61%) were represented by 3 generations and most nuclear families had 2 or 3 offspring (biological children.)

Table 3.2 Baseline characteristics of *SDHB* cohorts

	RNSH – Discovery cohort (n=279)	**SBH – Validation cohort (n=296)**	**RNSH and SBH – Combined cohort (n=575)**	**P-value**
Male; n (%)	133 (48)	149 (50)	282 (49)	0.48
Probands; n (%)	32 (11)	25 (8)	57 (10)	0.23
Genetic testing; n (%)	260 (93)	243 (82)	503 (88)	<0.001
Loss of function pathogenic variant; n (%)	172 (62)	179 (60)	351 (61)	0.77
Birth order				
First; n (%)	49 (18)	120 (41)	169 (29)	0.43
Second; n (%)	47 (17)	96 (32)	143 (25)	
Third or later; n (%)	39 (14)	64 (22)	103 (18)	
Not available; n (%)	144 (52)	16 (5)	160 (28)	
Family size: children				
One; n (%)	15 (5)	20 (7)	35 (6)	0.08
Two; n (%)	71 (25)	120 (41)	190 (33)	
Three or more; n (%)	188 (67)	156 (53)	345 (60)	
Not available; n (%)	5 (2)	0	5 (1)	
Family size: generations				
Two; n families	18 (44)	11 (27)	29 (35)	0.02
Three; n families	23 (56)	25 (61)	48 (59)	
Four; n families	0	5 (12)	5 (6)	

Table 3.3 Pathogenic variants of *SDHB* in the combined cohort

	Pathogenic variant	Families (n)
Missense	c.118A>G, p.(Lys40Glu)	6
	c.137G>A, p.(Arg46Gln)	7
	c.338G>A, p.(Cys113Tyr)	1
	c.380T>G, I127S	2
	c.418G>T, p.(Val140Phe)	2
	c.587G>A, p.(Cys196Tyr)	3
	c.590C>G, p.(Pro197Arg)	1
	c.600G>T, p.(Trp200Cys)	1
	c.688C>G, p.(Arg230Gly)	1
	c.688C>T, p.(Arg230Cys)	1
	c.689G>A, p.(Arg230His)	2
	c.725G>A, p.(Arg242His)	2
Nonsense	c.44_45dupCC, p.(Thr16Profs*62)	1
	c.79C>T, p.(Arg 27*)	3
	c.136C>T, p.(Arg46*)	4
	c.268C>T, p.(Arg90*)	10
	c.406delA, p.(Ile136*)	2
Insertion	c.311delAinsGG, p.(Asn104fs)	2
	c.761dup, p.(Pro254_Lys255insTer)	1
	details unavailable	1
Deletion	c.88delC, p.(Gln30fs)	2
	c.166delCCTCA	1
	c.283-285delA	1
	exon 1 deletion	6
	exon 2 deletion	1
	whole gene deletion	1
Splice	c.286+2T>G, IVS3+2T>G	1
	c.287-3C>G	1
	c.287-1G>C	1
	c.423+1G>A	1
	c.642+1G>A	1

	c.72+1G>T	5
	IVS1+1G>T	1
	IVS4+1G>A	1
	IVS4-1G>A	1
Unknown	details unavailable	1

The transmission ratio for *SDHB* PV was 0.59 (p=0.005) in the discovery cohort (Table 3.4), 0.67 (p<0.001) in the validation cohort and 0.63 (p<0.001) in the combined cohort (Table 3.5 and Figure 3.2). For the discovery cohort, TRD was apparent when analyzing for each of paternal inheritance, loss of function PV, mutation within the proximal region of the gene (exons 1-3 and up to IVS3), male sex, and the second generation from the proband (Table 3.4).

Table 3.4 Transmission Ratio in *SDHB* families in the discovery cohort

	Actual (95% CI)	**Expected**	**P-value**
Cohort that underwent genetic testing (n=260)	0.59 (0.53-0.65)	0.50	0.005**
Probands excluded (n=228)	0.53 (0.46-0.60)	0.50	0.40
Cohort with complete family size data (n=30)	0.63 (0.44-0.80)	0.50	0.20
Cohort excluding those <20 years of age without genetic test (n=269)	0.57 (0.51-0.63)	0.50	0.03*
Paternal inheritance (n=123)	0.62 (0.53-0.70)	0.50	0.01*
Maternal inheritance (n=124)	0.57 (0.47-0.65)	0.50	0.18
Loss of function pathogenic variant (n=157)	0.59 (0.51-0.67)	0.50	0.03*
Missense pathogenic variant (n=103)	0.58 (0.48-0.68)	0.50	0.12
Pathogenic variant in exons 1-3 or intronic region up to IVS3 (n=193)	0.59 (0.52-0.66)	0.50	0.01*
Pathogenic variant in exons 4-8 or intronic region from IVS3 to IVS6 (n=60)	0.57 (0.43-0.69)	0.50	0.37
Male sex (n=125)	0.59 (0.51-0.68)	0.50	0.04*
Female sex (n=135)	0.58 (0.49-0.67)	0.50	0.06
Second generation (n=156)	0.64 (0.56-0.72)	0.50	<0.001**
Third generation (n=104)	0.51 (0.41-0.61)	0.50	0.92
Birth order first ^ (n=13)	0.69 (0.39-0.91)	0.50	0.27
Birth order second ^ (n=10)	0.60 (0.26-0.89)	0.50	0.75
Birth order third or later ^ (n=6)	0.50 (0.12-0.88)	0.50	1.0
Family size one child ^ (n=1)	1.0 (0.25-1.0)	0.50	1.0
Family size two children ^ (n=12)	0.58 (0.28-0.85)	0.50	0.77
Family size three or more children ^ (n=17)	0.65 (0.38-0.86)	0.50	0.33

* P-value < 0.05; ** p-value < 0.01; ^ analysis in families with complete family size data.

Table 3.5 Transmission Ratio in *SDHB* families in the combined cohort

	Actual (95% CI)	Expected	P-value
Cohort that underwent genetic testing (n=503)	0.63 (0.58-0.67)	0.50	<0.001**
Probands excluded (n=446)	0.58 (0.53-0.63)	0.50	<0.001**
Cohort with complete family size data (n=273)	0.67 (0.61-0.72)	0.50	<0.001**
Cohort excluding those < 20 years of age without genetic test (n=539)	0.59 (0.54-0.63)	0.50	<0.001**
Paternal inheritance (n=234)	0.64 (0.57-0.70)	0.50	<0.001**
Maternal inheritance (n=252)	0.64 (0.57-0.69)	0.50	<0.001**
Loss of function pathogenic variant (n=297)	0.66 (0.60-0.71)	0.50	<0.001**
Missense pathogenic variant (n=206)	0.59 (0.52-0.66)	0.50	0.02*
Pathogenic variant in exons 1-3 or intronic region up to IVS3 (n=344)	0.63 (0.58-0.68)	0.50	<0.001**
Pathogenic variant in exons 4-8 or intronic region from IVS3 to IVS6 (n=152)	0.62 (0.54-0.70)	0.50	0.005**
Male sex (n=236)	0.66 (0.59-0.72)	0.50	<0.001**
Female sex (n=264)	0.60 (0.54-0.66)	0.50	0.001**
Second generation (n=294)	0.66 (0.60-0.71)	0.50	<0.001**
Third generation (n=194)	0.60 (0.53-0.67)	0.50	0.005**
Birth order first ^ (n=110)	0.75 (0.65-0.82)	0.50	<0.001**
Birth order second ^ (n=90)	0.67 (0.60-0.76)	0.50	0.002**
Birth order third or later ^ (n=56)	0.55 (0.42-0.69)	0.50	0.50
Family size one child ^ (n=15)	1.00 (0.78-1.00)	0.50	<0.001**
Family size two children ^ (n=112)	0.72 (0.63-0.80)	0.50	<0.001**
Family size three or more children ^ (n=146)	0.59 (0.51-0.67)	0.50	0.004**

* P-value < 0.05; ** p-value < 0.01; ^ analysis in families with complete family size data.

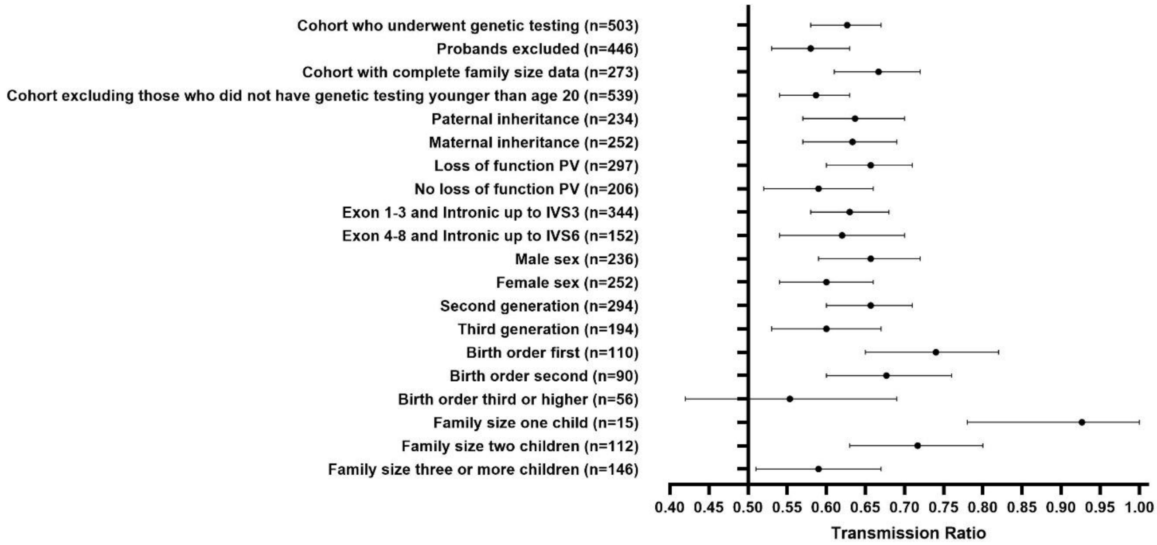

Figure 3.2 Forest plot of transmission ratio in *SDHB* families in the combined cohort

No parent-of-origin effect was observed in the combined cohort. TRD remained significant after adjusting for potential confounders: 0.67 ($p<0.001$) if families with incomplete family size data were excluded and 0.58 ($p<0.001$) if probands were excluded. Of the 72 individuals who did not have genetic testing, 36 were younger than 20 years of age; after excluding these participants, the transmission ratio was 0.59 ($p<0.001$). No factors predicted TRD on a generalized linear model with binary logistic regression (Tables 3.6 and 3.7).

Table 3.6 Generalized linear model with binary logistic regression of predictors of TRD in the combined cohort of participants that underwent genetic testing

Predictors of TRD	**P-value**	**OR (95% CI)**
Male	0.41	0.85 (0.69-1.24)
Loss of function pathogenic variant	0.21	1.29 (0.87-1.91)
PV in exon 1-3 or intronic region up to IVS3	0.79	0.94 (0.62-1.44)
Paternal inheritance	0.96	0.99 (0.68-1.44)
Test	**P-value**	**X^2**
Overall model likelihood ratio test (Omnibus Test)	0.99	2.39

Table 3.7 Generalized linear model with binary logistic regression of potential predictors of TRD in the combined cohort of families with complete family size data

Predictors of TRD	**P-value**	**OR (95% CI)**
Male	0.23	0.71 (0.41-1.24)
Loss of function pathogenic variant	0.06	1.73 (0.97-3.11)
PV in exons 1-3 or intronic region up to IVS3	0.83	0.93 (0.49-1.78)
Paternal inheritance	0.99	1.00 (0.56-1.80)
Birth order	0.67	1.06 (0.80-1.41)
Family size (number of children)	0.37	1.10 (0.88-1.41)
Test	**P-value**	**X^2**
Overall model likelihood ratio test (Omnibus Test)	0.12	10.05

Transmission ratio analysis was replicated for the *SDHD* cohort at St Bartholomew's Hospital: 81 patients from 13 families, most commonly of 3 generations (range 2-4) and with 2 children per nuclear family (range 1-5) (Table 3.8). Phenotype expression was dependent on paternal inheritance, as expected. Of the 61 participants with confirmed germline testing, 43 harboured an *SDHD* PV (Table 3.9), which represents a significant distortion in transmission ratio: 0.70 (p=0.0019). TRD in *SDHD* was upheld even when assuming that a Mendelian 50% of those with unknown genotypes were carriers (0.65, p=0.0073). Neither clinical centre had *SDHA* or *SDHC* cohorts of sufficient size for analysis.

Table 3.8 Baseline characteristics of *SDHD* cohort

***SDHD* Cohort; n**	**81**
Male; n (%)	45 (57)
Probands; n (%	13 (16)
Genetic testing; n (%)	61 (75)
Loss of function pathogenic variant; n (%)	18 (22)
Birth order	
First; n (%)	39 (48)
Second; n (%)	25 (31)
Third or later; n (%)	17 (21)
Family size: children	
One; n (%)	10 (12)
Two; n (%)	14 (17)
Three or more; n (%)	9 (11)
Family size: generations analysed	
Two; n (%)	41 (51) from 8 families
Three; n (%)	25 (31) from 3 families
Four; n (%)	15 (18) from 2 families

Table 3.9 Pathogenic variants of *SDHD* in the analysed cohort

	Pathogenic variant	**Families (n)**
Missense	c.242C>T, p.(Pro81Leu)	3
	c.274G>T, p.(Asp92Tyr)	1
	c.340T>A p.(Tyr114Asn)	1
Nonsense	c.14G>A, p.(Trp5*)	1
	c.46C>T, p.(Arg22*)	1
	c.325C>T p.(Gln109*)	1
Deletion	c.98_108del11,p.(Ala33Glyfs*32)	1
	c.171delT, p.(Gly58Alafs*28)	1
	c.276_278delCTA p.(Try93del)	1
	c.296delT p.(Leu99Profs*36)	1
Splice	c.314+5G>A, p.Ser57TrpfsTer30	1

3.4 Discussion

A transmission ratio distortion of 60% in favor of the *SDHB* PV being transmitted was evident in our cohort. In the discovery cohort, it appeared that TRD was associated with particular variables, but analysis was limited by incomplete family data and insufficient power. In the combined cohort, TRD was observed irrespective of sex, parent of origin, loss of function PV, or location of the mutation within the proximal or distal region of the gene. Given that rates of genetic testing differed between centres, we assessed complete family data. When families with incomplete family size data were excluded, TRD was still noted. When probands were excluded, TRD still occurred, suggesting TRD was not due to oversampling of cases (128). We considered the possibility of bias in young individuals not undergoing genetic testing due to being asymptomatic, since the median age of disease diagnosis is 37 years (129), but after exclusion of participants younger than age 20 years without a genetic test, TRD was still observed. Birth order third or later was not associated with TRD, likely due to insufficient power (n=56), given that heterogeneity was absent on Levene's test. As TRD was consistently observed across different variables, the finding that no particular factors predicted TRD on the generalized linear model was unsurprising.

The 60% distortion in transmission of pathogenic *SDHB* alleles is consistent with the magnitude of other examples of TRD: pathogenic variants of the tumour suppressor gene for retinoblastoma, *RB-1,* were found to have 63% transmission from affected males to sons (80); *STX16-GNAS* mutations in autosomal dominant pseudohypoparathyroidism type Ib were transmitted to 63% of offspring (81); mutated alleles in long-QT syndrome conferred 55% transmission to female offspring (125); and a study of embryos from preimplantation genetic testing for myotonic dystrophy type 1 found that 59% harboured the CTG nucleotide repeat expansion (130).

It has been suggested that the phenomenon of anticipation is apparent in *SDH*-deficient disease (131), albeit without a trinucleotide repeat expansion to facilitate this in the classical way. Whilst an earlier age of tumour diagnosis was documented in some subsequent generations, this was attributed to an early age of screening and surveillance; furthermore, this phenomenon was not borne out across our 82 families to suggest a genuine pattern of an underlying biological change in disease penetrance across the generations.

Limitations to this study included some uncollected data that could hypothetically influence interpretation of inheritance patterns, such as age of parenthood, miscarriage rate, and birth order. The sample size was robust relative to accessible cohorts of rare disease, but may limit extrapolation from statistical significance to biological significance, such as with the question of a parent-of-origin effect on transmission. However, to counter potential sources of bias, we tested the sensitivity of our results by excluding, in turn, possible confounders: families with incomplete family size data, probands, and untested participants less than 20 years old. None of these analyses significantly altered the main finding of TRD in favor of *SDHB* PVs.

The reason for a TRD in *SDH-B* and *-D* is unknown. We hypothesize the mechanism could occur at the post-fertilization stage and arise as a selective advantage, perhaps for adapting to hypoxia; however, assessing timing and mechanisms for TRD was beyond the scope of the present study. It is fascinating to consider how an embryonic survival advantage for hypoxia/pseudo-hypoxia might then be accompanied by variably penetrant tumour risk in postnatal life. Intriguingly, several tumour suppressor genes and oncogenes have also been demonstrated to manifest TRD in favour of the mutant allele (77), including *CDKN1C* (132), *HRAS1* and *SIRT3* (133). Moreover, *CDKN1C* has been implicated in the pathogenicity of *SDHAF2* and *SDHD* mutations when arising from loss of maternal chromosome 11 (134), whereas HRAS and sirtuin-3 both regulate mitochondrial function (135, 136).

A post fertilisation survival advantage is the hypothesised mechanism for TRD in *SDH-B* and *-D* because early embryos depend critically on oxygen sensing pathways for normal development and survival (137-140), and SDH function directly influences the hypoxia-signalling axis (46). Oxygen sensing pathways and physiological hypoxia are recognised as critical regulators of early embryonic development. In mammalian models, deletion of oxygen sensing transcription factors such as HIF-1α leads to severe cardiac and vascular malformations and embryonic lethality, underscoring the key role of hypoxia-inducible signalling in embryo survival (138, 140). Preimplantation embryos are sensitive to oxygen tension, so SDH function may have a protective role against hypoxic stress in the earliest stages of embryonic development. Both mouse and human data suggest that physiological low oxygen and intact HIF signalling are required for normal progression to the blastocyst (137, 139). These findings support the plausibility that alterations in hypoxia-sensing pathways such as those induced by heterozygous *SDH-B* and *-D* PVs, may confer a post-fertilisation survival advantage, leading to the TRD observed in our cohort.

The clinical relevance of distortion in the transmission of *SDH-B* and *-D* mutations is immediately apparent: a TRD that favors the potential for tumourigenesis has significant implications for genetic counselling of all carriers. The role for pre-implantation genetic diagnosis is arguably stronger when the odds are against the likelihood of healthy offspring. We encourage other centres to analyze their cohorts similarly to validate our findings, with a view to updating guidelines on genetic counselling. An understanding of the mechanism behind TRD in *SDH*, where the heterozygous state may have an advantage, might lead to insights that later allow interventions in carriers to decrease the risk of tumour development.

Chapter 4: Quality of life of SDHB PV carriers: a multicentre cross-sectional study

4.0 Preface

The following manuscript contains a multicentre cross-sectional study conducted with local ethics approval (Northern Sydney Local Health District Ethics Committee Ref: 2019/ETH12699; Tasmania Health and Medical Human Research Ethics Committee Ref: 24407). It assessed quality of life in *SDHB* PV carriers using validated tools. The findings demonstrated that carriers experienced significantly reduced quality of life, with anxiety and depression emerging as major contributors even in asymptomatic individuals. This provided the first quantitative evidence of the psychosocial cost of surveillance, informing the health economic modelling of surveillance (Chapter 6). The references for this manuscript can be found directly at the end of the manuscript.

This manuscript has been submitted to *Orphanet Journal of Rare Diseases* and is currently under review.

Title Page

Full title:

Quality of life in Australian *SDHB* pathogenic variant carriers compared to population normative values

Short running title:

Quality of life for *SDHB* carriers

Authors:

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Structured abstract:

Background

Carriers of germline pathogenic variants (PV) in *succinate dehydrogenase type B* (*SDHB*) are at increased risk of developing phaeochromocytomas and paragangliomas (PPGL). Understanding their health-related quality of life (HRQoL) can guide recommendations for multidisciplinary care approaches.

Objective

We performed a cross-sectional observational study of HRQoL in *SDHB* PV carriers attending genetics clinics at two tertiary centres in Australia.

Materials and Methods

SDHB PV carriers were sent a letter of invitation to complete the European Organisation for Research and Treatment Quality of Life Questionnaire (EORTC QLQ-C30), the EuroQol 5 Dimensions 5 Levels (EQ5D5L) questionnaire and the EQ visual analogue scale (EQ-VAS). Results were analysed and expressed as utilities. Utility scores were then compared to population normative values.

Results

47 *SDHB* PV carriers (20 with a history of *SDHB*-related tumours, 27 with no history of tumours) completed the quality of life questionnaires. The mean utility scores for EQ5D5L, EQ-VAS and QLU-C10D (for the QLQ-C30 questionnaire) were 0.776, 72.70 and 0.792 respectively. Utility scores for the complete cohort of *SDHB* PV carriers were lower than Australian normative values for both EQ5D5L (difference between means 0.13, $p<0.0001$) and EQ-VAS (difference between means 5.85, $p=0.017$). *SDHB* PV carriers were more likely to report issues with the EQ5D5L dimension of anxiety and depression compared to Australian normative values (OR 3.78, 95% CI 2.13-6.60, $p<0.001$). As Australian normative values were not available for the QLU-C10D utility score, a control group was obtained from UK population normative data; those utility scores did not differ from UK normative values.

Conclusion

SDHB PV carriers were more likely to have poorer quality of life and a higher prevalence of anxiety or depression using the EQ5D5L measure compared to population normative values. These findings underscore the importance of routine psychological screening and tailored support for *SDHB* PV carriers.

Disclosures:

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Disclosures: The authors have nothing to declare.

4.1 Introduction

Carriers of germline pathogenic variants (PV) in succinate dehydrogenase type B (*SDHB*) are at increased risk of developing phaeochromocytomas and paragangliomas (PPGL) (2). PPGL can present with symptoms of catecholamine excess including headaches, palpitations and sweats, cause local invasion into adjacent tissue and may metastasise (2), which carries a high five-year mortality of 18% (72, 141). Current guidelines recommend lifelong surveillance for *SDHB* PV carriers, starting as early as age 6 to 10 years, to facilitate early detection and potential surgical cure of PPGL (100).

Beyond the physical health implications, living with a hereditary predisposition to potentially life-threatening conditions like PPGL poses significant emotional and psychosocial challenges. Quality of life, defined as an individual's overall satisfaction with life and general sense of personal well-being (142), encompasses not only physical health but also psychological and social dimensions. For *SDHB* PV carriers, the burden of repeated medical evaluations, the uncertainty of disease development, and potential impacts on personal and professional life may profoundly influence their health-related quality of life (HRQoL).

Van Hulsteijn *et al.* demonstrated reduced HRQoL in patients with paragangliomas (PGL) through a case-control study that included 25 *SDHB* PV carriers (106). Using validated tools such as the Hospital Anxiety and Depression Scale, the Multidimensional Fatigue Index 20, and the Short Form 36, the study found that patients with PGL experienced impaired fatigue and physical condition. However, asymptomatic carriers showed quality of life comparable to the general population (106).

Despite these findings, the unique challenges faced by *SDHB* PV carriers, particularly those without active disease, remain underexplored. With lifelong surveillance often beginning in childhood, the psychosocial impact of living with an elevated hereditary cancer risk has not been systematically studied for affected and unaffected *SDHB* PV carriers. This represents a critical gap in our understanding that has direct implications for patient care and support.

The aim of this study was to assess the HRQoL of affected and unaffected carriers with known *SDHB* PVs enrolled in a long term surveillance program, in order to better understand the broader impacts of living with this hereditary condition and to identify opportunities for enhancing patient care.

4.2 Materials and Methods

Study procedure

This was a cross-sectional observational study of *SDHB* PV carriers attending genetics clinics at Royal North Shore Hospital (RNSH) and Royal Hobart Hospital (RHH) in Australia. Patients with confirmed *SDHB* PVs on molecular genetic testing were eligible to participate. *SDHB* PV carriers were sent a letter of invitation to complete an online questionnaire via the Research Electronic Data Capture (REDCap) system (112). Ethics approval was obtained from the Northern Sydney Local Health District Ethics Committee for RNSH (Ref: 2019/ETH12699) and from Tasmania Health and Medical Human Research Ethics Committee for RHH (Ref: 24407).

Demographic and clinical data

Demographic and clinical data were collected in addition to the HRQoL questionnaires. Data gathered from patients included age, gender, smoking status (non-smoker, current smoker, past smoker), occupation (student, professional, clerical and service worker, labourer, manager, tradesperson, retired, production and transport worker, disability support pension, unemployed), level of education (primary school graduate, high school graduate, vocational certificate, diploma, bachelor degree, masters degree, doctoral degree), clinical symptoms (sweats, heart racing or palpitations, headaches, flushing, chest pain, abdominal pain, shortness of breath), history of tumour, primary tumour location (head and neck paraganglioma (HNPGL), phaeochromocytoma (PC), PGL, renal cell carcinoma (RCC)), disease state (tumour excised, tumour localised, tumour metastatic) and tumour treatment (surgical removal, octreotide or lanreotide, 131-Iodine metaiodobenzylguanidine (MIBG), [177Lu]Lu-octreotate (lutate), chemotherapy, external beam radiotherapy).

Quality of life questionnaires

HRQoL was assessed using two well-validated quality of life instruments: the European Organisation for Research and Treatment Quality of Life Questionnaire (EORTC QLQ-C30) questionnaire validated in cancer research (143) and the EuroQol 5 Dimensions 5 Levels (EQ5D5L) questionnaire validated as a generic patient-reported outcome measure (144).

EORTC QLQ-C30 is a 30-item patient-reported outcome measure (143), categorised into five functional scales (physical functioning, role functioning, emotional functioning, cognitive functioning, social functioning), three symptom scales (fatigue, nausea and vomiting, pain), a global quality of life scale and six single-item scales (dyspnoea, insomnia, appetite loss, constipation, diarrhoea, financial difficulties) (145). A score for each domain is obtained from 0 to 100, with a higher score reflecting a higher level of functioning or higher level of symptoms using the scoring algorithm derived by the EORTC (145). Answers to 13 questionnaires in the EORTC QLQ-C30 can also be used to calculate utility scores (anchored on 0 to 1 scale) for use in economic evaluations using the EORTC QLU-C10D algorithm (146).

The EQ5D5L developed by the EuroQol Group has two components: the EQ5D descriptive system and the EQ visual analogue scale (EQ-VAS) (144). The descriptive system has five domains (mobility, self-care, usual activities, pain/discomfort and anxiety/depression). For each descriptive system domain, patients describe their health by rating against five response levels (no problems – a score of 1, slight problems, moderate problems, severe problems and extreme problems – a score of 5) (144). The EQ-5D-5L descriptive system scores are combined to produce utility values, anchored on a scale from 0 to 1, for use in economic evaluation. The EQ VAS is a vertical visual analogue scale where patients judge their overall health to be from zero to 100, with zero being ‘The worst health you can imagine’ and 100 being ‘The best health you can imagine’ (144).

Normative values

Normative values for comparison with HRQoL values from the study sample for the EQ5D5L questionnaire were drawn from Australian population values (147). As Australian normative values were not available for the QLU-C10D utility score, these were obtained from UK population normative data for the QLQ-C30 questionnaire (148).

Data analysis

Statistical analysis was performed using GraphPad Prism version 10.0.2 and IBM SPSS version 28. Descriptive statistics were performed with numerical data presented as mean and standard deviation. Groups were compared using the unpaired independent sample t-test. Fisher's exact test was used to determine odds ratios between groups. Univariate and multivariate analysis was carried out using binary logistic regression to assess determinants of reduced HRQoL and clinical symptoms. Pearson correlation was used to assess the correlation coefficient (r) and coefficient of determination (R^2) between the EQ5D5L utility score and QLU-C10D utility score. A p-value ≤ 0.05 was considered as statistically significant.

4.3 Results

Letters of invitation were provided to 24 patients at RNSH and 84 patients at RHH. A total of 49 of 108 (45%) eligible *SDHB* PV carriers participated in the study. 2 patients only completed the initial demographic questions and did not proceed with the HRQoL questionnaires. We therefore assessed the data of the 47 patients who completed the HRQoL questionnaires (17 RNSH, 30 RHH). Baseline characteristics of the 47 participants are shown in Table 4.1. Median age was 50 and 45% of respondents were male. Participants (n=20, 42.6%) with a history of *SDHB*-related tumours differed in their education and were more likely to report symptoms of sweats, heart racing or palpitations and flushing compared to participants with no history of *SDHB*-related tumours. Of the 20 participants with a history of *SDHB*-related tumours, these were: 6 HNPGL, 4 PC, 3 thoracic PGL, 9 abdominal PGL and 1 RCC. Two participants reported a history of PC and abdominal PGL and one participant reported a history of HNPGL and abdominal PGL. 14 participants reported disease remission following surgery, 5 reported ongoing localised disease and one participant reported metastatic disease.

Table 4.1 Baseline characteristics

	Complete cohort (n=47)	*SDHB*-related tumours (n=20)	No *SDHB*-related tumours (n=27)	P value
Age; median (range)	50 (14-84)	48 (20-76)	51 (14-84)	0.57
Male; n (%)	21 (45)	11 (55)	10 (37)	0.23
Smoking status				
Non-smoker; n (%)	36 (77)	17 (85)	19 (70)	0.22
Current smoker; n (%)	4 (8)	2 (10)	2 (7)	
Past smoker	6 (13)	1 (5)	5 (19)	
Not available; n (%)	1 (2)	0	1 (4)	
Occupation; n (%)				
Student	2 (4)	1 (5)	1 (4)	0.29
Professional	11 (23)	5 (25)	6 (22)	
Clerical, sales and service worker	6 (13)	3 (15)	3 (11)	
Labourer	2 (4)	1 (5)	1 (4)	
Manager	7 (15)	3 (15)	4 (15)	
Tradesperson	3 (6.5)	2 (10)	1 (4)	
Retired	6 (13)	1 (5)	5 (18)	
Production and transport worker	1 (2)	0	1 (4)	
Disability support pension	3 (6.5)	1 (5)	2 (7)	
Unemployed	3 (6.5)	1 (5)	2 (7)	
Not available	3 (6.5)	2 (10)	1 (4)	
Education; n (%)				
Primary school graduate	1 (2)	0	1 (4)	0.02**
High school graduate	20 (43)	6 (30)	14 (52)	
Vocational certificate	8 (17)	3 (15)	5 (18)	
Diploma	3 (6.5)	1 (5)	2 (7)	
Bachelor degree	8 (17)	4 (20)	4 (15)	
Masters degree	4 (8)	4 (20)	0	
Doctoral degree	1 (2)	0	1 (4)	
Not available	2 (4)	2 (10)	0	
Clinical symptoms; n (%)				
Sweats	12 (26)	9 (45)	3 (11)	0.01**
Heart racing or palpitations	9 (19)	7 (35)	2 (7)	0.03**
Headaches	18 (38)	8 (40)	10 (37)	0.84
Flushing	7 (15)	6 (30)	1 (4)	0.03**
Chest pain	6 (13)	3 (15)	3 (11)	0.71
Abdominal pain	9 (19)	4 (20)	5 (19)	0.90

Shortness of breath None	12 (26) 21 (44)	5 (25) 9 (45)	7 (26) 12 (44)	0.94 0.97
Primary tumour location; n (%) HNPGL PC Thoracic PGL Abdominal PGL RCC	 6 (13) 4 (9) 3 (6) 9 (19) 1 (2)	 6 (30) 4 (20) 3 (15) 9 (45) 1 (5)	 NA	 -
Disease state Tumor excised and in remission Localised Metastatic	 14 (30) 5 (11) 1 (2)	 14 (70) 5 (25) 1 (5)	 NA	 -
Tumor treatment Surgical removal Octreotide or lanreotide MIBG Lutate Chemotherapy Other External beam Radiotherapy	 18 (38) 2 (4) 0 1 (2) 1 (2) 2 (4)	 18 (90) 2 (10) 0 1 (5) 1 (5) 2 (10)	 NA	 -

* P value < 0.01; ** P value < 0.05. HNPGL head and neck paraganglioma; MIBG metaiodobenzylguanidine; NA not applicable; PC phaeochromocytoma; PGL paraganglioma; RCC renal cell carcinoma; *SDHB succinate dehydrogenase type B*

The mean EQ5D5L utility score for the complete cohort was 0.776 (SD 0.239, 95 % CI 0.709-0.841) and the mean EQ-VAS score was 72.70 (SD 19.69; 95 % CI 67.02-78.06). The mean QLU-C10D utility score was 0.792 (SD 0.14; 95 % CI 0.90-0.91).

The EQ5D5L utility score and QLU-C10D utility score correlation was r = 0.871 (95% CI 0.716-0.937), $p<0.001$ and $R^2 = 0.759$.

Subgroup analysis did not find a difference between the EQ5D5L utility scores and QLU-C10D utility scores when grouped by age, gender, smoking, occupation, education, centre, history of tumours, tumour functional status or disease state (Table 4.2). Subgroup analysis of EQ5D5L and QLU-C10D utility scores based on tumour location is shown in Table 4.3.

Table 4.2 Quality of life results: Age, gender, smoking, occupation, education, centre, history of tumours, tumour functional status and disease state

	Age		
	Age 50 years or less (n=22)	**Age more than 50 years (n=25)**	**P value**
EQ5D5L utility score; mean (SD)	0.798 (0.204)	0.758 (0.268)	0.57
QLU-C10D utility score; mean (SD)	0.814 (0.225)	0.774 (0.212)	0.54
	Gender		
	Male (n=21)	**Female (n=26)**	**P value**
EQ5D5L utility score; mean (SD)	0.826 (0.171)	0.737 (0.279)	0.21
QLU-C10D utility score; mean (SD)	0.831 (0.129)	0.733 (0.255)	0.25
	Smoking		
	Non-smoker (n=36)	**History of smoking (n=10)**	**P value**
EQ5D5L utility score; mean (SD)	0.787 (0.233)	0.741 (0.074)	0.70
QLU-C10D utility score; mean (SD)	0.806 (0.213)	0.736 (0.241)	0.38
	Occupation		
	Managers or professionals (n=18)	**Other professions (n=29)**	**P value**
EQ5D5L utility score; mean (SD)	0.829 (0.158)	0.744 (0.275)	0.24
QLU-C10D utility score; mean (SD)	0.822 (0.183)	0.775 (0.237)	0.47
	Education		
	School certificate (n=21)	**Vocational or higher education (n=24)**	**P value**
EQ5D5L utility score; mean (SD)	0.747 (0.308)	0.796 (0.172)	0.51

QLU-C10D utility score; mean (SD)	0.769 (0.259)	0.802 (0.181)	0.61
Centre			
	Royal North Shore Hospital (n=17)	**Royal Hobart Hospital (n=30)**	**P value**
EQ5D5L utility score; mean (SD)	0.792 (0.231)	0.768 (0.246)	0.75
QLU-C10D utility score; mean (SD)	0.783 (0.247)	0.798 (0.202)	0.82
Tumour functional status			
	Functional (n=8)	**Non-functional (n=12)**	**P value**
EQ5D5L utility score; mean (SD)	0.697 (0.184)	0.769 (0.236)	0.46
QLU-C10D utility score; mean (SD)	0.796 (0.173)	0.784 (0.247)	0.91

History of tumours				
	Complete cohort (n=47)	***SDHB*-related tumours (n=20)**	**No *SDHB*-related tumours (n=27)**	**P value**
EQ5D5L utility score; mean (SD)	0.776 (0.239)	0.740 (0.215)	0.804 (0.255)	0.36
QLU-C10D utility score; mean (SD)	0.792 (0.217)	0.789 (0.215)	0.796 (0.222)	0.91
Disease state				
	Tumour excised and in remission (n=14)	**Localised (n=5)**	**Metastatic (n=1)**	**P value**
EQ5D5L utility score; mean (SD)	0.712 (0.242)	0.830 (0.123)	0.675	0.19
QLU-C10D utility score; mean (SD)	0.739 (0.241)	0.816 (0.233)	0.880	0.23

EQ5D5L EuroQol 5 Dimensions 5 Levels; NA not applicable; QLU-C10D Quality of Life Utility-Core 10 Dimensions

Table 4.3 Quality of life results: Tumour location

	EQ5D5L utility score; mean (SD)	**QLU-C10D utility score; mean (SD)**	**P value**
	HNPGL		
HNPGL excised and in remission (n=3)	0.882 (0.067)	0.869 (0.038)	0.79
HNPGL localised (n=3)	0.845 (0.163)	0.899 (0.027)	0.57
	PC		
PC excised and in remission (n=3)	0.721 (0.121)	0.781 (0.270)	0.64
PC metastatic disease (n=1)	0.675	0.880	NA
	Thoracic PGL		
Thoracic PGL excised and in remission (n=2)	0.272 (0.170)	0.352 (0.221)	0.26
Thoracic PGL localised (n=1)	0.860	0.877	NA
	Abdominal PGL		
Abdominal PGL excised and in remission (n=6)	0.747 (0.173)	0.782 (0.196)	0.66
Abdominal PGL localised (n=2)	0.715 (0.056)	0.928 (0.070)	0.03**
Abdominal PGL metastatic (n=1)	0.675	0.880	NA
	RCC		
RCC and in remission (n=1)	1.000	1.000	NA

* P value < 0.01; ** P value < 0.05. EQ5D5L EuroQol 5 Dimensions 5 Levels; HNPGL head and neck paraganglioma; NA not applicable; PC phaeochromocytoma; PGL paraganglioma; QLU-C10D Quality of Life Utility-Core 10 Dimensions; RCC renal cell carcinoma

Utility scores for the complete cohort of *SDHB* PV carriers were lower than Australian normative values for both EQ5D5L (difference between means 0.13, p<0.0001) and the EQ-VAS (difference between means 5.85, p=0.017) (147). *SDHB* PV carriers were more likely to report issues with the EQ5D5L dimension of anxiety and depression compared to Australian normative values (OR 3.78, 95% CI 2.13-6.60, p<0.001, Table 4.4) (147). There were no other differences in the frequency of issues reported in the other EQ5D5L dimensions (mobility, personal care, usual activities or pain and discomfort) for *SDHB* PV carriers compared to Australian normative values.

Table 4.4 Frequency of issues reported in each EQ5D5L dimension compared to Australian normative values

SDHB PV carriers (n=47)		
Dimension	**No issue n (%)**	**There is an issue (from slight to extreme) n (%)**
Mobility	38 (80.9)	9 (19.1)
Personal care	43 (91.5)	4 (8.5)
Usual activities	35 (74.5)	12 (25.5)
Pain and discomfort	22 (46.8)	25 (53.2)
Anxiety and depression	21 (44.7)	26 (55.3)
Normative values from McCaffrey et al. (n=2908)		
Dimension	**No issue n (%)**	**There is an issue (from slight to extreme) n (%)**
Mobility	2161 (74.3)	747 (25.7)
Personal care	2774 (95.4)	134 (4.6)
Usual activities	2405 (82.7)	503 (17.3)
Pain and discomfort	1617 (55.6)	1291 (44.4)
Anxiety and depression	2190 (75.3)	718 (24.7)

EQ5D5L EuroQol 5 Dimensions 5 Levels; EQ-VAS EuroQol Visual Analogue Scale; *SDHB succinate dehydrogenase type B*

The QLU-C10D utility score for the complete cohort of *SDHB* PV carriers did not differ to UK normative values (difference between means 0.06, p=0.121) (148). UK normative values were not available for the component dimensions in the instrument (physical functioning, role functioning, social functioning, emotional functioning, pain, fatigue, sleep disturbance, appetite, nausea, and bowel problems) so no comparison could be made with *SDHB* PV carriers.

Given participants with a history of *SDHB*-related tumours were more likely to report symptoms of sweats, heart racing or palpitations or flushing compared to participants with no history of tumours, we examined whether HRQoL differed between those who reported the symptoms (Table 4.5). The EQ5D5L utility score was lower in those who reported sweats (difference between means 0.40, p<0.0001), heart racing or palpitations (difference between means 0.17, p=0.048) or flushing (difference between means 0.21, p=0.028) compared to those who did not report these symptoms. The EQ-VAS score was lower in those who reported sweats (difference between means 25.18, p<0.0001), but did not differ for those who reported heart racing or palpitations or flushing, compared to those without those respective symptoms. The QLU-C10D utility score was lower in those who reported sweats (difference between means 0.30, p<0.0001), but did not differ for those who reported heart racing or palpitations or flushing, compared to those without those respective symptoms. We examined whether any quality of life dimensions were associated with the symptoms of sweats, heart racing or palpitations or flushing. On multivariate analysis, impaired social functioning was the only factor associated significantly with the symptom of sweats (data not shown).

Table 4.5 Quality of life results: Symptoms

	Sweats (n=12)	**No sweats (n=35)**	**P value**
EQ5D5L utility score; mean (SD)	0.481 (0.295)	0.878 (0.087)	<0.0001*
EQ-VAS score; mean (SD)	53.92 (27.28)	79.14 (10.79)	<0.0001*
QLU-C10D utility score; mean (SD)	0.571 (0.287)	0.869 (0.117)	<0.0001*
	Heart racing or palpitations (n=9)	**No heart racing or palpitations (n=38)**	**P value**
EQ5D5L utility score; mean (SD)	0.636 (0.276)	0.810 (0.220)	0.048**
EQ-VAS score; mean (SD)	64.56 (30.81)	74.63 (16.02)	0.17
QLU-C10D utility score; mean (SD)	0.724 (0.258)	0.809 (0.207)	0.68
	Flushing (n=7)	**No flushing (n=40)**	**P value**
EQ5D5L utility score; mean (SD)	0.595 (0.301)	0.808 (0.215)	0.03**
EQ-VAS score; mean (SD)	60.71 (34.45)	74.80 (15.63)	0.08
QLU-C10D utility score; mean (SD)	0.709 (0.289)	0.808 (0.203)	0.27

* P value < 0.01; ** P value < 0.05; EQ5D5L EuroQol 5 Dimensions 5 Levels; EQ-VAS EuroQol Visual Analogue Scale; QLU-C10D Quality of Life Utility-Core 10 Dimensions

4.4 Discussion

Our study is the first to report HRQoL exclusively for carriers of *SDHB* PVs, whether affected or unaffected by *SDHB*-related tumours. We found quality of life using the EQ5D5L measure was lower in *SDHB* PV carriers compared to population norms. We found anxiety or depression in the EQ5D5L measure was more common in *SDHB* PV carriers compared to population normative values, and those reporting symptoms of sweats, heart racing or palpitations and flushing had lower quality of life compared to those who did not report these symptoms. More than half our cohort had no personal history of *SDHB*-related tumours, yet anxiety and depression was still more common in those unaffected by tumours than in population normative values.

More than a decade ago, van Hulsteijn *et al.* examined quality of life in 174 patients with PGL (106). The study included 25 *SDHB* PV carriers of whom 13 were asymptomatic (106). The investigators found that quality of life was lower for PGL patients compared to controls across 3 measures: Hospital Anxiety and Depression Scale (HADS), Multidimensional Fatigue Index 20 (MFI-20) and Short Form 36 (SF-36) (106). *SDHB* PV carriers with PGL did not have worse quality of life compared to other PGL patients and asymptomatic *SDHB* PV carriers did not have worse quality of life compared to controls and age-adjusted reference values (106). Our study had approximately double the number of participants comparatively, and we found lower quality of life and higher reported anxiety or depression using the EQ5D5L measure compared to population norms, even in *SDHB* PV carriers unaffected by *SDHB*-related tumours.

In the Van Hulsteijn *et al.* study, 60% of *SDHB* PV carriers had HNPGL, whereas only 13% of our cohort reported HNPGL (106). Their study found that among HNPGL patients, those experiencing symptoms such as tinnitus and dysphonia had poorer quality of life compared to asymptomatic individuals (106). Similarly, our study found that *SDHB* PV carriers with catecholamine excess symptoms also reported poorer quality of life. These findings emphasize the impact of tumour-related symptoms on quality of life.

Quality of life has been assessed for other hereditary cancer syndromes including multiple endocrine neoplasia type-1 (MEN1), multiple endocrine neoplasia type-2 (MEN2), BRCA1 and BRCA2 (149-158). More than 10 instruments have been used to assess quality of life for other hereditary cancer syndromes. We used the EORTC QLQ-C30 measure similar to Rodrigues *et al.* (156), but others have not used the EQ5D5L measure to our knowledge. Instruments such as Patient-Reported Outcomes Measurement Information System 29-item profile measure (PROMIS-29) and Short Form 12 (SF-12) asked similar questions to the instruments we used, about general physical, emotional and social quality of life (149, 152-154). We did not address quality of life attributes of optimism, post-traumatic stress and cancer worry that have been studied in other hereditary cancer syndromes (150, 151, 157). In other studies, living with hereditary cancer syndromes was associated with physical symptoms, role limitations, social function impairment and emotional symptoms (149, 150, 152, 153, 158). However, there were some studies that found those with hereditary cancer syndromes generally had good QOL compared with population norms (151, 156). Anxiety and depression were common in our cohort and are also common in other studies for those living with other hereditary cancer syndromes (149, 151, 152, 156). The prevalence of anxiety and depression for individuals with clinical manifestations of MEN1 was 28% and 8% respectively (151) and anxiety or depression was more common in individuals with MEN1 compared to population norms (152). The prevalence of depression for individuals with clinical manifestations of MEN2 was 26% (156). The prevalence of anxiety in women with BRCA 1 or 2 irrespective of history of cancer was 44%, and these women had poorer mental health compared to population norms (149).

Fear of cancer, or Damocles syndrome, could explain the reduced quality of life and higher prevalence of anxiety and depression in *SDHB* PV carriers compared to the general population. Damocles syndrome is based on the Ancient Grecian tale, where the king Dionysius invited Damocles to sit in his throne (159). Initially Damocles enjoyed the throne, until he realised a sword was hanging by a single hair above his head and could strike him at any moment (159).

Similarly, those at risk of cancer may feel relieved to be in remission or absent of cancer diagnosis, but worry and uncertainty about future cancer can preoccupy their thoughts and feelings. For those with a history of cancer, fear of cancer recurrence is defined as "Fear, worry, or concern relating to the possibility that cancer will come back or progress" (160). *SDHB* PV carriers enrolled in a long term surveillance program may have annual reminders of this fear when they are advised to attend their annual clinical reviews.

Worry about cancer has certainly been described in other hereditary cancer syndromes such as familial adenomatous polyposis, MEN1, lynch syndrome and Li-Fraumeni Syndrome (161-164). In a study of 334 adults with familial adenomatous polyposis compared to 130 proven non-carriers but with a family history of FAP, those with FAP thought more about their chance of developing cancer (p=0.02) and worried more about the possibility of needing surgery (p= 0.00) compared to non-carriers (161). Qualitative studies have shed light on the lived experience of hereditary cancer syndromes. An analysis of interviews with twelve MEN1 patients found that leading up to screening appointments, patients started paying more attention to physical symptoms and worrying about upcoming screening test results (163). Those with children worried what would happen to their children if they died (163). Patients describe having an uncertain future and how this could influence life choices such as moving house and make them appreciate the life they currently have (163). Kohut *et al.* interviewed twenty lynch syndrome patients, half of whom were unaffected by cancer (164). They described carriers as living on "high alert" due to expecting cancer to develop and that cancer worry changed "from panic to back of my mind" (164). There was worry about not only themselves but their children and other family members such as parents (164). Forbes Shepherd interviewed thirty young adults with confirmed Li Fraumeni syndrome and described how those with personal or strong family history of cancer felt an obligation to kin, where they not only worried about passing the pathogenic variant to their children but also about exposing their children to anxiety and grief that they themselves had experienced (162).

'Scanxiety' is another factor that may explain the reduced quality of life in *SDHB* PV carriers compared to the population. Scanxiety is defined as distress or anxiety which can occur before, during, and after procedures related to imaging (such as discomfort and claustrophobia) and receipt of scan results (such as uncertainty about results and implications for disease status) (165). Current surveillance recommendations for *SDHB* PV carriers are for annual clinical reviews, biennial biochemical assessments and MRI every 2-3 years and/or functional imaging in adulthood (100). In a recent systematic review, scanxiety prevalence leading up to cancer patients' follow-up visits was 34% to 85% (166). Risk factors for scanxiety included sociodemographic factors, cancer-related factors, coping self-efficacy and pre-existing anxiety (166). A study of 218 patients with metastatic cancer found that those who reported scanxiety had lower quality of life on the EORTCQLQ-30 compared to those who did not report it ($p = 0.004$) (167). To our knowledge, scanxiety has not been researched deliberately in hereditary cancer syndromes.

Limitations of our research include that this was a cross-sectional rather than longitudinal observational study, which did not allow assessing changes of HRQoL over time. As the study was cross-sectional we could not determine causal relationships between variables. While we assessed sociodemographic factors that can influence quality of life, we did not control for all potential confounders e.g. comorbid medical conditions. Recruiting patients via hospital-based surveillance clinics might result in a selection of patients and result in different findings compared to a population-based study. Data was not necessarily timed in relation to surveillance imaging, so further research into scanxiety in this population is warranted. While patients with a history of tumours were more likely to report flushing, we acknowledge that flushing is not a common symptom of PPGL as pallor associated with vasoconstriction during catecholamine excess is more common. Our study tried to mitigate ascertainment bias by ensuring that the background medical and social history was unknown to the investigator inviting participants to complete the survey. We invited all *SDHB* carriers who attend clinic irrespective of whether they had history of being diagnosed with *SDHB* associated tumours. This was a quantitative quality of life study, but qualitative research is warranted to understand and provide insights into the challenges facing this population.

4.5 Conclusion

This is the first known study to investigate the quality of life for *SDHB* PV carriers with and without a history of tumours. *SDHB* PV carriers were more likely to have poorer quality of life and a higher prevalence of anxiety or depression using the EQ5D5L measure compared to population norms, particularly those who reported symptoms of sweats, heart racing or palpitations and flushing. In addition, increased anxiety and depression was observed among *SDHB* PV carriers, relative to the general population, indicating the negative impact of knowing carrier status on individual's HRQOL.

These findings underscore the importance of routine psychological screening and tailored support for *SDHB* PV carriers. The results align with broader observations of psychological impacts in individuals with hereditary cancer syndromes (149, 150, 152, 153, 158), highlighting a critical need for multidisciplinary care approaches. Healthcare systems must prioritize the integration of mental health support into the care pathways for *SDHB* PV carriers to improve their overall quality of life. Further research is needed to investigate the impact of specific interventions, such as lifestyle modifications, counselling, or pharmacotherapy, on the quality of life and mental health outcomes in this population.

Chapter 5: Outcomes for SDHB PV carriers: a systematic review and meta-analysis

5.0 Preface

This chapter contains a systematic review and meta-analysis of original research studies documenting outcomes of *SDHB* PV carriers. The manuscript was published in the *Journal of Clinical Endocrinology and Metabolism* in 2024 with the text in the thesis identical to the published paper. The full pdf version is available in the appendix.

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Title Page

Full title:

Outcomes of *SDHB* pathogenic variant carriers: a systematic review and meta-analysis

Short running title:

Outcomes of *SDHB* carriers

Authors:

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Structured abstract:

Context

Carriers of germline pathogenic variants (PV) in *succinate dehydrogenase type B* (*SDHB*) are at increased risk of developing phaeochromocytomas and paragangliomas (PPGL). Understanding their outcomes can guide recommendations for risk assessment and early detection.

Objective

We performed a systematic review and meta-analysis of the following outcomes in *SDHB* PV carriers: age-specific risk of developing tumours, metastatic progression, second primary tumour development, and mortality.

Materials and Methods

Pubmed, MEDLINE and EMBASE were searched. Sixteen studies met the inclusion criteria and were sorted into four outcome categories: age-specific penetrance, metastatic disease, risk of second tumour and mortality. We assessed heterogeneity and performed a meta-analysis across studies using a random effects model with the DerSimonian and Laird method.

Results

Penetrance of PPGL for non-proband/ non-index *SDHB* PV carriers by age 20 was 4% (95% CI, 3%-6%), 11% (95% CI, 8%-15%) by age 40, 24% (95% CI, 19%-31%) by age 60 and 35% (95% CI, 25%-47%) by age 80. The overall risk of metastatic disease for non-proband/ non-index carriers with PPGL was 9% (95% CI, 5-16%) per lifetime. In all affected cases (combining both proband/ index and non-proband/ non-index carriers with tumours), the risk of a second tumour was 24% (95% CI, 18-31%) and all cause 5-year mortality was 18% (95% CI 6-40%).

Conclusion

Penetrance for PPGL in *SDHB* PV carriers increases linearly with age. Affected carriers are at risk of developing and dying from metastatic disease, or of developing second tumours. Lifelong surveillance is appropriate.

Disclosures:

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Disclosures: The authors have nothing to declare.

5.1 Introduction

Carriers of germline pathogenic variants (PV) in *succinate dehydrogenase type B* (*SDHB*) are at increased risk of developing phaeochromocytomas and paragangliomas (PPGL) (2). Phaeochromocytomas arise from chromaffin cells in the adrenal medulla and paragangliomas arise from chromaffin cells in sympathetic, or chief cells in parasympathetic, ganglia (168). Overall, around 20% of all PPGL cases are attributed to germline PVs in genes encoding *SDH* subunits (*SDHx*), with the most frequent being *SDHB* (100). *SDHB* PV carriers are typically identified after a proband/ index patient presents with PPGL and undergoes genetic testing, followed by family risk notification and predictive testing.

SDHB PV carriers have a significantly increased risk of metastatic progression and mortality. A recent analysis of 448 proband cases from a registry dataset noted that metastatic disease affected 27% of *SDHB* PV carriers (169). In another paper, proband patients were four times more likely to develop metastatic disease compared with non-proband cases (129). Recent analysis of PPGLs from the Cancer Genome Atlas (TCGA) found that patients with PPGLs associated with a germline *SDHB* PV had significantly higher 15-year mortality (HR 4.7), compared to non-*SDHB* cases (73).

Understanding the outcomes facing this group is clinically important, as it can help to inform patients, their families and healthcare providers regarding risk assessment, early detection, and intervention strategies. Previous systematic reviews and meta-analyses (72, 170, 171) have not focused on outcomes facing non-proband/non-index *SDHB* PV carriers. The risk of metastatic progression for non-index *SDHB* PV carriers was last assessed in a systematic review and meta-analysis more than a decade ago (118), and in light of subsequent studies deserves an update. The aim of this study was to assess the following outcomes in *SDHB* PV carriers by meta-analysis of published studies in the field: age-specific risk of developing tumours, risk of metastatic progression, risk of developing a second primary tumour, and risk of death. An understanding of these risks is crucial for optimizing patient management and improving clinical outcomes, because these four risks are key factors in driving the high burden of disease facing *SDHB* PV carriers and health care utilization. Tumour development is associated with symptoms of catecholamine excess and/ or mass-effect symptoms to surrounding structures (1); each subsequent tumour increases the likelihood of morbidity and mortality; metastatic progression is associated with morbidity from tumour symptoms (1) and is a risk factor for mortality (51, 74).

5.2 Materials and Methods

Eligibility criteria

We searched for original retrospective and prospective observational studies reporting on outcomes for *SDHB* PV carriers whose carrier status was confirmed on genetic testing. Studies were included if they reported on any of the following outcome measures: (1) age-specific penetrance (age 20, 40, 60 and/ or 80), (2) number or proportion of *SDHB* PV carriers who developed metastatic disease, (3) number or proportion of *SDHB* PV carriers who developed second tumour(s), and (4) mortality of *SDHB* PV carriers with metastatic disease. Penetrance was defined as the proportion of *SDHB* PV carriers who developed PPGL. Metastasis was defined by WHO classification as the presence of chromaffin tissue in non-chromaffin organs such as lymph nodes, liver, lungs and bone (168). In the event a study reported metastasis on imaging without a biopsy diagnosis, we categorized this as metastasis and not as a second tumour. We classified second tumour only when a study used the phrases 'second tumour' or 'multifocal' or provided individual patient data on the particular second tumour diagnoses (the type of head or neck paraganglioma (HNPGL) or thoraco-abdominal paragangliomas and phaeochromocytomas (TAPPGL)). Mortality was defined as the number of patients who died from the disease. For mortality estimates we included studies that reported 5-year mortality of *SDHB* PV carriers with metastatic disease to minimise bias from studies with unclear or short duration of follow-up. We did not exclude studies that reported on *SDHB* PV carriers who presented with symptomatic disease. We also did not exclude studies with probands, defined as the first individual in a family to be diagnosed with a *SDHB* PV after presenting with a tumour, nor did we exclude studies with index cases, defined as the first identified case of *SDHB* PV. However, if data on non-proband/ non-index *SDHB* PV carriers was available, we assessed these results preferentially to data on probands/ index carriers. We only included results for non-probands/non-index cases for the outcomes of age-specific penetrance and risk of metastatic progression. However for risk of second tumour, we found only one study that reported non-probands/ non-index carrier data, so we could not assess non-probands/ non-index carrier data preferentially. Similarly for mortality risk, we found only one study that reported non-probands/ non-index carrier 5-year mortality data, so we could not assess non-probands/ non-index carrier data preferentially.

As a systematic review and meta-analysis has been performed looking at the risk of metastatic disease for the combined group of proband/ index and non-proband/ non-index *SDHB* PV carriers (171), we performed a meta-analysis of the risk of metastatic progression for non-proband/ non-index *SDHB* PV carriers with disease only. Studies with fewer than 10 *SDHB* PV carriers were excluded. Where studies reported on the same cohort of patients, we assessed the study with the larger number of *SDHB* PV carriers addressing the outcome of interest.

Search strategy

In March 2022 the databases Pubmed, Ovid MEDLINE and Ovid EMBASE were searched by DFD. Studies were limited to those in humans but there was no limit on language. Additional records were identified through primary article references. The Pubmed search was as follows: ((paraganglioma or phaeochromocytoma) and (succinate or SDHB or SDH)) limit to humans. The Ovid MEDLINE search was as follows: (succinates/ or succinate.mp. or SDHB.mp.) and (exp paraganglioma/ or paraganglioma.mp.) limit to humans. The Ovid EMBASE search was as follows: (exp succinate dehydrogenase/ or succinate.mp. or sdhb.mp. or sdh.mp) and (exp paraganglioma/ or exp phaeochromocytoma/ or paraganglioma.mp or phaeochromocytoma) limit to human.

Data selection

Studies from the search were entered into the reference management software (Endnote X9). Duplicates were removed and articles were screened for eligibility based on title and abstract by DFD. Basic science reports, case reports, review articles, editorials, conference abstracts with insufficient data, non-original research and unrelated articles (those that did not address the research questions or meet the inclusion criteria) were removed. Once relevant full text articles were obtained a full text review of screened articles was conducted by authors DFD, DEB, VHMT and RCB with disagreements determined by group consensus with RDAL. We performed an analysis of the penetrance data from our cohort (129) (Appendix 1) and included the data in the pooled penetrance assessment.

Articles were separated into four categories according to penetrance for non-proband/ non-index *SDHB* PV carriers, risk of metastatic disease for *SDHB* PV carriers with disease, risk of a second tumour for *SDHB* PV carriers with disease and 5-year mortality for *SDHB* PV carriers with metastatic disease.

Data extraction

The following data were extracted from eligible articles: first author, year of publication, observational study design, country, data collection, duration of follow-up, number of participants who were non-proband/ non-index *SDHB* PV carriers, number of non-proband/ non-index *SDHB* PV carriers who developed disease by age 20, 40, 60 and/ or 80 years based on reported age-specific penetrance, total number of *SDHB* PV carriers who developed disease, number of *SDHB* PV carriers who developed metastatic disease, number of *SDHB* PV carriers who developed a second tumour and number of *SDHB* PV carriers with metastatic disease who died within 5 years. Studies were classified as either cohort studies (with follow-up of a cohort of *SDHB* PV carriers to observe who developed outcomes of interest (172)) or cross-sectional; as retrospective or prospective; and as single or multicentre.

Risk of bias assessment

The quality of the observational studies were assessed independently by authors DFD, DEB and RCB using a modified Newcastle-Ottowa tool described by Hamidi *et al*. (72) and further adapted to this study. The following were described: (1) how the sample represented the population of interest, (2) how genetic information was assessed, (3) how the outcome measures were assessed, (4) sufficient duration of follow-up and, (5) adequacy of follow-up. Detailed description of the tool is listed in Supplementary material. The tool used by Hamidi *et al*. (72) was adapted by replacing their question 'how the data on metastatic PPGL was collected' with 'how genetic information was assessed' because we assessed *SDHB* PV carriers only and we assessed four outcomes rather than the one outcome of metastatic disease risk.

Statistical analyses

Penetrance in our cohort of *SDHB* PV carriers (129) was performed using the Kaplan-Meier method. The outcome of the meta-analysis was the pooled penetrance of PPGL in *SDHB* PV carriers. The proportion of patients with PPGL by age 20, 40, 60 and 80 was calculated as the number of patients with PPGL at those ages divided by the total number of *SDHB* PV carriers. Probands/ Index cases were excluded to reduce ascertainment bias (21). For all four outcomes of interest, a meta-analysis of proportions was performed by using a random effects generalised linear mixed model due to expected differences in the populations from which data was pooled. The random effects model was determined using the DerSimonian and Laird method (173), with the estimate of heterogeneity taken from the inverse variance method. Confidence intervals were obtained using the Jackson method. Prediction intervals were calculated to denote the penetrance which may be observed in future studies. Publication bias assessment was attempted but ultimately could not be assessed with Egger's regression test, funnel plot asymmetry or the meta-regression "weightr" package in R, because there were too few studies and insufficient data to run these tests, defined as fewer than 10 studies for each pooled assessment (174). Analyses were performed with R version 4.2.2.

5.3 Results

Study selection

The search of publications produced 4035 references. Duplicates were removed and abstracts reviewed leaving 241 publications for assessment. The manuscripts were reviewed and a further 224 publications were removed: 12 publications were eliminated due to being review articles, 9 publications were conference abstracts, 29 studies were case reports, 13 studies were case reports on *SDHB* PV carriers, 2 were pathology studies, 132 publications were unrelated and 19 were basic science publications. One study was an identical cohort presented in another publication by the same authors. Two studies reported risk of metastatic disease but were not included in the assessment because there were fewer than 10 non-proband/ non-index *SDHB* PV carriers with disease. Five studies that reported risk of metastatic disease were excluded because there was insufficient data on non-proband/ non-index *SDHB* PV carriers. One study reported 5-year mortality but did not report the number of *SDHB* PV carriers with metastatic disease. Altogether, 16 studies met the inclusion criteria (19-21, 51, 52, 56-60, 67, 74-76, 129, 175). The study inclusion flow diagram is shown in Figure 5.1.

Study characteristics

Of the 16 studies, 10 were cohort studies (19, 20, 51, 57-60, 74, 129, 175), and 6 were cross-sectional studies (21, 52, 56, 67, 75, 76) (Table 5.1). The follow-up duration of cohort studies was variable and median follow up ranged between 1 year and 5.9 years. Twelve studies were retrospective, three were prospective and one study was retrospective with ongoing prospective follow-up. 9 were single centre and 7 were multicentre. 7 studies were suitable for the penetrance assessment, 5 for assessing risk of metastatic disease assessment, 5 for assessing risk of a second tumour and 6 for the 5-year mortality of *SDHB* PV carriers with metastatic disease assessment. The number of *SDHB* PV carriers in each study ranged from 11 to 317.

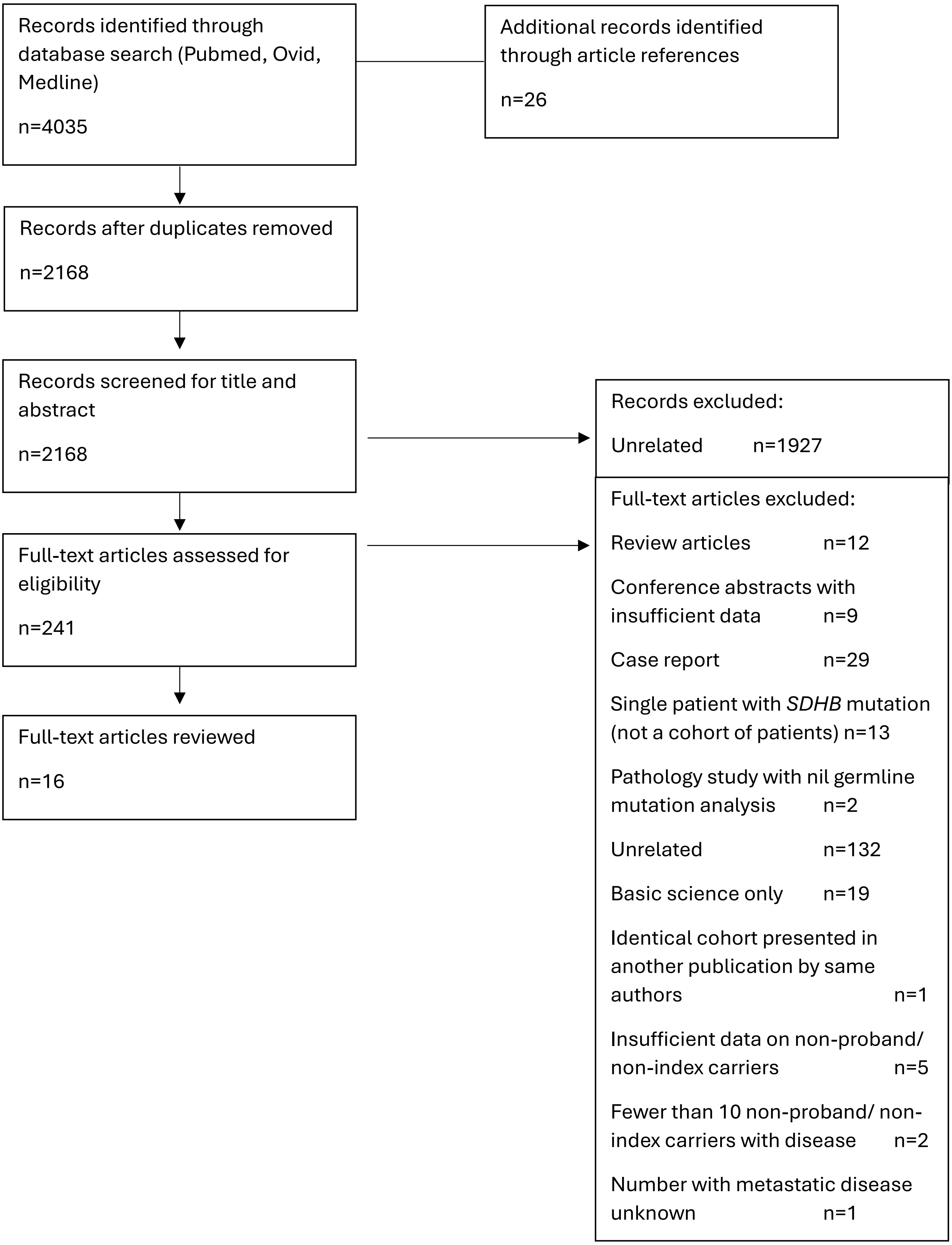

Figure 5.1 Study flow diagram

Table 5.1 Characteristics of studies (part 1)

Study	Country	Study design	Population studied	Data collection	Follow up	Outcome category of this meta-analysis
Amar *et al*. (2007) (74)	France	Retrospective multicentre cohort study	Patients with metastatic PPGL	NA-2005	For the *SDHB* carrier the median follow-up was 28 months	Mortality
Andrews *et al*. (2018) (21)	UK	Retrospective multicentre cross-sectional study	*SDHB/SDHC/SDHD* carriers	NA	NA	Penetrance, Metastatic disease
Bausch *et al*. (2014) (58)	Germany, Italy, Poland, France, UK, Hungary, Ukraine, Latvia, Argentina, USA	Prospective multicentre cohort study	Patients with symptomatic paraganglial tumours	NA-2013	NA-2013	Second tumour
Daniel *et al*. (2016) (60)	UK	Retrospective single centre cohort study	*SDHx* PV carriers	2005-2015	2005-2015	Second tumour
Davidoff *et al*. (2022) (129)	Australia	Retrospective and ongoing prospective multicentre cohort study	*SDHB* PV carriers	1994-2021	1994-2021	Penetrance, Metastatic disease, Second tumour, Mortality
Eijkelenkamp *et al*. (2017) (20)	The Netherlands	Retrospective single centre cohort study	*SDHB* PV carriers	2008-2015	2008-2015	Penetrance
Jafri *et al*. (2013) (56)	UK	Prospective multicentre cross-sectional study	*SDHB* probands who presented with PPGL and/ or HNPGL and non-proband carriers	2001-2011	2001-2011	Penetrance
Jochmanova *et al*. (2017) (57)	USA	Retrospective single centre cohort study	Family members of *SDHB* index cases who presented with PPGL	2004-2016	2004-2016	Penetrance, Metastatic disease
Jochmanova *et al*. (2020) (52)	USA	Retrospective single centre cross-sectional study	*SDHB* PV carriers with PPGLs	2000-2019	2000-2019	Mortality
King *et al*. (2011) (75)	USA	Retrospective single centre cross-sectional study	Patients with metastatic PPGL	2000-2010	2000-2010	Mortality
Niemeijer *et al*. (2017) (19)	The Netherlands	Retrospective multicentre cohort study	*SDHB* PV carriers	Prior to 2014	Prior to 2014	Penetrance, Metastatic disease
Schovanek *et al*. (2014) (67)	USA	Retrospective single centre cross-sectional study	Patients with *SDHB*-related PPGL	NA	NA	Mortality
Srirangalingam *et al*. (2008) (59)	UK	Retrospective multicentre cohort study	*SDHB* PV carriers	NA	NA	Second tumour
Tufton *et al*. (2017) (51)	UK	Prospective single centre cohort study	*SDHB* PV carriers	1975-2015	1975-2015	Second tumour, Metastatic disease
Turkova *et al*. (2016) (76)	USA	Retrospective single centre	Patients with metastatic PPGL	2000-2015	2000-2015	Mortality

		cross-sectional study				
White *et al*. (2022) (175)	UK	Retrospective single centre cohort study	*SDHx* carriers who presented with PPGL and first-degree relatives with *SDHx* PV	2000-2020	2000-2020	Penetrance

HNPGL head and neck paraganglioma, NA not applicable, na not available, PPGL phaeochromocytoma and paraganglioma, PV pathogenic variant, SDHB succinate dehydrogenase type B, SDHx succinate dehydrogenase

Table 5.1 Characteristics of studies (part 2)

Study	Number of non-proband/ non-index *SDHB* PV carriers	Penetrance of *SDHB* PV carriers with PPGL and/ or HNPGL (probands/ index cases excluded where known)	Total number of *SDHB* PV carriers with PPGL and/ or HNPGL	Number of *SDHB* PV carriers with multifocal disease or a second tumour (excluding metastatic disease)	Total number of *SDHB* PV carriers with metastatic disease	5-year survival of *SDHB* PV carriers with metastatic disease
Amar *et al.* (2007) (74)	NA	NA	23	NA	23	36%
Andrews *et al.* (2018) (21)	371	Age 60: 83; Age 80: 145[a]	NA	NA	9	NA
Bausch *et al.* (2014) (58)	NA	NA	25	6	2	NA
Daniel *et al.* (2016)	27	NA	11	2	1	NA
Davidoff *et al.* (2022) (60)	148	Age 20: 7; Age 40: 17; Age 60: 25; Age 80: 29[b]	62	18	3	69%
Eijkelenkamp *et al.* (2017) (20)	70	Age 40: 1; Age 60: 8[c]	NA	NA	8	NA
Jafri *et al.* (2013) (56)	187	Age 20: 9; Age 40: 30; Age 60: 75[d]	NA	NA	NA	NA
Jochmanova *et al.* (2017) (57)	241	Age 20: 8; Age 40: 30; Age 60: 64; Age 80: 118[e]	143	NA	7	NA
Jochmanova *et al.* (2020) (52)	NA	NA	64	NA	45	100%
King *et al.* (2011) (75)	NA	NA	23	NA	23	96%
Niemeijer *et al.* (2017) (19)	129	Age 20: 3; Age 40: 13; Age 60: 32; Age 80: 43[f]	83	NA	15	NA
Schovanek *et al.* (2014) (67)	NA	NA	NA	NA	77	76%
Srirangalingam *et al.* (2008) (59)	NA	NA	16	3	NA	NA
Tufton *et al.* (2017) (51)	65	NA	40	8	8	NA
Turkova *et al.* (2016) (76)	NA	NA	73	NA	73	92%
White *et al.* (2022) (175)	56	Age 20: 1; Age 40: 3; Age 60: 16[g]	NA	NA	NA	NA

HNPGL head and neck paraganglioma, NA not applicable, na not available, PPGL phaeochromocytoma and paraganglioma, PV pathogenic variant, *SDHB succinate dehydrogenase type B*, *SDHx* succinate dehydrogenase;[a-g] penetrance figures are based on the assumption that all non-proband/ non-index *SDHB* PV carriers were included in the age-specific penetrance proportions reported for non-proband/ non-index carriers in each study. [a]Andrews *et al*. (2013): Age 60: 22.5%; Age 80: 39% (21), [b]Davidoff *et al*. (this study): Age 20: 5%; Age 40: 11%; Age 60: 17%; Age 80: 20%, [c]Eijkelenkamp *et al*. (2017): Age 40: 2%; Age 60: 12% (20), [d]Jafri *et al*. (2013): Age 20: 5%; Age 40: 16%; Age 60: 40% (56), [e]Jochmanova *et al*. (2017): Age 20: 3.3%; Age 40: 12.4%; Age 60: 26.4%; Age 80: 48.8% (57), [f]Niemeijer *et al*. (2017): Age 20: 2%; Age 40: 10%; Age 60: 25%; Age 80: 33% (19), [g]White *et al*. (2022): Age 20: 2.5%; Age 40: 5%; Age 60: 28.7% (175)

Risk of bias assessment

Risk of bias assessments were performed for each study and results are summarised in Figure 5.2. Patient selection assessment was assessed as having low risk of bias for 10 studies and high risk of bias for 6 studies. Genetic diagnosis (14/16) and clinical outcomes (15/16) were assessed by our authors as having low risk of bias. Follow up duration (12/16 high or unclear risk) and follow up completeness (15/16 high or unclear risk) were assessed as having high risk of bias.

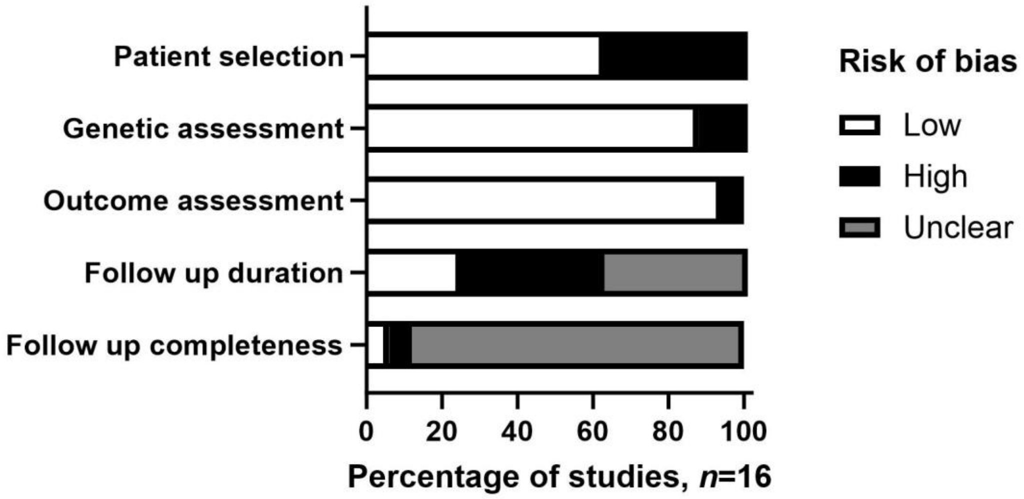

Figure 5.2 Risk of bias assessment

Penetrance for non-proband/ non-index SDHB PV carriers: meta-analysis

Results for age-specific penetrance for non-proband/non-index *SDHB* PV carriers are shown in Figure 5.3. The pooled penetrance of PPGL by age 20 was 4% (95% CI, 3%-6%; prediction interval, 2%-7%; n=761, 5 studies) with I^2 of 0%. By age 40 the pooled penetrance was 11% (95% CI, 8%-15%; prediction interval, 5%-25%; n=831, 6 studies). There was moderate variability between studies with I^2 of 52%. By age 60 pooled penetrance was 24% (95% CI, 19%-31%; prediction interval, 9%-50%; n=1202, 7 studies). There was high variability between studies with I^2 of 83%. By age 80 pooled penetrance was 35% (95% CI, 25%-47%; prediction interval, 5%-84%; n=889, 4 studies). There was high variability between studies with I^2 of 91%. The overall pooled penetrance is summarised in Figure 5.4. Excluding the penetrance data from our cohort produced similar results for pooled penetrance and heterogeneity at age 20, 40 and 60 but gave a higher penetrance for age 80 (Appendix 1 and 2).

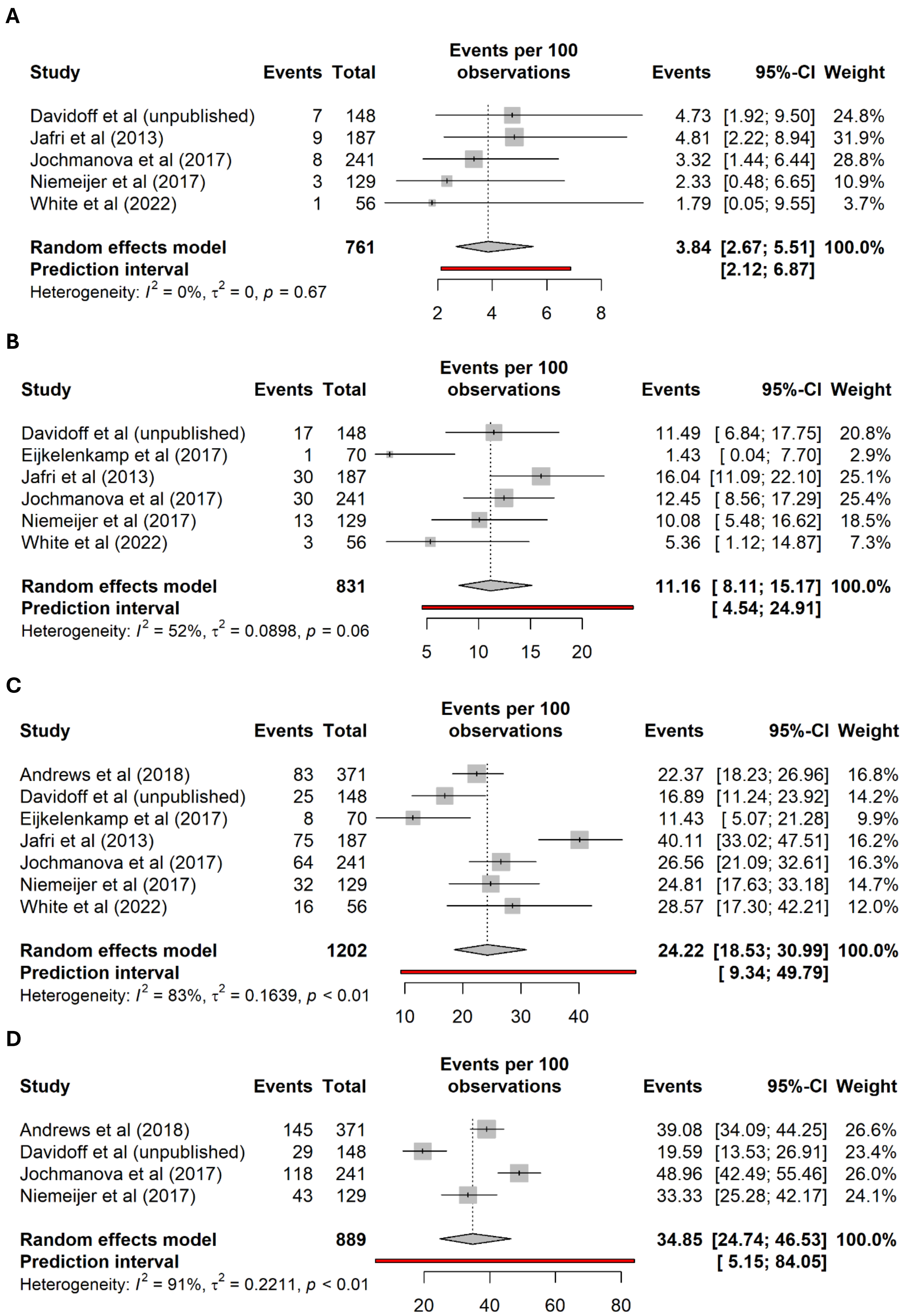

Figure 5.3 Penetrance of PPGL for non-proband/ non-index *SDHB* PV carriers

Panel A: Age 20. Panel B: Age 40. Panel C: Age 60. Panel D: Age 80.

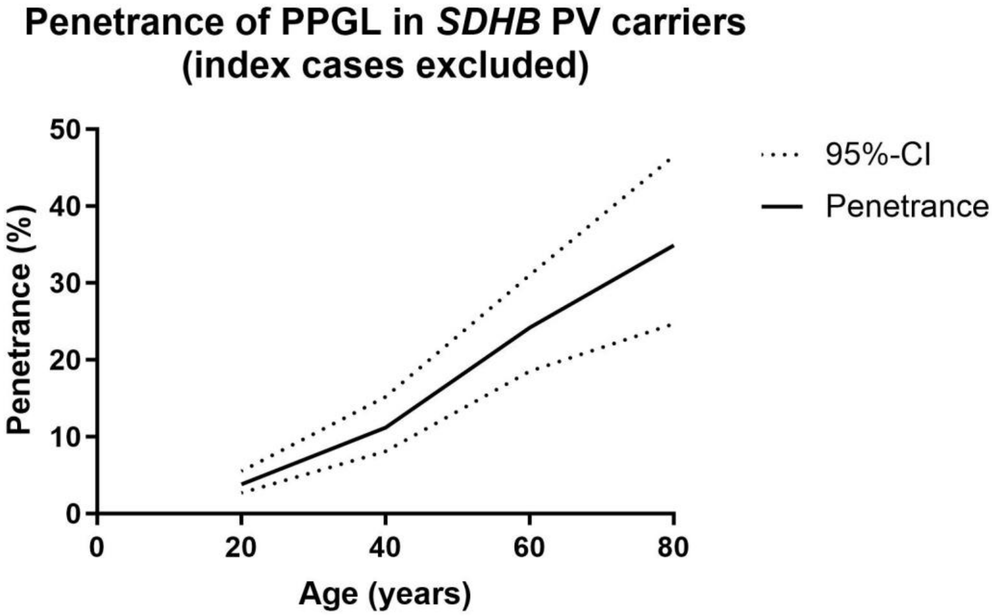

Figure 5.4 Overall pooled penetrance of PPGL for *SDHB* PV carriers, excluding proband/ index cases

Risk of metastatic disease for non-proband/ non-index SDHB PV carriers with disease: meta-analysis

Results for the risk of metastatic disease for *SDHB* PV carriers with disease excluding proband/ index cases, are shown in Figure 5.5. The pooled risk of metastatic disease for non-proband/ non-index *SDHB* PV carriers with tumours was 9% (95% CI, 5-16%; prediction interval 2-34%; n=251, 5 studies). There was mild variability between studies with I^2 of 33%. The subgroup analysis of risk of metastatic disease in non-proband/ non-index carriers with HNPGL and TAPPGL are provided in Figures 5.6 and 5.7.

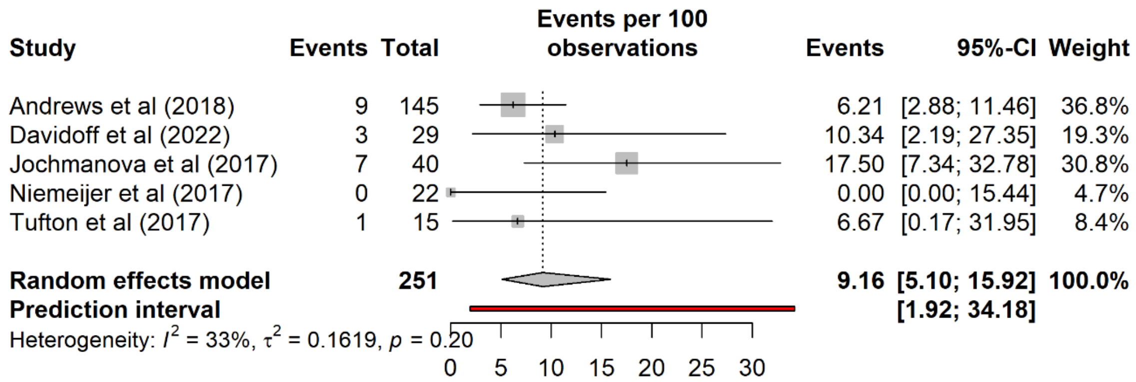

Figure 5.5 Risk of metastatic disease for SDHB PV carriers with disease, excluding proband/ index cases

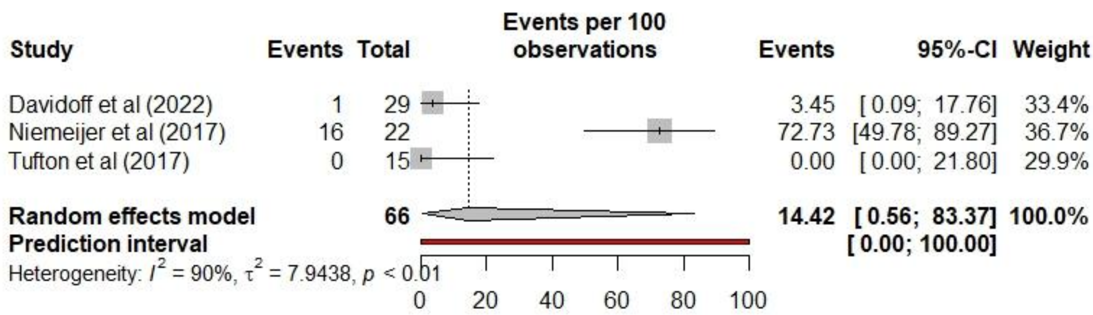

Figure 5.6 Risk of metastatic disease in non-proband/ non-index carriers with HNPGL

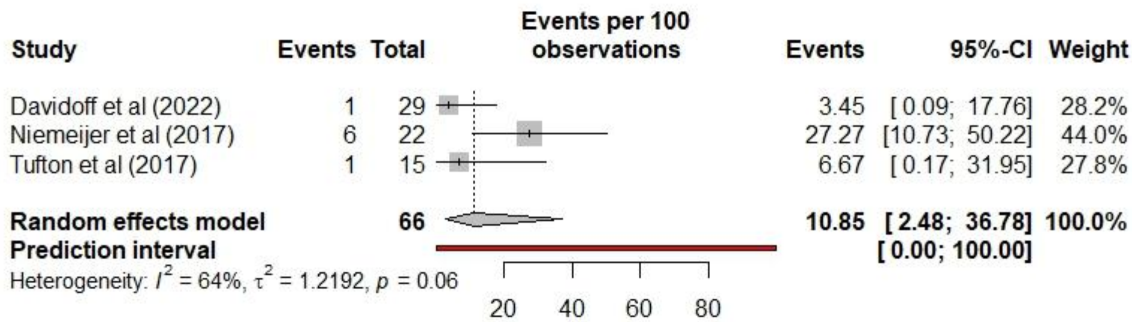

Figure 5.7 Risk of metastatic disease in non-proband/ non-index cases with TAPPGL

Risk of a second tumour for SDHB PV carriers with disease: meta-analysis

The risk of developing a second primary tumour could not be determined for non-index carriers alone due to insufficient data in the literature. Considering outcomes for probands and non-probands/non-index cases with disease combined, the risk of a second primary tumour is shown in Figure 5.8. The pooled risk of a second tumour was 24% (95% CI, 18-31%; prediction interval 15-37%; n=156, 5 studies) with I^2 of 0%.

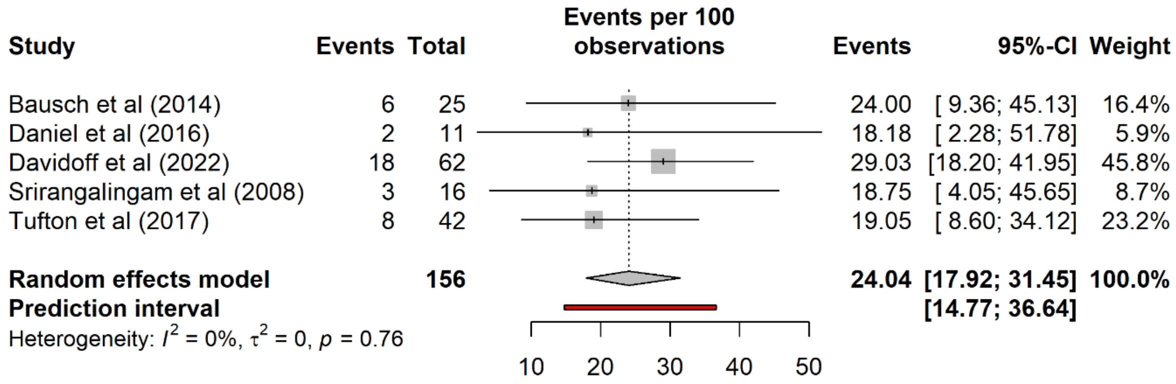

Figure 5.8 Risk of a second tumour for *SDHB* PV carriers with disease

Five-year mortality for SDHB PV carriers with metastatic disease: meta-analysis

Five-year mortality could not be determined for non-index carriers alone due to insufficient data in the literature. Combining outcomes for probands and non-probands/non-index cases with metastatic disease is shown in Figure 5.9. Five-year mortality of *SDHB* PV carriers with metastatic disease was 18% (95% CI 6-40%; prediction interval 1-90%; n=254, 6 studies). There was high variability between studies with I^2 of 86%.

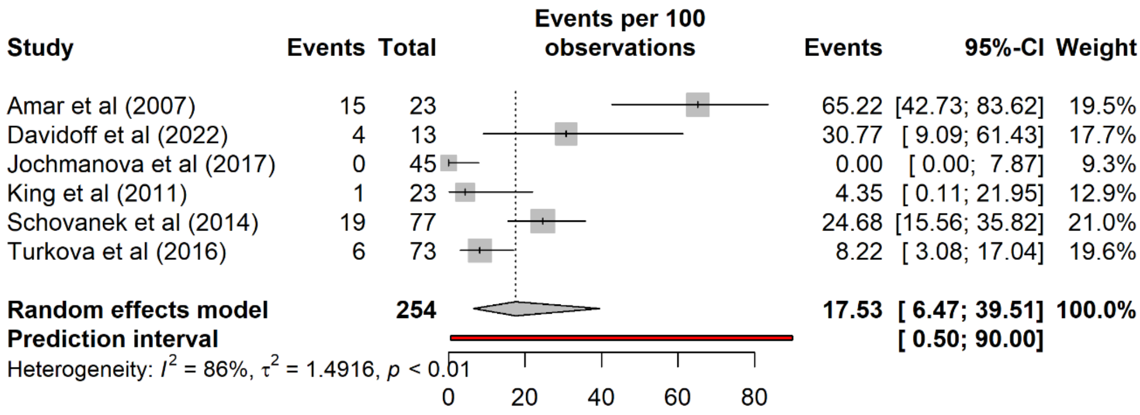

Figure 5.9 5-year mortality of *SDHB* PV carriers with metastatic disease

5.4 Discussion

In this systematic review and meta-analysis, we aimed to assess four clinically relevant outcomes facing *SDHB* PV carriers namely, penetrance of disease, risk of metastatic progression, risk of developing a second tumour and mortality following a diagnosis of metastatic disease. These outcomes were selected due to their relevance to patients, families and healthcare providers conducting surveillance and treatment.

To our knowledge, this is the first systematic review and meta-analysis to examine penetrance for *SDHB* PV carriers excluding proband/ index cases, to assess the risk of developing second tumour and to report on 5-year mortality of *SDHB* PV carriers with metastatic disease altogether.

We found penetrance of non-proband/ non-index *SDHB* PV carriers rose from 4% at age 20 to 24% by age 60 and to 35% by age 80. For non-proband/ non-index *SDHB* PV carriers who had developed PPGL, overall risk of metastatic progression was 9% per lifetime. Carriers with a history of tumours (proband/ index carriers or non-probands/ non-index carriers) had a 24% risk of a second tumour. Finally, all cause 5-year mortality was 18% for both proband/ index and non-proband/ non-index carriers with metastatic disease.

Our meta-analysis updates the risk of metastatic progression for *SDHB* PV carriers excluding proband/ index cases. The most recent systematic review and meta-analysis of metastatic disease risk in *SDHx* PV carriers by Lee *et al*. differed to our assessment, as they included symptomatic and proband/ index cases (171) while we excluded proband/ index cases in the analysis of risk of metastatic progression for *SDHB* PV carriers who had developed PPGL. Lee *et al*. found a higher risk of 23% of metastatic progression compared to our estimate of 9% in non-proband/ non-index carriers with tumours (171). We speculate finding higher risk of metastatic progression when including proband/ index cases is likely due to delayed diagnosis and therefore a longer period of ‘tumour incubation’. In addition, symptomatic patients may have larger tumours, and tumour size is a risk factor for metastatic progression (52, 68, 129).

Over a decade ago, van Hulsteijn *et al*. reported on the risk of malignant (metastatic) paraganglioma in *SDHB* PV carriers and found the pooled risk in non-index carriers to be 13% (118), broadly in keeping with our risk estimate of 9%. In their systematic review and meta-analysis, the heterogeneity of studies was not stated and the investigators cautioned the generalisability of applying the findings, citing the paucity of cohort studies and high risk of bias (118). Our meta-analysis was strengthened by finding only mild variability between studies reporting metastatic progression, with an I^2 of 33%; and 4 of the 5 studies in our assessment were cohort studies, as compared to 2 of 12 studies in van Hulsteijn *et al*. (118).

Hamidi *et al*. performed a systematic review and meta-analysis of overall mortality following a diagnosis of metastatic disease with subgroup analysis of two studies reporting on *SDHB* PV carriers (72). In our updated analysis we assessed 5-year mortality in three times more participants than this previous study (72). We found lower pooled mortality of 18%, compared to their mortality estimate of 35-55% (72). Changes in surveillance practices and treatment options for patients with advanced disease might account for the decrease in mortality estimates.

There were several limitations of the evidence in the pooled analysis. The I^2 result of 0% for the studies on pooled penetrance at age 20 and risk of developing a second tumour suggests there was potential sampling error (176). While some studies reported their surveillance protocols (19, 20, 51, 52, 59, 60, 129, 175), others did not and differences in follow-up across centres may have modified outcomes reported for *SDHB* PV carriers. Some of the variants in earlier studies have subsequently been reclassified as variants of uncertain significance (VUS) or likely benign. Of 344 *SDHB* PV carriers studied by Jochmanova *et al*., two index cases in total may be VUS and one index case and one non-index carrier without disease may have been likely benign variants (57).

Of 16 *SDHB* PV carriers with disease studied by Srirangalingam *et al*., one may be VUS (59). There was high heterogeneity in the pooled penetrance at age 80. There was also high heterogeneity in the 5-year mortality rate of *SDHB* PV carriers with metastatic disease, and given many of the studies did not describe treatment protocols for carriers with metastatic disease (57, 67, 75, 76, 129), potential treatment differences may have been a factor in variable mortality outcomes. Quality of individual studies included in the systematic review and meta-analysis varied, as shown in the risk of bias assessment. For example, in some studies follow up duration or completeness was unclear at an individual patient level. Therefore in the pooled analysis of age-specific penetrance, we assumed all non-proband/ non-index *SDHB* PV carriers were included in the age-specific penetrance proportions reported in each study. Similarly in the pooled analysis of 5-year mortality, we assumed all patients with metastatic disease were included in the 5-year mortality assessment for each study. While these assumptions would not have affected the penetrance or 5-year mortality proportions, the combined weighting of the studies may have been different. We tried to assess studies that reported on non-proband/ non-index carriers, but there were limited eligible studies to assess of risk of developing a second tumour and 5-year mortality for non-proband/ non-index carriers alone. Six studies included highly selected populations such as *SDHB* PV carriers with advanced or metastatic disease whereas a typical group of *SDHB* PV carriers undergoing surveillance often includes asymptomatic or non-proband/ index carriers. Therefore, while this was the first meta-analysis to assess risk of developing second tumour and 5-year mortality, we acknowledge risk of ascertainment bias as we included proband/ index cases in these analyses.

Limitations to this study included the potential that search terms missed articles that were not indexed under those terms or if they were published in sources not included in the search. To counter this possibility, we also included articles identified in references. Similarly, there was the possibility of language bias; while our search was not limited to the English language, it transpired the articles selected for eligibility were all in English. Inclusion and exclusion criteria used in the review were narrow, but we felt this was appropriate given we were interested in outcomes of this specific cohort.

Surveillance for *SDHB* PV carriers aims to detect PPGL before metastasis has occurred, to facilitate surgical cure (100). Our meta-analysis has potential implications for surveillance approaches among *SDHB* PV carriers. We found that penetrance appears to increase linearly across the lifespan up to and including 80 years of age, suggesting that there is utility in ongoing screening up to 80 years of age. Current guidelines recommend less screening after 70 years of age (100). *SDHB* PV carriers with a history of tumours (either proband/ index or non-proband/ non-index carriers) have a 24% risk of second tumour, so surveillance must continue even following surgical excision of the primary tumour. The frequency of such surveillance requires further research. Moreover, the risk of metastatic progression for non-proband/ non-index carriers is 9%, which although lower than previously estimated for index cases (169) still highlights the need for careful follow-up of these cases. Apart from tumour size (52, 67, 68, 129), risk factors for metastatic progression are not currently well known. As risk factors for metastatic progression are identified in the future, healthcare professionals may wish to consider more frequent surveillance for patients with these risk factors. Finally, our meta-analysis confirms poor prognosis for *SDHB* PV carriers once metastatic disease has occurred, with a 5-year mortality of 18%.

5.5 Conclusion

This review has clinical relevance for genetic counselling and surveillance of all *SDHB* PV carriers. Non-proband/ non-index carriers have a 4% chance of having developed PPGL by age 20 y, rising to 35% by age 80 y. For carriers who develop PPGL, there is a 9% chance of metastatic progression. Since *SDHB* PV carriers who have developed one tumour (probands and non-proband/ non-index cases combined) have a 24% chance of developing a second tumour, follow up surveillance is important even when the first tumour is surgically removed. In the setting of a lifelong risk of developing PPGL and subsequent risks of metastatic progression, developing a second tumour and mortality from disease, our review supports lifelong surveillance as an important recommendation for all centres, as is advocated by clinical practice guidelines (100).

Chapter 6: Cost effectiveness of surveillance for SDHB PV carriers: a Markov model

Preface 6.0

The following manuscript contains a health economic Markov model that assessed the cost effectiveness of annual, 3 and 5 yearly surveillance intervals for *SDHB* PV carriers. It provided an integrated analysis that combined patient-level outcomes, quality of life data and meta-analysis data to inform economic modelling. The references for this manuscript can be found directly at the end of the manuscript.

The manuscript is intended for submission to a peer reviewed journal.

Title Page

Full title:

Cost-effectiveness of surveillance strategies to detect phaeochromocytoma and paraganglioma in asymptomatic Australian *SDHB* pathogenic variant carriers

Short running title:

Cost-effectiveness of surveillance for *SDHB* carriers

Authors:

Dahlia F. Davidoff[1,2,3], Roderick J. Clifton-Bligh[1,2,3], Venessa H. M. Tsang[1,2,3], John R. Burgess[5,6], Richard De Abreu Lourenco[4]

Structured abstract:

Background

Surveillance for carriers of SDHB pathogenic variants (PVs) aims to detect phaeochromocytoma and paraganglioma at a curable stage. Current international guidelines recommend annual biochemical testing with clinical review and imaging every 2 years; in our model, this approach was defined as the base case. The cost-effectiveness of extending surveillance intervals has not been evaluated. Balancing early detection with costs, resource use and patient anxiety associated with screening is crucial for refining recommendations.

Methods

We developed a Markov model to compare the base case, 3-yearly and 5-yearly surveillance strategies incorporating clinical review, plasma metanephrines, whole-body MRI, and ^{68}Ga-DOTATATE PET/CT. Transition probabilities were informed by meta-analyses and patient-level data. Costs, utilities, and disutilities were sourced from a cross-sectional quality of life study and the published literature. Outcomes included average costs, quality-adjusted life years (QALYs) and incremental cost-effectiveness ratios (ICERs) states as cost per QALY, and cost per tumour detected. Deterministic sensitivity analyses explored uncertainty in key parameters, including test performance and disutility from scan-associated anxiety (scanxiety).

Results

The base case cost $69,595 with 14.41 QALYs, compared with $56,538 and 14.35 QALYs for 3-yearly surveillance and $49,458 and 14.29 QALYs for 5-yearly surveillance. ICERs for less frequent strategies exceeded the $50,000/QALY threshold per QALY lost. Results were sensitive to the assumed disutility of the base case; when scan-associated anxiety ("scanxiety") was modelled at 0.038 per year, the base case was dominated by less frequent strategies.

Conclusion

Less frequent surveillance for asymptomatic *SDHB* PV carriers reduced costs and patient burden with potentially acceptable health losses. Incorporating anxiety disutility rendered extended surveillance intervals cost-effective relative to the base case. Results were robust to cost assumptions but sensitive to test accuracy, highlighting the importance of advances in imaging and the need for prospective studies quantifying scanxiety in *SDHB* PV carriers.

Disclosures:

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Disclosures: The authors have nothing to declare.

6.1 Introduction

Patients who are carriers of succinate dehydrogenase type B (*SDHB*) pathogenic variant (PV) are at risk of rare neuroendocrine tumours from a young age (1, 2). These tumours are phaeochromocytomas (PC), which arise from the adrenal medulla, and paragangliomas (PGL) which arise from ganglia in the head and neck (head and neck paraganglioma or HNPGL), thoracic or abdominal (TA) location (1, 2). Tumours may secrete catecholamines, leading to symptoms such as hypertension, palpitations, and sweating (2). Others are biochemically silent and detected incidentally or through surveillance imaging but may cause symptoms through local progression and invasion (3). *SDHB*-related phaeochromocytomas and paragangliomas (PPGL) are often aggressive with a significant potential for metastatic progression (2, 15). Metastatic disease is incurable, requiring life-long systemic therapy and leading to substantial physical and psychological burden in addition to a 5-year mortality of 18% (141). Morbidity arises not only from tumour mass effect and catecholamine excess, but also from repeated interventions and monitoring. Tumours can occur from as young as age 6 y (49, 115). However, penetrance is incomplete, and meta-analysis data suggest that approximately 24% of carriers develop PPGL by age 60 y (141). Surveillance aims to detect tumours early enough for curative surgical resection, preventing metastatic progression and thereby improving long-term outcomes, reducing treatment burden, and preserving quality of life (100).

Biochemical testing with annual assessment of catecholamine metabolites is important to detect PPGL in *SDHB* PV carriers, as PPGL produce and metabolize catecholamines (22, 93). Production of catecholamines is best assessed by measuring O-methylated metabolites, namely free plasma normetanephrine, metanephrine and methoxytyramine respectively, which are commonly elevated in *SDHB*-associated PPGL(93). However biochemically silent tumours remain challenging to recognise without imaging (3, 59). Imaging is therefore a mainstay of surveillance in this population. Computed tomography (CT) and magnetic resonance imaging (MRI) offer high anatomical resolution, with MRI preferred due to the absence of ionising radiation (22, 83). Among functional imaging techniques, ^{68}Ga-DOTATATE PET/CT and ^{18}F- FDG-PET/CT are considered useful tools for detection of small tumours and metastatic disease for *SDHB* PV carriers (88, 90).

Newly detected asymptomatic mutation carriers are recommended to enter lifelong surveillance from as young as age 6 to 10 y (100). Baseline assessment should include clinical history for catecholaminergic symptoms, blood pressure, either plasma or urinary metanephrine and normetanephrine, and MRI of head and neck, thorax, abdomen and pelvis and the option of functional imaging in adulthood (100). Currently, surveillance consists of annual biochemical testing with clinical review, while imaging is performed biennially (every 2 years) (100).

While the base case of annual biochemical testing with clinical review and biennial imaging may provide timely detection of disease, it can also be resource intensive. In addition, there are concerns beyond cost that support moving to less frequent surveillance intervals. One key consideration is the psychological burden associated with repeated imaging known as scan-associated anxiety (scanxiety). Scanxiety is the distress or anxiety that occurs before, during, and after surveillance imaging, encompassing fears about scan procedures, potential results, and disease detection or progression (165, 166). This phenomenon is well-documented in cancer populations, with distress particularly heightened during the waiting period for scan results (165). A scoping review by Derry-Vick *et al*. reported scanxiety prevalence ranging from 34% to 85% in adults living with different types of cancer undergoing follow-up imaging (165). The review included cross-sectional, longitudinal, qualitative, and quantitative studies, but the frequency and timing of follow-up varied and was not consistently reported (165). Similarly, Khatri *et al*. highlighted that even when scans show no disease, the cumulative emotional toll of frequent surveillance can lead to anticipatory anxiety and reduced mental wellbeing (166). Therefore, if longer surveillance intervals can be implemented without increasing the risk of late stage or metastatic disease and while also reducing the cumulative harms of surveillance itself (including scanxiety and potential imaging side effects), they may provide a safer balance between clinical effectiveness and patient quality of life.

Despite international consensus that surveillance is necessary, the cost-effectiveness of alternative surveillance strategies in *SDHB* PV carriers remains uncertain. To date, no studies have comprehensively evaluated the comparative value of different surveillance intervals and imaging strategies in this high-risk population. International guidelines continue to recommend lifelong surveillance consisting of annual biochemical testing with clinical review and biennial imaging (100, 101), yet imaging carries a cumulative burden including costs, radiation exposure and psychological distress such as scanxiety. Conversely, there are circumstances wherein surveillance has low yield: for instance, in subjects younger than 10 y in whom penetrance of PPGL is still low (141); and for subjects with small, solitary HNPGL which are unlikely to metastasize (22). Surveillance might therefore be more precisely tailored to individual risk, to improve safety and cost-effectiveness without compromising sensitivity. Our study therefore aimed to model whether extended intervals could achieve comparable clinical outcomes at reduced cost without compromising clinical and quality of life outcomes.

This study investigated cost-effectiveness of less frequent surveillance (3 and 5 year) compared with the base case, which consisted of annual biochemical testing with clinical review and biennial imaging, for early detection of PPGL in asymptomatic individuals with a germline *SDHB* pathogenic variant using costs and utility values derived from the Australian health system.

6.2 Methods

Model structure

To assess the cost-effectiveness of the base case strategy (annual biochemical testing with clinical review and biennial imaging) compared with 3-yearly and 5-yearly biochemical and imaging surveillance, a decision-analytic model was constructed using R version 4.2.2. The model commenced with patients receiving one of 3 follow-up strategies: (1) base case: routine annual biochemistry and biennial imaging, (2) 3-yearly biochemistry and imaging, and (3) 5-yearly biochemistry and imaging. All strategies included specialist clinical review, plasma metanephrines and whole-body MRI at the time of surveillance in accordance with standard clinical practice(100). For the 3-yearly and 5-yearly strategies, clinical review, biochemical testing and imaging were performed at the same interval; annual clinical assessment or biochemical testing was not modelled independently of imaging. ^{68}Ga-DOTATATE PET/CT was assumed to be performed in adults every 5 (for base case or 5-yearly surveillance) or 6 years (3-yearly surveillance) as no consensus was available in current guidelines (100). MRI and ^{68}Ga-DOTATATE PET/CT were modelled as independently scheduled investigations, with each modality occurring at its specified interval and not synchronised within the same model year unless their cycles coincided by chance.

Stage 1 of the model represented the 3 surveillance strategies as distinct alternatives to determine disease status (Figure 6.1). The model began with asymptomatic *SDHB* PV carriers with no detected disease. Outputs from the model, detected tumours, were categorised as true positive (TP) with alternative outcomes categorised as false negative

(FN), false positive (FP) or true negative (TN). Based on those outputs, individuals in the model were characterised into no detected disease state or no surgical remission state.

Individual disease/carrier status in Stage 1 determined the health state in which they entered a subsequent Markov model. Stage 2 estimated costs and outcomes for ongoing condition management (Figure 6.2). Health states included no detected state, no surgical remission state, surgical remission state, metastatic disease and dead.

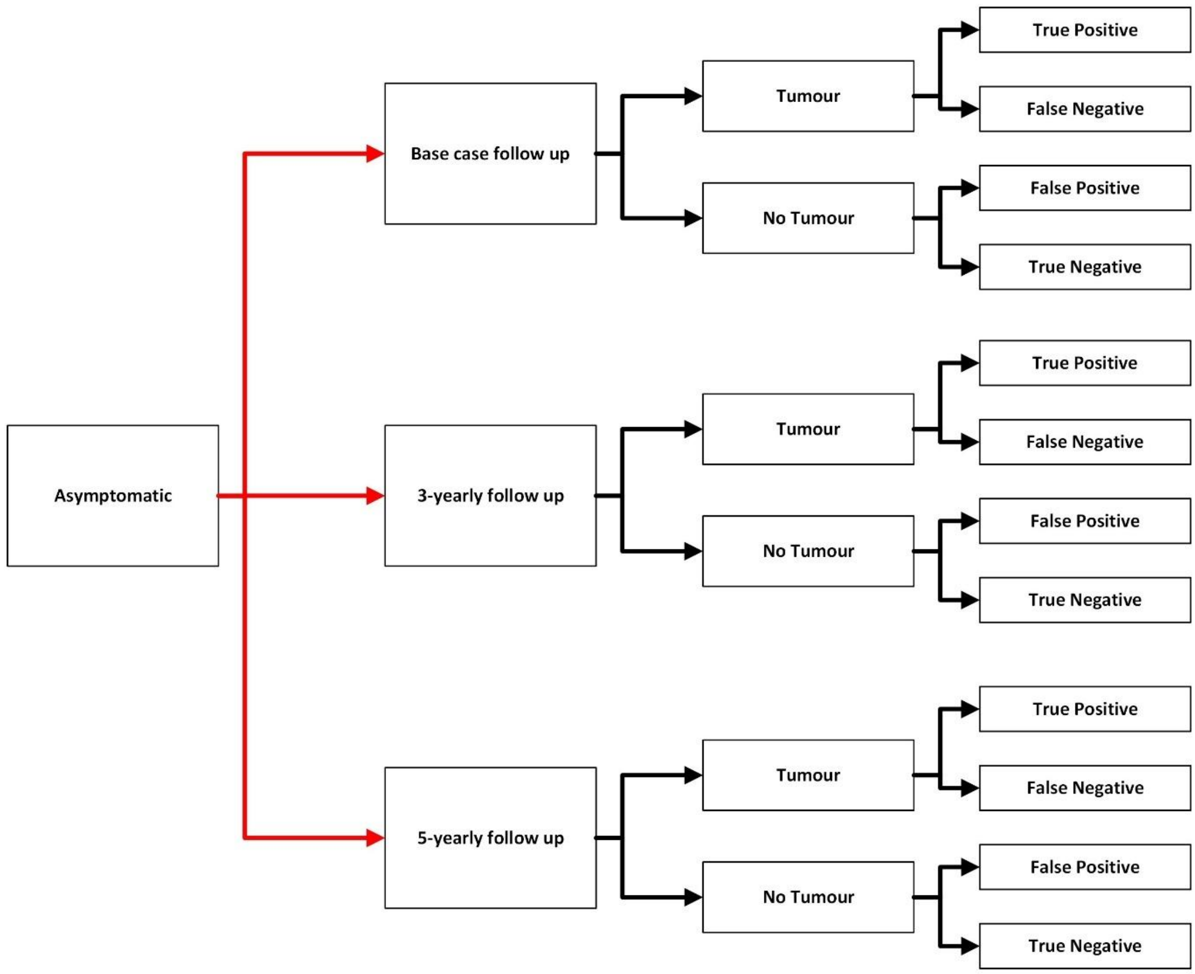

Figure 6.1 Stage 1 of the decision tree

Asymptomatic *SDHB* PV carriers with no detected disease received one of 3 follow-up strategies: (1) base case: routine annual biochemistry and biennial imaging, (2) 3-yearly biochemistry and imaging, and (3) 5-yearly biochemistry and imaging. Detected tumours were categorised as true positive (TP) with alternative outcomes categorised as false negative (FN), false positive (FP) or true negative (TN). Based on those outputs, individuals in the model were characterised into no detected state or no surgical remission state.

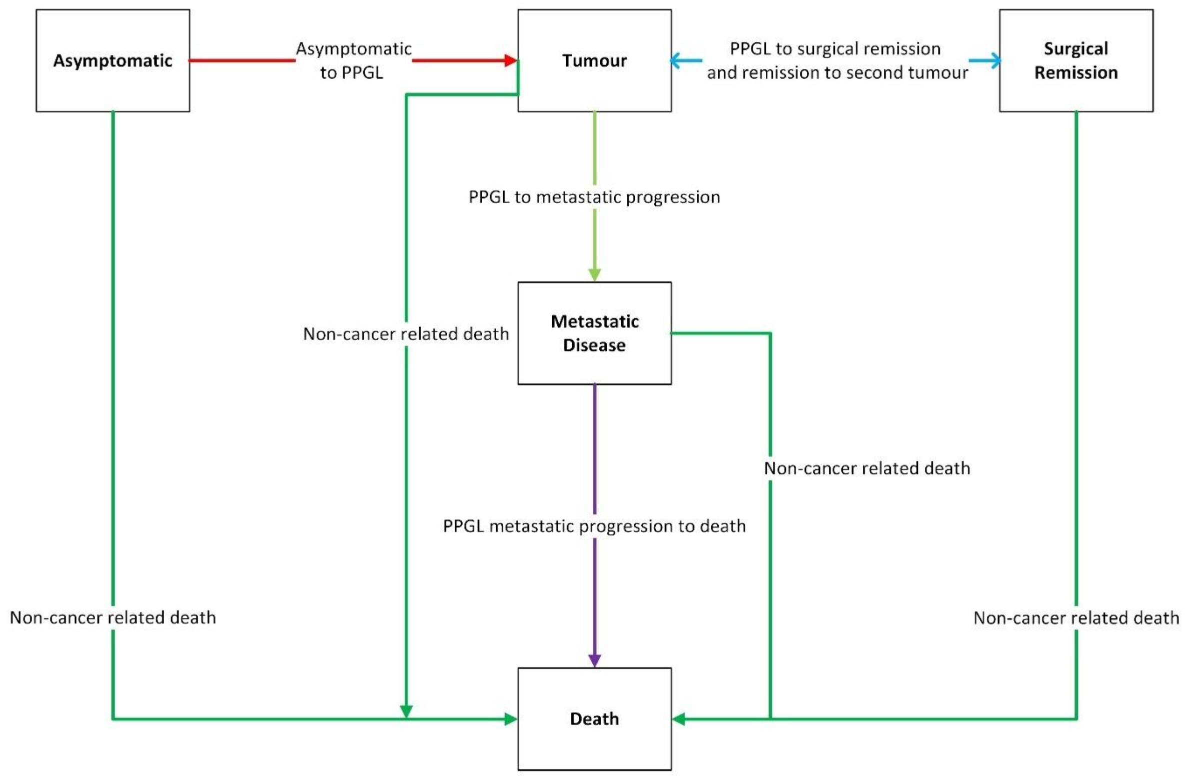

Figure 6.2 Stage 2 of the decision tree

Ongoing disease states were characterised as no detected state, no surgical remission state, surgical remission state, metastatic disease and dead. Asymptomatic *SDHB* PV carriers who had tumour detected could enter into surgical remission. Once carriers were in surgical remission there was potential for locoregional recurrence of tumour. Detected tumour could develop metastatic progression. Metastatic disease could progress to death from disease. At any stage *SDHB* PV carriers could die from non-cancer related death.

Asymptomatic *SDHB* PV carriers who had tumour detected could enter into surgical remission. Once carriers were in surgical remission there was potential for locoregional recurrence of tumour. Detected tumour could develop metastatic progression. Metastatic disease could progress to death from disease. At any stage *SDHB* PV carriers could die from non-cancer related death.

A lifetime time horizon was applied. The model cycle length was one year. Both costs and health outcomes were discounted at 5% annually.

<u>Patients and data source</u>

Surveillance practices and costs were obtained from our multicentre cohort study of *SDHB* PV carriers attending genetics clinics at Royal North Shore Hospital, Prince of Wales Hospital, and Royal Hobart Hospital, all located in Australia (129). Data were collected retrospectively and prospectively from medical records (129). Surveillance protocols at each site included annual clinical review, biochemical testing, biennial MRI, and functional imaging as clinically indicated (129). Data for the probabilities of health states were from our multicentre cohort study and our systematic review and meta-analysis (141).

Data for quality of life estimates were sourced from our cross-sectional observational study of health-related quality of life (HRQoL) in *SDHB* PV carriers attending genetics clinics at Royal North Shore Hospital and Royal Hobart Hospital (129, 177). Patients with confirmed *SDHB* PVs were invited to complete the EuroQol 5 Dimensions 5 Levels (EQ5D5L) and European Organisation for Research and Treatment Quality of Life Questionnaire (EORTC QLQ-C30) questionnaires during a surveillance episode (177). Data for quality of life in metastatic disease estimated based on the CLARINET trial (178).

Model assumptions

In the model, PPGL were identified through surveillance that included physical examination, plasma metanephrines, whole-body MRI and ^{68}Ga-DOTATATE PET/ CT. ^{68}Ga-DOTATATE PET/ CT imaging were used as baseline PET/ CT imaging based on current international guidelines (100) and ^{18}F-FDG-PET/CT was explored in sensitivity analyses given its use in our cohort. Patients in the no detected disease state incurred costs related to medical appointments, biochemistry and imaging tests (Table 6.1). If tumours were detected but not surgically cured patients entered the no surgical remission state, where costs reflected continued appointments, biochemical testing, imaging and pharmacotherapies. Patients who achieved surgical remission state accrued costs from surgery, pharmacotherapy, medical appointments, biochemical testing and imaging. Progression to metastatic disease was associated with costs from pharmacotherapies, chemotherapy, radiotherapy, nuclear medicine therapy alongside medical appointments, biochemical testing and imaging surveillance. Longer surveillance intervals were assumed to reduce the likelihood of surgical remission due to larger tumour size at detection and to increase the risk of metastatic disease at diagnosis due to later-stage presentation.

Table 6.1 Cost inputs for the model, 2025 Australian dollars

Test	Site	Cost (AUD)	Source
Medical appointments			
Clinical review	na	87.30	MBS #116
Biochemistry			
Urinary catecholamines	Urine	39.95	MBS #66779
Plasma catecholamines	Blood	39.95	MBS #66779
Chromogranin A	Blood	32.10	Non-MBS (private)(179)
Imaging and diagnostic procedures			
Ultrasound	Neck	122.40	MBS #55032
Ultrasound	Echocardiogram	258.70	MBS #55126
Ultrasound	Abdomen	124.70	MBS #55036
Ultrasound	Renal tract	51.60	MBS #58700
Ultrasound	Pelvis	110.20	MBS #55065
Xray	Abdomen	40.10	MBS #58900
Xray	Pelvis	68.30	MBS #57715
CT	Brain	218.75	MBS #56001
CT	Neck	257.85	MBS #56101
CT	Chest	330.80	MBS #56301
CT	Abdomen	280.35	MBS #56401
CT	Abdomen and Pelvis	431.60	MBS #56501
CT	Neck, chest, abdomen and pelvis	523.25	MBS #57001
MRI	Skull base	441.45	MBS #63007
MRI	One region spine	392.40	MBS #63154
MRI	Full spine	490.50	MBS #63204
^{68}Ga-DOTATATE PET	Whole Body	953.00	MBS #61647
^{123}I-MIBG scitigraphy	Whole Body	1,523.92	Historic MBS pricing (180)
^{18}F-FDG-PET	Whole Body	953.00	MBS #61604
Octreoscan	Whole Body	2,015.75	MBS #61369
Cystoscopy with biopsy		261.90	MBS #36836
Gastroscopy		201.75	MBS #30473
Surgery			
Surgery D02A and D02C costed weights (HEAD & NECK INTERVENTIONS)	Head and neck	20,412.15	IHACPA
Surgery D14A and D14B costed weights (MOUTH&SALIVRY GLAND INTERVTN)	Mouth and salivary gland	5,432.43	IHACPA
Surgery E01A and E01C costed weights (MAJOR CHEST INTERVENTIONS)	Chest	27,029.72	IHACPA

Surgery E71A and E71B costed weights (RESPIRATORY NEOPLASMS)	Chest	8,072.13	IHACPA
Surgery F19A and 19B costed weights (TRNS-VSCLR PERC CRDC INTERVTN	Chest	17,586.60	IHACPA
Surgery G60A and G60B costed weights (DIGESTIVE MALIGNANCY)	Abdomen	6,129.46	IHACPA
Surgery H01A and H01B costed weights (PANCREAS,LIVER&SHUNT INTERVTN)	Abdomen	28,883.85	IHACPA
Surgery I09A and I09B costed weights (SPINAL FUSION)	Spine	32,658.29	IHACPA
Surgery I10A and I10B costed weights (OTHER BACK & NECK INTERVTN)	Spine	14,832.79	IHACPA
Surgery I65A and I65B costed weights (MUSCULOSK MALIG NEOPLASM)	Spine	10,420.97	IHACPA
Surgery K03Z costed weight (ADRENAL INTERVENTIONS)	Abdomen	20,769.00	IHACPA
Surgery L62A and L62C costed weights (KDNY&UNRY TR NEOPLASMS)	Abdomen	4,034.82	IHACPA
Surgery costed weight Q01Z Splenectomy	Abdomen	25,713.65	IHACPA
Pharmacotherapies			
Amitriptyline 10mg oral, box of 50		21.60	PBS
Capecitabine 500 mg oral, box of 120		31.60	PBS
Dexamethasone 4mg oral, box of 30		23.61	PBS
Duloxetine 30mg oral, box of 28		22.63	PBS
Everolimus 10mg oral, box of 30		1,738.38	Non-PBS (private)
Granisetron 2 mg oral, box of 5		31.60	PBS
Ibuprofen 400mg oral, box of 90		26.44	PBS
Imatinib 400mg oral, box of 30		31.60	PBS
Lanreotide 60mg injection, box of 4		31.60	PBS
Lanreotide 90mg injection, box of 4		31.60	PBS
Metoclopramide 10mg oral, box of 100		29.13	PBS

Metoprolol 25mg oral, box of 100		21.07	PBS
Morphine 7.5mg oral liquid		31.60	PBS
Octreotide 50microgram subcutaneous, box of 90		31.60	PBS
Oxycodone 5mg oral, box of 20		27.13	PBS
Oxycodone 20mg oral, box of 56		31.60	PBS
Paracetamol 1000mg oral, box of 100		7.70	PBS
Phenoxybenzamine 10mg oral, box of 100		31.60	PBS
Pregabalin 75mg oral, box of 56		23.45	PBS
Ripretinib 50mg oral, box of 90		31.60	PBS
Sunitinib 25mg oral, box of 28		1,150.57	Non-PBS (private)
Sunitinib 50mg oral, box of 42		31.60	PBS
Sunitinib 50mg oral, box of 42		2255.28	Non-PBS (private)
Tapentadol 100mg oral, box of 28		31.60	PBS
Zoledronic acid 4mg infusion		31.60	PBS
Radiotherapy			
Radiation Oncology-Megavoltage planning		725.45	MBS #15902
Radiation Oncology-Megavoltage treatment		91.25	MBS #15930
Nuclear medicine therapy			
Lutate cost per cycle		8,000	RNSH contract pricing (historic)
Chemotherapy			
5-Fluorouracil 5 g injection		31.60	PBS
Portacath insertion for chemotherapy		405.30	MBS #35317
Temozolomide 100 mg oral, box of 5		31.60	PBS

^{18}F-FDG-PET/CT fluorodeoxyglucose (18F) positron emission tomography/computed tomography; ^{68}Ga-DOTATATE PET/CT Gallium-68 DOTATATE PET CT; ^{123}I-MIBG scintigraphy Iodine-123 meta-iodobenzylguanidine scintigraphy; AUD Australian dollars; CT computed tomography; IHACPA Independent Health and Aged Care Pricing Authority; Lutate [177Lu]Lu-octreotate; MBS medicare benefit scheme; MRI magnetic resonance imaging; na not applicable; PBS pharmaceutical benefit scheme; RNSH Royal North Shore Hospital

Data required for the model

Transition probability data

Probabilities of transition between health states were obtained from published literature including our cohort study (129, 141). The probability of transitioning from no detected state to no surgical remission state was based on age cohort data for prevalence and lead-in times to diagnosis. Age-specific prevalence based on meta-analysis data was 3.8% prevalence by age 20 y, 11.2% prevalence by age 40 y, 24.2% prevalence by age 60 y, 34.9% prevalence by age 80 y, 37.6% by age 85 y (141). Age-specific average lead-in times to diagnosis based on our cohort study were 8.0 years by age 20 y, 7.9 years by age 40 y, 6.5 years by age 60 y, 4.5 years by age 80 y and 4.5 years by age 85 y (129).

The probability of transition from no surgical remission state to surgical remission state was based on average tumour size in our cohort, the expected tumour doubling time of 7 years, and data that tumours greater than 40 mm were less likely to be surgically cured (69, 129). The probability of transitioning from surgical remission into no surgical remission was based on meta-analysis data (141).

The probability of transitioning from no surgical remission state to metastatic disease and from metastatic disease to death from disease were obtained from 5 and 10-year metastatic progression data in our cohort study (129). The probability of transitioning from metastatic disease to death from disease were obtained from 5 and 10-year survival data in our cohort study (129). The probability of non-cancer related death was from the Australian Bureau of Statistics (181).

A lifetime time horizon was applied, with age 90 y as the maximum follow-up age in the model. Therefore the number of years in the model varied by age group. Younger cohorts such as those age 10-20 y had a longer duration of follow-up while older cohorts such as those age 85 y were followed for a shorter duration.

Test Accuracy data

Diagnostic performance was based on data from our cohort study (129). The following diagnostic accuracy values were used:

- Plasma metanephrines: Sensitivity 72.7%, Specificity 96.4%
- Whole-body MRI: Sensitivity 82.6%, Specificity 98.3%
- ^{68}Ga-DOTATATE PET/CT: Sensitivity 93.3%, Specificity 100%
- ^{18}F-FDG-PET/CT: Sensitivity 100%, Specificity 100%

Cumulative sensitivity for each strategy and age group was calculated by combining the sensitivities of the tests included in that strategy, weighted by how often each test was performed. For example, in the base case strategy metanephrines were done every year, MRI every 2 years and PET every 5 years, while in the 3-yearly strategy tests were done once every 3 years and PET every 6 years. These frequencies were used to give a weighted average sensitivity for each strategy.

Healthcare Resource Use and Costs

Costs of each health state were estimated as mean annual per-patient costs, based on data from our cohort study of 181 *SDHB* PV carriers, of whom 148 were non-probands (129) (Table 6.2).

Table 6.2 Mean costs for health states in the model

	Asymptomatic	Surgical remission	No surgical remission	Metastatic disease
Cost (AUD)	796.92	17,938.14	3,885.02	11,804.62

AUD Australian dollars

Costs were valued using Australian health system sources, including the Medicare Benefits Schedule, Pharmaceutical Benefits Scheme and Australian Refined Diagnosis Related Groups, and stated in 2025 Australian dollars.

Health Outcomes

Utility values for the no detected, no surgical remission, and surgical remission states were derived from our cross-sectional HRQoL study, while the metastatic disease utility was estimated using proportional disutility from the CLARINET trial (177, 178). In that study, HRQoL was assessed in patients with neuroendocrine tumours, comparing stable disease with progressive disease. Meng *et al.* described several approaches for modelling this decrement and found that progression was associated with a mean utility reduction of 0.05. We applied this proportional decrement to the stable state utility values from our *SDHB* PV cohort to derive an estimated utility for the metastatic disease state in the model (Table 6.3).

Table 6.3 Mean utilities based on the EQ5D5L questionnaire

	Asymptomatic	Surgical remission	No surgical remission	Metastatic disease
EQ5D5L utility score, mean	0.804	0.712	0.712*	0.662**
Source	Davidoff *et al.* 2025 (177)	Davidoff *et al.* 2025 (177)	Davidoff *et al.* 2025 (177)	Meng *et al.* 2017 (178)

EQ5D5L EuroQol 5 Dimensions 5 Levels * Assume same utility as surgical remission as insufficient participants in this health state; ** Proportion disutility based on the CLARINET trial (Meng *et al*. 2017). Meng *et al.* described different ways of modelling the decrement in (health-related quality of life) HRQoL utility between stable and progressive neuroendocrine tumour. They found utility decreased by 0.05.

Analysis

The model estimated the cost per case of tumour identified for each surveillance strategy and the longer-term cost per quality adjusted life year (QALY). Incremental cost-effectiveness ratios (ICERs) were calculated for the 3 and 5 year strategies relative to the base case. Analyses were conducted separately in each age cohort and the overall result was obtained based on the proportion of patients who were in each age group from our cohort (129). We assumed an indicative willingness to pay (WTP) threshold of $50,000 per QALY gained.

Sensitivity Analyses

Deterministic sensitivity analyses were conducted by testing the impact on the ICER of varying a wide range of model parameters, including PET type (^{18}F-FDG-PET/CT versus ^{68}Ga-DOTATATE PET/CT), tumour type (HNPGL, TA PPGL), test sensitivity and specificity, disease prevalence, remission probability, metastatic progression, mortality, surveillance frequency, unit costs, and utility values. Given that our cohort observed 100% sensitivity and specificity for ^{18}F-FDG-PET/CT, which likely reflected small sample size, we ran a sensitivity analysis using more conservative estimates from Saie *et al*. (sensitivity 87%, specificity 99%) (83). We tested scenarios where extending the surveillance interval either reduced or increased the number of tumours detected. Finally, we modelled a scenario where the base case surveillance caused disutility (a reduction in the quality of life) in asymptomatic carriers due to scanxiety, whereas less frequent surveillance was assumed to cause less disutility.

Ethics and Dissemination

Ethics approval was obtained from the Northern Sydney Local Health District Ethics Committee for Royal North Shore and Prince of Wales Hospitals (Ref: 2019/ETH09870) and from the Tasmania Health and Medical Human Research Ethics Committee for the Royal Hobart Hospital (Ref: H0018520). The study was conducted and reported in line with the CHEERS checklist (182).

6.3 Results

Base Case Analysis

Primary cost-effectiveness analysis demonstrated the base case of annual clinical review and plasma metanephrines with 2-yearly whole-body-MRI and 5-yearly ^{68}Ga-DOTATATE PET/CT cost an average of $69,595 with a QALY of 14.41 and a cost per case of tumour appropriately identified of $2,518,582. Three-yearly surveillance with the same modalities cost an average of $56,538 with a QALY of 14.35 and a cost per case of tumour appropriately identified of $2,046,060 and 5-yearly surveillance with the same modalities cost an average of $49,458 with a QALY of 14.29 and a cost per case of tumour appropriately identified of $1,789,845 (Table 6.4).

Table 6.4 Base Case Analysis

	Base case	3-yearly surveillance	5-yearly surveillance
Cost (AUD)	$69,595	$56,538	$49,458
Effectiveness (QALYs)	14.41	14.35	14.29
Cost per tumour detected (AUD)	$2,518,582	$2,046,060	$1,789,845
ICER	Reference group	$217,135 per QALY lost	$160,488 per QALY lost

AUD Australian dollars; ICERs incremental cost-effectiveness ratios; QALYs quality adjusted life years

The disaggregated results showed differences in average costs and QALYs per patient across strategies and health states. For the base case strategy, average costs per patient were $11,142.73 for the no detected state, $5,450.69 for no surgical remission, $48,945.18 for surgical remission and $4,056.38 for metastatic disease. Corresponding average QALYs per patient were 11.24, 1.00, 1.94, and 0.23 respectively. For the 3-yearly strategy, average costs per patient were $3,714.24 (no detected), $6,360.82 (no surgical remission), $40,417.08 (surgical remission) and $6,045.81 (metastatic disease). QALYs for this strategy were 11.24, 1.17, 1.60, and 0.34 respectively. For the 5-yearly strategy, average costs per patient were $2,228.55 (no detected), $7,209.36 (no surgical remission), $31,846.29 (surgical remission) and $8,173.88 (metastatic disease). QALYs were 11.24, 1.32, 1.26, and 0.46 respectively across the 4 non-death health states.

The ICER for 3-yearly surveillance was $217,135 per QALY lost, and $160,488 per QALY lost in 5-yearly surveillance, both for the comparison with base case surveillance. These ICERs exceeded the nominal WTP threshold of $50,000 per QALY gained; the analysis did not nominate a willingness to accept threshold.

Sensitivity analysis

Results from our sensitivity analyses and one way sensitivity analysis are shown in Table 6.5 and Table 6.6 respectively. Results were sensitive to the parameter "disutility from frequent surveillance". We determined that the disutility cut-off to achieve an ICER under the WTP threshold was 0.038. When we assumed a disutility of 0.038, both 3- and 5-yearly surveillance strategies dominated base case surveillance.

Table 6.5 Sensitivity analysis

Sensitivity analysis	Strategy	Cost (AUD)	QALY	ICER
Base case	base	69,595	14.41	
Base case	three	56,538	14.35	217,135
Base case	five	49,458	14.29	160,488
^{18}FDG-PET/CT	base	70,667	14.40	
^{18}FDG-PET/CT	three	57,547	14.34	213,715
^{18}FDG-PET/CT	five	50,367	14.27	158,470
Lower sensitivity ^{18}FDG-PET/CT	base	68,573	14.42	
Lower sensitivity ^{18}FDG-PET/CT	three	55,576	14.36	220,521
Lower sensitivity ^{18}FDG-PET/CT	five	48,592	14.30	162,483
Low detection probability for the 3- and 5-yearly strategies	base	69,595	14.41	
Low detection probability for the 3- and 5-yearly strategies	three	48,568	14.40	665,014
Low detection probability for the 3- and 5-yearly strategies	five	42,279	14.37	695,469
High detection probability for the 3- and 5-yearly strategies	base	69,595	14.41	
High detection probability for the 3- and 5-yearly strategies	three	63,693	14.28	46,178
High detection probability for the 3- and 5-yearly strategies	five	55,903	14.21	67,464
Disutility from frequent surveillance 0.038	base	69,595	13.88	
Disutility from frequent surveillance 0.038	three	56,538	14.35	Dominant
Disutility from frequent surveillance 0.038	five	49,458	14.29	Dominant
HNPGL low metastatic potential	base	69,595	14.41	
HNPGL low metastatic potential	three	56,800	14.37	346,075
HNPGL low metastatic potential	five	49,765	14.35	307,234

* sensitive result. HNPGL head and neck paraganglioma

Table 6.6 One way sensitivity analysis

Parameter	Bound	Strategy	Cost (AUD)	QALY	ICER
p_detect	min	base	63,418	14.46	
p_detect	min	three	50,726	14.41	239,436
p_detect	min	five	44,223	14.35	173,558
p_detect	max	base	75,295	14.37	
p_detect	max	three	61,902	14.30	200,429
p_detect	max	five	54,290	14.23	150,638
surv	min	base	64,038	13.88	
surv	min	three	51,660	13.82	226,545
surv	min	five	45,126	13.76	165,898
surv	max	base	76,690	15.06	
surv	max	three	62,786	14.99	207,188
surv	max	five	55,010	14.92	154,821
p_remission	min	base	68,529	14.41	
p_remission	min	three	55,444	14.35	210,584
p_remission	min	five	48,433	14.28	156,205
p_remission	max	base	70,568	14.41	
p_remission	max	three	57,553	14.35	223,437
p_remission	max	five	50,425	14.29	164,522
p_metastatic	min	base	69,617	14.41	
p_metastatic	min	three	56,579	14.35	226,804
p_metastatic	min	five	49,490	14.29	167,348
p_metastatic	max	base	69,573	14.41	
p_metastatic	max	three	56,497	14.35	208,403
p_metastatic	max	five	49,426	14.28	154,293
p_metastatic_mort	min	base	70,028	14.43	
p_metastatic_mort	min	three	57,020	14.39	352,339
p_metastatic_mort	min	five	49,811	14.35	249,812
p_metastatic_mort	max	base	70,025	14.43	
p_metastatic_mort	max	three	56,935	14.39	304,166
p_metastatic_mort	max	five	49,677	14.34	216,080
cost_no_detected	min	base	68,481	14.41	
cost_no_detected	min	three	56,167	14.35	204,782
cost_no_detected	min	five	49,235	14.29	153,383
cost_no_detected	max	base	70,709	14.41	
cost_no_detected	max	three	56,909	14.35	229,488
cost_no_detected	max	five	49,681	14.29	167,592
cost_no_surgical_remission	min	base	66,870	14.41	
cost_no_surgical_remission	min	three	53,358	14.35	224,703
cost_no_surgical_remission	min	five	45,853	14.29	167,496
cost_no_surgical_remission	max	base	72,320	14.41	
cost_no_surgical_remission	max	three	59,718	14.35	209,567

cost_no_surgical_remission	max	five	53,063	14.29	153,480
cost_surgical_remission	min	base	66,332	14.41	
cost_surgical_remission	min	three	53,843	14.35	207,679
cost_surgical_remission	min	five	47,335	14.29	151,402
cost_surgical_remission	max	base	72,858	14.41	
cost_surgical_remission	max	three	59,233	14.35	226,591
cost_surgical_remission	max	five	51,581	14.29	169,574
cost_metastatic_disease	min	base	69,145	14.41	
cost_metastatic_disease	min	three	55,867	14.35	220,804
cost_metastatic_disease	min	five	48,552	14.29	164,127
cost_metastatic_disease	max	base	70,045	14.41	
cost_metastatic_disease	max	three	57,208	14.35	213,466
cost_metastatic_disease	max	five	50,365	14.29	156,848
utility_no_detected	min	base	69,595	13.55	
utility_no_detected	min	three	56,538	13.49	217,135
utility_no_detected	min	five	49,458	13.42	160,488
utility_no_detected	max	base	69,595	15.28	
utility_no_detected	max	three	56,538	15.22	217,135
utility_no_detected	max	five	49,458	15.15	160,488
utility_no_surgical_remission	min	base	69,595	14.23	
utility_no_surgical_remission	min	three	56,538	14.14	145,112
utility_no_surgical_remission	min	five	49,458	14.05	109,951
utility_no_surgical_remission	max	base	69,595	14.59	
utility_no_surgical_remission	max	three	56,538	14.56	431,099
utility_no_surgical_remission	max	five	49,458	14.52	296,996
utility_surgical_remission	min	base	69,595	14.20	
utility_surgical_remission	min	three	56,538	14.18	544,008
utility_surgical_remission	min	five	49,458	14.15	379,734
utility_surgical_remission	max	base	69,595	14.62	
utility_surgical_remission	max	three	56,538	14.52	135,636
utility_surgical_remission	max	five	49,458	14.42	101,744
utility_metastatic_disease	min	base	69,595	14.32	
utility_metastatic_disease	min	three	56,538	14.22	124,638
utility_metastatic_disease	min	five	49,458	14.10	92,440
utility_metastatic_disease	max	base	69,595	14.50	
utility_metastatic_disease	max	three	56,538	14.49	842,024
utility_metastatic_disease	max	five	49,458	14.47	608,182

AUD Australian dollars; ICER incremental cost effectiveness ratio; QALY quality adjusted life years

6.4 Discussion

This is the first study to model surveillance interval cost-effectiveness for asymptomatic *SDHB* PV carriers. We utilised patient-level and meta-analysis data to determine the cost-effectiveness of alternative surveillance strategies for *SDHB* PV carriers. The base case surveillance was more expensive but offered marginal gains in QALYs compared to 3- or 5-yearly surveillance. Cost per tumour detected was lowest for 5-yearly surveillance. Although 5-yearly surveillance was the least costly strategy it may have resulted in more late stage and metastatic disease, potentially presenting a loss in QALYs. This pattern was consistent across several sensitivity analyses, with longer intervals leading to more time spent in worse health states. Even when test sensitivity varied disease could not be detected if surveillance was delayed. However when we assumed a disutility of 0.038 or higher per year from anxiety of more frequent base case surveillance, this was sufficient to offset any potential increase in metastatic disease from a QALY perspective.

Cost-effectiveness analyses in other hereditary cancer syndromes, such as BRCA1/2, familial adenomatous polyposis (FAP) and Lynch syndrome (HNPCC) have largely focused on risk-reducing surgery rather than surveillance intervals (108-110, 183). In FAP, Greenblatt *et al*. modelled prophylactic surgery for duodenal cancer and found that pancreaticoduodenectomy performed at Spigelman stage IV duodenal polyposis was the most cost-effective strategy compared with surgery at cancer diagnosis (109). In Lynch syndrome, Alblas *et al*. modelled prophylactic hysterectomy, showing a 98% reduction in endometrial cancer incidence and a gain of 0.5 QALYs per woman when performed between age 40-80 y, with earlier surgery reducing QALYs due to premature menopause (110). For BRCA1/2, risk-reducing salpingo-oophorectomy and prophylactic mastectomy were consistently cost-effective, particularly when timed in the early 40s and combined with imaging surveillance (108, 183). Overall in BRCA, FAP and Lynch syndrome models, earlier and more aggressive interventions were often favoured despite QALY trade-offs. In contrast for *SDHB* PV carriers, extending surveillance intervals appeared cost-effective and could have dominated the base case strategy if the anxiety burden was substantial.

A key benefit of changing from base case to 5-yearly surveillance was a potential reduction in anxiety. This burden can partly be explained by scanxiety, defined as distress occurring before, during and after cancer-related imaging (165). It is linked to fears about the procedure and the potential results and is a well-recognised cause of reduced HRQoL in people with risk of cancer. Multiple studies have shown associations between scanxiety and poorer HRQoL.

Derry-Vick *et al.* summarised findings from 36 studies investigating the occurrence of scanxiety and noted consistent associations with somatic symptoms and poorer quality of life (QoL) (165). For some it hindered engagement in follow-up care. The waiting period for results was frequently described as the most stressful time and distress often recurred with each surveillance cycle (165). In a study of patients with non small cell lung cancer, Bauml *et al*. found that 83% reported some degree of scan-associated distress using the Impact of Event Scale 6 (IES-6) Instrument (184). Severity of distress was significantly associated with lower QoL, with each unit increase in IES-6 score linked to approximately one unit decrease in the Functional Assessment of Cancer Therapy Lung (FACT-L) score ($p = 0.004$) (184). The Oizys study in Greece utilised the Impact of Event Scale-Revised (IES-R) and reported that 71% of metastatic cancer patients experienced scanxiety with symptoms often peaking while awaiting results (167). Scanxiety was significantly associated with decreased QoL in patients (p = 0.004) (167). In a multicentre survey, Bui *et al*. reported that 55% of patients with advanced cancers experienced scanxiety assessed by a self-reported survey and a distress thermometer (185). Distress was most intense when waiting for scan results and those with higher state anxiety or fear of progression reported greater severity (185). Participants described how scans placed their lives on hold, exacerbated feelings of despair, and had a tangible negative effect on wellbeing (185). Although these studies did not report utility values, the frequency and severity of scanxiety suggested that the disutility of 0.038 per year assumed in our model was plausible.

Petrou *et al*. highlighted challenges in interpreting cost-effectiveness results fall into the South-West quadrant of the cost-effectiveness plane, where costs are lower but some health benefits are lost (186). In this setting, standard WTP thresholds provided little guidance and decisions instead required weighing trade-offs between health losses and cost reduction (186). In our study the assumed disutility of 0.038 per year from scanxiety provided a rationale for adopting less frequent surveillance, as it converted the modest health loss into a net QALY gain. This highlighted scanxiety as a meaningful loss of quality of life for patients at risk of cancer.

Our findings suggested that less frequent surveillance strategies for asymptomatic *SDHB* PV carriers reduced costs and patient burden with only small losses in health outcomes. The direction of effect appeared consistent across contexts. However, the precise choice of interval in other countries would depend on local imaging costs, service capacity and WTP thresholds. Importantly, when base case surveillance was associated with a disutility of 0.038 or higher from scanxiety, less frequent strategies become cost-effective or even dominant.

Our sensitivity analyses indicated that results were relatively robust to cost assumptions but sensitive to test accuracy. Advances in imaging technology that improve sensitivity and specificity would strengthen the value of surveillance overall, by detecting more tumours earlier. However higher accuracy may also narrow the differences between base case and less frequent strategies, potentially narrowing the relative benefit of shortening intervals. If novel imaging modalities deliver greater accuracy with lower patient burden (such as reduced radiation or faster testing times), the balance may shift in favour of less frequent surveillance by reducing disutility and minimising missed tumours.

This study had several limitations. As with all modelling studies, results depended on assumptions about transition probabilities, test accuracy, costs and utilities. Although we varied these in sensitivity analyses residual uncertainty remained. Utility values were derived from our cross-sectional quality of life study and did not capture longitudinal changes, while metastatic disease utilities were adapted from external sources rather than directly measured in *SDHB* PV carriers. The model assumed perfect adherence to surveillance across all strategies, however differential adherence by surveillance intensity could influence both costs and outcomes. The model was based on Australian costs, limiting generalisability to other health systems. In our model, less frequent surveillance translated into potential QALY gains by reducing anxiety burden. However, this remained an assumption as direct data on the impact of different surveillance intervals on anxiety in *SDHB* PV carriers are not available. The extent to which less frequent imaging truly improved quality of life was uncertain and required prospective evaluation. Finally we were unable to address a clinically relevant question: if a carrier remained tumour-free into later life (such as beyond age 60 or 70 y), could surveillance intervals be safely extended or even ceased? Testing this would require data on conditional probabilities of remaining disease-free after multiple negative cycles, which are currently unavailable.

6.5 Conclusion

This study provides the first cost-effectiveness analysis of surveillance intervals for asymptomatic *SDHB* PV carriers. We found that extending surveillance from the base case to 3- or 5-yearly intervals reduced costs and patient burden with only small losses in health outcomes. The incorporation of disutility from scanxiety highlighted that less frequent surveillance could become cost-effective or even dominant when patient anxiety is significant. While results were robust to cost assumptions, they were sensitive to test accuracy, underscoring the importance of advances in imaging technology. Further research should prospectively quantify the anxiety burden of base case surveillance in asymptomatic *SDHB* PV carriers, as this will be critical to refining cost-effectiveness estimates and informing guideline recommendations. In addition, studies addressing conditional risks in older tumour-free carriers are needed to guide decisions on whether, and when, surveillance can be safely spaced or ceased.

Chapter 7: Discussion

7.1 Overview

Phaeochromocytomas and paragangliomas (PPGL) are rare neuroendocrine tumours that may secrete catecholamines or present silently (2, 3). Germline PVs in the *succinate dehydrogenase* (*SDHx*) gene family are the commonest predisposition drivers and occur in 10-15% of all PPGL cases (2, 13). Carriers of a *succinate dehydrogenase subunit B* (*SDHB*) pathogenic variant (PV) are at high risk of extra-adrenal tumours, recurrence and metastasis which confer poor survival (19, 72). International guidelines recommend lifelong surveillance with annual biochemistry and regular imaging (1, 14, 103), but these are consensus-based, with uncertainties in penetrance, predictors of outcome, quality of life and cost-effectiveness (15, 22, 91).

This thesis aimed to evaluate clinical outcomes, heritability, penetrance, patient experience and health economics of surveillance strategies to inform evidence-based surveillance for *SDHB* PV carriers. **Chapter 2** presented a multicentre cohort study of *SDHB* PV carriers undergoing current routine surveillance. This study demonstrated that regular surveillance enabled earlier detection and improved outcomes compared to symptom-detected disease. **Chapter 3** investigated transmission ratio distortion (TRD) in *SDHB* and succinate dehydrogenase subunit D (*SDHD*) PV carriers. The findings demonstrated a higher than expected transmission of PVs to offspring which carried implications for genetic counselling and family planning.

Chapter 4 examined health-related quality of life (HRQoL) in carriers and showed the psychosocial impact of surveillance. Utility scores for the cohort were significantly lower than Australian norms on both EuroQol 5 Dimensions 5 Levels (EQ5D5L) and EQ visual analogue scale (EQ-VAS), with more than half of carriers also reporting anxiety or depression.

Chapter 5 presented a systematic review and meta-analysis which showed that penetrance rose steadily with age, metastatic risk in non-probands was lower than previously reported, and *SDHB* PV carriers remained at substantial risk of developing second tumours even after a first was treated. **Chapter 6** applied a health economic Markov model and showed that while annual surveillance for PPGL achieved slightly higher QALYs, less frequent strategies reduced costs and patient burden with only marginal health losses, and results were highly sensitive to scanxiety disutility. Together, these studies integrated clinical, genetic, epidemiological, patient-reported, and economic perspectives to update surveillance recommendations in *SDHB* PV carriers.

7.2 Clinical outcomes and heritability in *SDHB* PV carriers

Chapter 2 presented a large multicentre cohort study of *SDHB* PV carriers reviewed the detection rates of tumours in current surveillance programs. In this cohort, surveillance consisted of annual clinical and biochemical review, biennial whole-body magnetic resonance imaging (MRI) and functional imaging from age 18 y. This study showed that carriers could develop PPGL from childhood, with the youngest case a bladder paraganglioma diagnosed at age 9 y. Many tumours (34%) affecting non-probands were asymptomatic and only identified through surveillance. Tumours detected through surveillance were smaller (median 29 mm vs 45 mm) and more often curable by surgery. In contrast, probands who presented symptomatically had larger tumours, a higher rate of metastatic disease and increased risk of death with 10-year disease survival of 79% compared with 100% in non-probands. Importantly, surveillance altered the natural history of *SDHB*-related tumours by enabling earlier detection and treatment, leading to improved outcomes compared with symptomatic diagnosis.

The study also demonstrated that metastatic progression remained the main determinant of survival. Non-probands identified through surveillance were less likely to progress to metastatic disease, with probands having a hazard ratio of 4.13 compared with non-probands. This difference showed that earlier detection through surveillance allowed curative surgery and lowered the chance of metastatic progression. These multicentre data therefore strengthened the rationale for systematic surveillance in *SDHB* PV carriers.

The findings of this cohort study directly informed international consensus guidelines on the management of *SDHB* PV carriers. To date, multiple studies have referenced this work (5, 187-195), including being incorporated into two clinical practice guidelines (101, 102). The 2024 International Consensus Statement on the management of *SDHB*-related PPGL cited this study to support systematic family based surveillance, stating that physicians needed to ensure all at-risk relatives were identified and counselled, and that active surveillance should be performed for all asymptomatic carriers (102). Active surveillance was defined as regular clinical, biochemical, and imaging assessments unless disease progression occurred, with patients who had low tumour burden or indolent behaviour being particularly suited to this approach (102). In addition, the 2024 International Consensus on PPGL in children and adolescents cited this study as evidence that early diagnosis and therapeutic intervention were expected to reduce morbidity and mortality, strengthening recommendations for surveillance to begin in childhood (101). These guidelines demonstrated how outcome data from this multicentre cohort helped move surveillance in *SDHB* PV carriers from expert opinion to an evidence-based international standard of care.

Chapter 3 was a multicentre cohort study of 575 participants which demonstrated that *SDHB* and *SDHD* PVs were inherited more frequently than predicted by Mendelian genetics, with transmission rates significantly above the expected 50% in multiple pedigrees. This suggested a biological process favouring survival or transmission of the mutant allele. The effect was consistent across centres and robust to sensitivity analyses.

This finding had major implications for clinical practice. It was featured on the Pheo Para Alliance website, an internationally recognised patient advocacy group (196). TRD increased the number of carriers in each generation, thereby amplifying the disease burden in families. For genetic counselling, this meant that recurrence risk for children was higher than the standard 50% quoted in autosomal dominant conditions. Families planning children faced an even greater likelihood of passing on the PV, compounding both medical and psychological burden. TRD therefore added an additional layer to the challenges of managing *SDHB*-related disease, beyond tumour surveillance alone.

The observation of TRD in *SDHB* and *SDHD* PV carriers influenced the scientific literature. Gebhardt *et al.* built on this work, suggesting that skewed inheritance of *SDHB* variants might provide developmental advantages in the heterozygous state (190). Halpern *et al.* also cited this research and reported TRD in Li–Fraumeni syndrome, showing that the phenomenon extended beyond *SDHB* and *SDHD* to other hereditary cancer syndromes (197). These citations demonstrated that my research contributed to a broader recognition of TRD as an important mechanism in hereditary cancer, with implications for both biology and genetic counselling.

Taken together, my cohort study and TRD analysis highlighted both clinical and heritable dimensions of disease burden. TRD increased the likelihood of transmission to offspring but surveillance enabled earlier tumour detection and improved outcomes. These findings reinforced the importance of structured surveillance protocols with defined clinical, biochemical, and imaging schedules alongside careful genetic counselling. They also raised questions about the biological basis of TRD, which remains poorly understood and warrants further research.

7.3 Quality of life and psychological impact of surveillance

Chapter 4 assessed HRQoL in *SDHB* PV carriers using EQ5D5L, EQ-VAS and the European Organisation for Research and Treatment Quality of Life Questionnaire (EORTC QLQ-C30) with QLU-C10D utilities. Carriers had lower scores than population norms, with mean EQ5D5L at 0.778 (difference between means 0.13) and EQ-VAS at 72.7 (difference between means 5.85). This meant that, on average, carriers rated their overall health and wellbeing lower than expected for the general population. The differences were statistically significant, confirming that the burden of living with a pathogenic variant affected daily life. For the QLU-C10D utilities Australian normative values were not available, so carriers were compared with UK norms. The mean utility score was similar, but because the reference group came from a different population this finding should be interpreted with caution.

The most striking finding was in domains reflecting mental health. More than half of carriers reported problems with anxiety or depression on the EQ5D5L. Compared with Australian population norms, carriers were almost four times more likely to report anxiety or depression (OR 3.78, $p<0.001$). Importantly, this pattern was present even in carriers who had never developed a tumour. This suggested that the psychological impact was linked not only to disease, but also to the lifelong risk and the experience of ongoing surveillance.

Specific symptoms also had a marked effect. Carriers who experienced sweats, palpitations, or flushing had much lower quality of life scores. For example, those with sweats reported substantially lower utility values (difference between means 0.40 and 0.30 for EQ5D5L and QLU-C10D utilities respectively) and worse social functioning on the EORTC QLQ-C30. This showed that catecholamine symptoms could have a major effect on socialising and interactions with others. Other studies of hereditary cancer syndromes found that carriers experienced physical symptoms, role limitations, impaired social functioning, and emotional distress (149, 150, 152, 153).

These results had important implications. They demonstrated that surveillance while critical for improving outcomes, carried a real psychosocial cost. The presence of anxiety and reduced wellbeing in tumour free carriers highlighted the need for psychological support to be integrated into surveillance protocols. Education about risk, regular psychosocial screening and targeted counselling could help address anxiety and improve long term HRQoL.

7.4 Optimising surveillance through modelling and cost-effectiveness analysis

Chapter 5 presented a systematic review and meta-analysis of outcomes in *SDHB* PV carriers. The study quantified age-related penetrance, recurrence risk and survival. By age 20 y penetrance was 4%, rising to 11% by age 40 y, 24% by age 60 y and 35% by age 80 y. For non-probands with tumours, the risk of metastatic progression was 9% per lifetime. Carriers who had developed one tumour had a 24% risk of a second tumour. Five-year mortality for carriers with metastatic disease was 18%. These results provided the most precise estimates to date for penetrance and outcomes in non-probands, which are essential for counselling families and designing surveillance protocols.

Chapter 6 built on data from across the other studies in the thesis to construct a health economic Markov model. The model incorporated penetrance and recurrence risk from Chapter 5, outcome differences between probands and non-probands from Chapter 2, quality of life utility scores from Chapter 4, and inputs from the literature for tumour doubling time (69) and test performance data (83). Annual, three yearly and five yearly surveillance strategies were compared. Annual surveillance cost $69,595 and achieved 14.41 QALYs. Three yearly surveillance cost $56,538 with 14.35 QALYs, and five yearly surveillance cost $49,458 with 14.29 QALYs. Less frequent surveillance reduced costs and patient burden with only small health losses.

Results were highly sensitive to the inclusion of scanxiety disutility; when a disutility of 0.038 per year was applied, annual surveillance was no longer favoured. In this scenario, 3- or 5-yearly surveillance became cost effective or even dominant, showing how psychological burden could alter the preferred surveillance strategy.

Together, the meta-analysis and health economic modelling provided an evidence base for evaluating surveillance strategies. They confirmed the importance of lifelong surveillance, but suggested that the frequency of imaging could be reconsidered based on health outcomes and patient experience.

7.5 Limitations

This thesis had several limitations. In the multicentre cohort study some clinical data were missing and reporting varied between centres. The study was not nationwide and ascertainment bias may have influenced symptom reporting. The TRD analysis was limited by uncollected data such as age of parenthood and miscarriage history. The sample size was large for a rare disease but still constrained the ability to assess biological mechanisms in detail.

The quality of life study was cross-sectional. It did not allow assessment of changes over time or causal relationships. Recruitment from hospital surveillance clinics may have introduced selection bias. Data were not always collected in relation to timing of imaging, limiting conclusions about scanxiety. Not all possible confounders were controlled for and the study did not include qualitative methods. The meta-analysis was limited by possible publication bias and language bias. Narrow inclusion criteria also reduced generalisability, although they ensured focus on *SDHB* PV carriers.

The Markov model relied on assumptions about risks, test performance, costs and utilities. These were varied in sensitivity analyses, but uncertainty remained. Utilities came from a cross-sectional survey and some were taken from external sources. The model assumed perfect adherence and used Australian costs, limiting generalisability. It also could not test whether surveillance could be reduced or stopped in older tumour-free *SDHB* PV carriers.

7.6 Policy implications and recommendations

This thesis generated several findings with direct implications for clinical practice and policy. The multicentre cohort study in **Chapter 2** supported systematic surveillance beginning in childhood, with annual clinical and biochemical review and biennial MRI with functional imaging from age

18. These data were cited in international guidelines and helped move surveillance for *SDHB* PV carriers from consensus opinion toward evidence-based standards.

The demonstration of TRD in **Chapter 3** highlighted that inheritance risk is greater than the 50% expected in autosomal dominant inheritance. This finding should be incorporated into genetic counselling, so that families are informed of higher than usual risk when making reproductive decisions. Policy frameworks for counselling and reproductive support should be updated to reflect this evidence.

The quality of life study in **Chapter 4** showed that surveillance imposes a psychosocial burden, with carriers nearly four times more likely to report anxiety or depression compared with population norms. This finding supported the integration of psychosocial screening and support into surveillance programs. Policymakers should consider resourcing mental health support as part of standard care for *SDHB* PV carriers.

The systematic review and meta-analysis in **Chapter 5** provided the most precise estimates to date for penetrance and survival in *SDHB* PV carriers. These data could inform genetic counselling, allowing families to receive age-specific risk information to guide surveillance, reproduction and long-term planning. The quantified risks of second tumours showed that surveillance should continue even after treatment of a first tumour, as carriers remain at ongoing risk. These estimates also demonstrated that while metastatic progression is the leading determinant of survival, the absolute risk was lower than some earlier reports. This provides a more balanced evidence base for counselling families, setting expectations and shaping guideline recommendations.

The health economic model in **Chapter 6** demonstrated that less frequent surveillance could reduce costs and patient burden with only marginal health losses. Results were highly sensitive to assumptions about disutility from surveillance anxiety. While the need for ongoing surveillance was reinforced, the optimal frequency of imaging is still uncertain. Further research to better quantify the psychosocial burden of surveillance will be essential before firm policy recommendations can be made.

7.7 Future directions

Future work could evaluate diagnostic modalities in *SDHB*-associated tumours. Larger multicentre and prospective studies would clarify the sensitivity and specificity of biochemical tests, MRI and functional imaging. These data could strengthen surveillance recommendations and improve early tumour detection.

The observation of TRD could be validated in other cohorts. Analysis by additional centres would confirm if inheritance skew is consistent across populations. Understanding the mechanism, including a possible advantage in the heterozygous state, may provide biological insights and guide future interventions to reduce tumour risk.

Quality of life research would benefit from longitudinal and qualitative study designs. These could track changes over time and capture lived experience in greater depth. Such work could guide supportive care and help address scanxiety and psychosocial burden.

Risk estimates for outcomes in non-probands remain limited and deserve dedicated research. International collaborations could refine estimates for second tumour risk, metastatic progression and mortality. Future modelling studies could incorporate longitudinal HRQoL data, adherence and conditional survival. The question of whether surveillance could be reduced or stopped in older tumour-free carriers remained uncertain. Exploration of plasma succinate and fumarate could offer non-invasive biomarkers. Perhaps an international registry of *SDHB* PV carriers would allow larger sample sizes and harmonised data. Taken together these directions point to more precise, patient-centred and evidence-based care for this cohort who face significant lifelong risk.

7.8 Conclusion

This thesis evaluated outcomes, heritability, quality of life and cost effectiveness in *SDHB* PV carriers. The work showed that surveillance enabled earlier tumour detection and improved survival compared with symptomatic presentation. It also demonstrated that TRD increased the burden of disease within families and carried major implications for genetic counselling. Carriers experienced reduced quality of life with anxiety and surveillance burden emerging as important contributors. These findings highlighted the need for psychological support and integrated care alongside medical surveillance. A systematic review and meta-analysis provided precise estimates of penetrance, recurrence, and survival. These results informed a health economic model that compared surveillance strategies. The model supported the need for surveillance but showed that surveillance schedules could be balanced against health outcomes, costs and patient experience. Overall these studies moved the rationale for surveillance for *SDHB* PV carriers from expert opinion toward an evidence base. They provided clinical, genetic, psychosocial and economic data to assist genetic counsellors, psychologists, nurses, physicians and researchers in the field of hereditary endocrine tumours. The work contributed to international consensus recommendations and set the stage for more precise and patient-centred care. This thesis provided the first integrated evidence base for surveillance in *SDHB* PV carriers, influencing international guidelines and highlighting priorities for future research.

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Chapter 8: Supplementary Material

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Surveillance Improves Outcomes for Carriers of *SDHB* Pathogenic Variants: A Multicenter Study

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Abstract

Context: Carriers of succinate dehydrogenase type B (SDHB) pathogenic variants (PVs) are at risk of pheochromocytoma and paraganglioma (PPGL) from a young age. It is widely recommended carriers enter a surveillance program to detect tumors, but there are limited studies addressing outcomes of surveillance protocols for *SDHB* PV carriers.

Objective: The purpose of this study was to describe surveillance-detected (s-d) tumors in *SDHB* PV carriers enrolled in a surveillance program and to compare their outcomes to probands.

Methods: This was a multicenter study of *SDHB* PV carriers with at least 1 surveillance episode (clinical, biochemical, imaging) in Australian genetics clinics. Data were collected by both retrospective and ongoing prospective follow-up. Median duration of follow-up was 6.0 years.

Results: 181 *SDHB* PV carriers (33 probands and 148 nonprobands) were assessed. Tumors were detected in 20% of nonprobands undergoing surveillance (age range 9-76 years). Estimated 10-year metastasis-free survival was 66% for probands and 84% for nonprobands with s-d tumors ($P = .027$). S-d tumors were smaller than those in probands (median 27 mm vs 45 mm respectively, $P = .001$). Tumor size ≥40 mm was associated with progression to metastatic disease (OR 16.9, 95% CI 2.3-187.9, $P = .001$). Patients with s-d tumors had lower mortality compared to probands: 10-year overall survival was 79% for probands and 100% for nonprobands ($P = .029$).

Conclusion: *SDHB* carriers with s-d tumors had smaller tumors, reduced risk of metastatic disease, and lower mortality than probands. Our results suggest that *SDHB* PV carriers should undertake surveillance to improve clinical outcomes.

Key Words: SDHB, succinate dehydrogenase, pheochromocytoma, paraganglioma

Abbreviations: ^{18}F-FDG-PET/CT, ^{18}F-fludeoxy glucose positron emission tomography computed tomography; APGL, abdominal paraganglioma; GIST, gastrointestinal stromal tumor; HNPGL, head and neck paraganglioma; MRI, magnetic resonance imaging; PC, pheochromocytoma; PoWH, Prince of Wales Hospital; PPGL, pheochromocytoma and paraganglioma; PV, pathogenic variant; RCC, renal cell carcinoma; RHH, Royal Hobart Hospital; s-d, surveillance-detected; RNSH, Royal North Shore Hospital; ROC, receiver operated curve; SDHB, succinate dehydrogenase type B; TPGL, thoracic paraganglioma.

Carriers of *succinate dehydrogenase type B* (*SDHB*) pathogenic variants (PVs) are at risk of pheochromocytoma and paraganglioma (PPGL), renal cell carcinomas (RCCs), and gastrointestinal stromal tumors (GISTs) from a young age (1). It is widely recommended carriers enter a surveillance program to detect tumors, as metastatic disease is associated with high mortality (2, 3). However, there are limited studies addressing outcomes of surveillance protocols for *SDHB* PV carriers.

A recent international Delphi consensus study recommended asymptomatic *SDHB* PV carriers commence surveillance for tumors from as young as age 6-10 years; baseline assessment should include clinical history for catecholaminergic symptoms, blood pressure, either plasma or urinary metanephrine and normetanephrine, and magnetic resonance imaging (MRI) of head and neck, thorax, abdomen, and pelvis and the option of functional imaging in adulthood (4). These guidelines recommended ongoing assessment, after a first negative initial surveillance, with annual clinical examination, biennial biochemical testing, and MRI every 2 to 3 years and/or functional imaging in adulthood (4).

The goal of surveillance is to detect *SDHB*-associated tumors early enough for curative surgical resection, since large

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tumor size is associated with metastatic progression (5-9). A multicenter European study of patients with metastatic PPGL reported primary tumor size was ≥5 cm in 76% of cases (5). Single-center studies from the United States have reported PPGL cut-offs of 4 cm (6), 4.5 cm (7), 5 cm (8), and 6.1 cm (9) are associated with metastatic disease in *SDHB* PV carriers. Tumor location also impacts on risk of metastatic disease. Head and neck paragangliomas (HNPGLs) tend to have a lower risk of metastatic progression than *SDHB*-associated abdominal or pelvic PPGLs (12% vs 33%) (10), and when metastases occur the disease course is often indolent (11).

The purpose of this study was to describe surveillance-detected (s-d) tumors in *SDHB* PV carriers undergoing surveillance compared with probands by (1) describing the nature of *SDHB*-associated tumors detected during surveillance; and (2) investigating case detection strategies that are helpful in detecting *SDHB*-related tumors and whether these translate into improved outcomes.

Materials and Methods

This was a multicenter observational cohort study of *SDHB* PV carriers with at least 1 surveillance episode (clinical, biochemical, imaging) in genetics clinics at Royal North Shore Hospital (RNSH), Prince of Wales Hospital (PoWH), and Royal Hobart Hospital (RHH) in Australia. Clinical data from medical records were collected by both retrospective and ongoing prospective follow-up using a bespoke data extraction form developed in Research Electronic Data Capture (REDCap) (12) software. Retrospective data were collected from July 2000 at RNSH, September 1994 at PoWH, and February 2002 at RHH up to September 29, 2019. Ongoing prospective data collection has been performed since September 30, 2019, with most recent data collection performed on August 10, 2021.

The data extraction form was tailored to the surveillance protocol at each site, but also had capacity to record types and frequencies of biochemical or imaging surveillance that differed to the standard protocol if relevant to that participant. Annual clinical assessment of symptoms, blood pressure, and biochemical measurements of plasma metanephrines or urinary catecholamines were included in surveillance protocols of all 3 centers. At RHH, biochemical assessment also included annual chromogranin A measurement, and imaging surveillance consisted of biennial MRI from base of skull to coccyx and, after age 18, of 4 yearly ^{18}F-fludeoxy glucose positron emission tomography computed tomography (FDG-PET/CT) alternating with 4 yearly neck and abdominal ultrasounds (13). RNSH and PoWH followed the Cancer Institute NSW guidelines for biennial MRI from base of skull to coccyx (14). After age 18, 5-yearly functional imaging with either ^{68}Ga-DOTATATE PET/CT or ^{18}F-FDG-PET/CT was included as clinically appropriate.

Probands were defined as the first individual in a family to be diagnosed with a *SDHB* PV after presenting with a tumor, and for this cohort were always the index case. S-d tumors were defined as tumors detected in nonprobands during surveillance. These were classified either as "clinical" s-d tumors if detected as part of familial surveillance prior to formal genetic diagnosis of *SDHB* PV, in other words before the advent of routine *SDHB* genetic testing, or "genetic" s-d tumors if diagnosed following predictive genetic diagnosis. Incidence density of s-d tumors was defined as the total number of new tumors over the person-time at risk contributed by each participant during the period of surveillance. Incidence was reported as number of tumors per 100 person-years. Genotypes were classified as loss of function (nonsense, splicing, deletion, or frameshift) or missense variants. Tumor diagnosis and size were obtained from histopathology reports, or from radiological appearance in combination with biochemistry if the tumor was not resected. "Classic" symptoms (ie, those typically associated with catecholamine excess) were defined as headaches, palpitations, or excessive sweating and were recorded systematically in the clinical records at each site: The majority of clinic visits recorded presence or absence of headaches, palpitations, or sweats and where absent were classified as not available. Poor adherence was defined as either attending only 1 follow-up visit or 2 or more years between surveillance episodes. Loss to follow-up was defined as 2 or more years since the last follow-up episode. Ethics approval was obtained from the Northern Sydney Local Health District Ethics Committee for RNSH and PoWH (ref. 2019/ETH09870) and from Tasmania Health and Medical Human Research Ethics Committee for RHH (ref. H0018520).

Statistical analysis was performed using GraphPad Prism version 7.03 and IBM SPSS version 26. Descriptive statistics were performed with numerical data presented as median and range. Groups were compared using the Mann–Whitney test for nonparametric data. Cumulative frequency analysis was conducted on s-d tumors to represent the timing of tumor diagnosis during surveillance. Sensitivity, specificity, positive predictive value, and negative predictive value of clinical, biochemical, and imaging methods of tumor detection were calculated using the Wilson–Brown method to compute 95% CI and Fisher's exact test to determine statistical significance. Predictors of disease diagnosis, metastatic disease, and mortality were assessed using a generalized linear model with binary logistic regression to perform a multivariate analysis. Explanatory variables included in this model were sex, genotype, history of smoking, classic symptoms, hypertension, age at first surveillance episode, tumor size, Ki-67, tumor location, tumor functional status (defined as production of catecholamines noradrenaline, adrenaline, dopamine or nonfunctioning), multifocality, and synchronous metastases. Significant predictors in the main effects model were then assessed for interaction. We estimated metastasis-free survival and overall survival of probands and nonprobands by Kaplan–Meier analysis in patients with any *SDHB*-associated tumors and in a subgroup of patients with thoraco-abdominal PPGLs. Receiver operated curve (ROC) analysis of tumor size for prediction of metastatic disease was presented as area under the curve with an area of 0.7 considered acceptable and a Youden's index determined based on the optimal value that maximized the sum of sensitivity and specificity. Cox proportional hazards regression was also performed to examine the impact of tumor size and risk of probands compared with nonprobands for metastatic progression and risk of a second primary tumor. $P \leq .05$ was considered to be statistically significant.

Results

Baseline Characteristics

This cohort includes 181 *SDHB* PV carriers from 59 families undergoing routine clinical surveillance (Table 1): 92 (51%)

from RHH, 50 (27%) from RNSH, and 39 (22%) from PoWH. There were 33 (18%) probands and 148 (82%) nonprobands. Median age at first surveillance for nonprobands was 33 years (range 1-81 years) and 84 (46%) were male. One proband and 3 nonprobands had poor adherence to surveillance. Median duration of follow-up was 6.0 years and was not different between probands and nonprobands. Nonprobands had 1059 person-years of follow-up. Of the total cohort, 110 (61%) had at least 5 years of follow-up, 54 (30%) had at least 10 years of follow-up, 24 (13%) had 15 years, and 5 (3%) had 20 years follow-up. Twenty (11%) carriers were lost to follow-up.

The Nature of *SDHB*-associated Tumors Detected During Surveillance

Surveillance-detected tumors

Tumors were detected in 29 (20%) of 148 nonprobands during surveillance (Table 2 and Fig. 1A). The incidence of s-d tumors was 1.3 cases per 100 person-years (95% CI 0.7-2.3 per 100 person-years). Twenty-two (15%) of 148 nonprobands had their first surveillance aged ≤10 years. The youngest age of first surveillance was at age 1 year. The youngest age of a s-d tumor was a bladder paraganglioma diagnosed at age 9 years. Seventeen (11%) of the 148 nonprobands had a final age of follow-up after 70 years of age, and 3 (2%) nonprobands had follow-up after age 80 years. The oldest age of a s-d tumor was an asymptomatic gastric GIST detected at age 76. The age of case detection of the 29 nonprobands with s-d tumors is represented in Fig. 2.

Clinical surveillance-detected tumors

Twelve (41%) of 29 nonprobands with disease had "clinical" s-d tumors, defined as tumor detected as part of familial surveillance but prior to formal predictive genetic diagnosis of an *SDHB* PV (Table 2). Four (33%) patients with clinical s-d tumors had tumors smaller than 40 mm and 1 (8%) had a tumor under 20 mm. Of the 8 nonprobands with clinical s-d tumors whose records on symptoms were available, 5 (63%) had classic symptoms consistent with elevated catecholamines, 2 had hearing loss in the context of HNPGL, and only 1 patient was asymptomatic.

Six (50%) of 12 patients with clinical s-d tumors had tumors resected and are in remission at most recent follow-up. One (8%) patient has a persistent localized HNPGL despite surgical excision. Four (33%) patients had metachronous tumors: 1 (8%) had a metachronous abdominal paraganglioma (APGL) resected and is in remission, 2 (17%) patients had an abdominal PGL resected but have persistent localized HNPGLs, and 1 (8%) patient has persistent localized HNPGLs. One (8%) patient developed recurrence of HNPGL and subsequently metastatic disease. One (8%) patient with clinical s-d tumor died from disease.

Genetic surveillance–detected tumors

Seventeen (57%) of 30 nonprobands with disease had "genetic" s-d tumors, defined as tumors diagnosed following predictive genetic diagnosis (Table 2 and Fig. 1B). Six patients had tumors diagnosed at the initial surveillance (35% of all genetic s-d tumors; Table 2). The remaining 11 (65%) patients with genetic s-d tumors had tumors detected at 3 to 152 months following the initial surveillance episode, of which 1 was a synchronous GIST and APGL. Notably 11 patients (65%) with genetic s-d tumors had tumors smaller than 40 mm and 7 (41%) patients had tumors smaller than 20 mm. Ten (59%) patients with genetic s-d tumors were asymptomatic. One patient had a 14 mm prolactinoma without suprasellar or cavernous sinus extension. Eleven patients (65%) with genetic s-d tumors had tumors resected and are in remission at most recent follow-up. Five (29%) have persistent localized disease (1 thoracic paraganglioma [TPGL], 1 pituitary adenoma, 2 HNPGL, 1 APGL) and 1 patient has metastatic disease. None of the patients with genetic s-d tumors experienced recurrence, a metachronous tumor, or death from disease at most recent follow-up.

Case Detection Strategies Helpful in Detecting *SDHB*-related Tumors and Translating Into Improved Clinical Outcomes

Method of detection of tumors and tumor size

To describe the methods by which tumors were detected, we then reviewed data on all 82 tumors detected in these 62 patients (33 probands and 29 nonprobands; Supplemental Table 1 (15)). Forty (65%) patients had 49 diagnoses of thoraco-abdominal PPGL (9 pheochromocytoma [PC], 4 TPGL, and 38 APGL). Data on tumor functional status were available for 38 thoraco-abdominal PPGLs. Twenty-nine (76%) secreted noradrenaline of whom 21 (72%) had classic symptoms. Three (8%) secreted adrenaline of whom 2 had classic symptoms and 6 (16%) were apparently nonfunctional of whom 1 reported classic symptoms. Of those whose tumor functional status were unavailable, 9 (18%) had unavailable data from the 1990s and 2 (4%) patients were probands who entered surveillance following surgery at another center where PPGL had not been suspected (of whom 1 survived an intraoperative hypertensive crisis). Median maximal tumor diameter was 40 mm (range 25-90 mm), 43 mm (range 35-75 mm), and 41 mm (range 8-190 mm) for PC, TPGL, and APGL respectively. Tumors were detected by functional imaging followed by directed anatomical imaging in 3 HNPGL, 2 PC, 1 TPGL, and 8 APGL (Supplemental Table 1 (15)). When we compared the size of tumors detected before and after the introduction of functional PET imaging, the difference was not significant albeit possibly limited by power (median 34 vs 23 mm, $P = .15$; Supplemental Table 2 (15)). The sensitivity and specificity of diagnostic modalities for detection of sympathetic PPGL are shown elsewhere (Supplemental Table 3 (15)).

Twenty (32%) patients had 23 diagnoses of HNPGL of whom 5 (25%) reported classic symptoms typically associated with catecholamine excess. Two (10%) patients with classic symptoms had concomitant APGL, but 3 (15%) curiously had classic symptoms of headaches, palpitations, or sweats despite absence of biochemical catecholamine excess. Median HNPGL tumor diameter was 23 mm (range 3-65 mm). Seven (35%) HNPGLs were detected smaller than 20 mm and imaging modalities of detection for these small tumors were 1 ultrasound, 5 MRI, and 4 ^{68}Ga-DOTATATE PET/CT scans. The sensitivity and specificity of diagnostic modalities in this cohort for detection of HNPGL are shown elsewhere (Supplemental Table 4 (15)).

Other *SDHB*-associated tumors are shown in Table 2. GIST tumor size was median 55 mm (range 36-60 mm). The imaging modalities for detection of GIST were 1 ultrasound, 2 CT, 2 MRI, and 2 ^{18}F-FDG-PET/CT scans. RCC tumor size was median 41 mm (range 25-167 mm) and CT-detected RCC in these cases. Finally, 1 pituitary adenoma was detected on MRI measuring 14 mm diameter.

Table 1. Baseline characteristics

	Complete cohort (n = 181)	Probands (n = 33)	Nonprobands (n = 148)	*P* value
Age at first surveillance, years; median (range)	33 (1-81)	35 (9-70)	33 (1-81)	.68
Male; n (%)	84 (46)	17 (52)	67 (45)	.50
Probands; n (%)	33 (18)	—	—	—
Genotype; n (%)				
Missense	57 (31)	9 (27)	48 (32)	.52
Nonsense	43 (24)	10 (30)	33 (22)	
Splicing	35 (19)	3 (9)	32 (22)	
Deletion	43 (24)	10 (30)	33 (22)	
Duplication	2 (1)	1 (3)	1 (1)	
Not available	1 (0.5)	0	1 (1)	
Country of birth; n (%)				
Australia	155 (86)	26 (79)	129 (87)	.86
UK	5 (3)	1 (3)	4 (3)	
Malaysia	1 (0.5)	1 (3)	0	
Philippines	2 (1)	2 (6)	0	
Brazil	2 (1)	0	2 (1)	
The Netherlands	2 (1)	1 (3)	1 (1)	
South Africa	1 (0.5)	0	1 (1)	
Not available	13 (7)	2 (6)	11 (7)	
Smoking status				
Nonsmoker; n (%)	95 (52)	19 (58)	76 (51)	.76
Current smoker; n (%)	29 (16)	4 (12)	25 (17)	
Past smoker	27 (15)	7 (21)	20 (14)	
Not available; n (%)	30 (17)	3 (9)	27 (18)	
Occupation; n (%)				
Student	49 (27)	3 (9)	46 (31)	.22
Professional	33 (18)	9 (27)	24 (16)	
Clerical, sales and service worker	12 (7)	2 (6)	10 (7)	
Laborer	11 (6)	3 (9)	8 (5)	
Manager	10 (5.5)	3 (9)	7 (5)	
Tradesperson	10 (5.5)	3 (9)	7 (5)	
Retired	7 (4)	0	7 (5)	
Full time parent	4 (2)	0	4 (3)	
Production and transport worker	2 (1)	0	2 (1)	
Disability support pension	1 (0.5)	1 (3)	0	
Not available	42 (23)	9 (27)	33 (22)	
Duration of ongoing follow-up, years; median (range)	6.0 (1 month-25.6 years)	8.1 (14 month-25.6 years)	5.9 (1 month-23.9 years)	.08

Predictors of disease diagnosis

Male gender, classic symptoms (headaches, palpitations, or sweats), absence of hypertension, and young age were associated with tumors on a generalized linear model with binary logistic regression (Table 3). However, there were multiple interactions in the model. Male gender was associated with absent symptoms (*P* = .023) and normal blood pressure (*P* = .010) while female gender was associated with younger age at first presentation (*P* = .007). There was no interaction between classic symptoms and hypertension (*P* = .810). Younger age was associated with normal blood pressure (*P* = .003), suggesting that young age rather than normal blood pressure was a predictor of disease. The age of disease diagnosis for females was median 34 years (range 9-72) and for males was median 41 years (range 9-76). Overall median age of diagnosis was 37 years (range 9-76). A generalized linear model with binary logistic regression for predictors of thoraco-abdominal PPGL was unable to be obtained due to sample size and therefore quasi-complete separation in the data. Probands had higher numbers of APGLs and overall *SDHB*-associated tumors than nonprobands with disease (*P* = .002 and *P* = .03 respectively, Table 2). Probands were more likely to develop multifocal disease over time than nonprobands (OR 3.28, 95% CI 1.02-9.74, *P* = .046) but were not more likely to have multifocal disease at baseline (*P* = .68). Cox proportional hazard assessment found the risk of a second tumor was not determined by primary tumor size 40 mm or larger (HR 2.61, 95% 0.69-9.87, *P* = .157). No other factors were able to predict

Table 2. *SDHB* PV carriers with disease diagnoses (n = 62): tumor types

	Probands (n = 33)	Nonprobands with s-d tumors (n = 29)	*P* value
HNPGL (n)	9	14	.16
PC (n)	7	2	.11
Thoracic PGL (n)	1	3	.25
Abdominal PGL (n)	27	11	.002[a]
RCC (n)	3	0	.21
GIST (n)	1	3	.25
Pituitary adenoma (n)	0	1	.29
Total tumors (n)	48	34	.03[b]

13 probands and 5 nonprobands had multifocal tumors. For probands, 1 patient had a synchronous APGL and GIST, 1 patient had synchronous APGL and PC followed by a metachronous APGL, 1 patient had synchronous bilateral RCC, 1 patient had synchronous bilateral APGL; 10 probands had metachronous tumors (31%). For nonprobands, 1 patient had synchronous APGL and GIST and 1 patient with synchronous HNPGL and APGL; 3 patients developed a metachronous tumor (2 HNPGL, 1 PC). Abbreviations: GIST, gastrointestinal stromal tumor; HNPGL, head and neck paraganglioma; PC, pheochromocytoma; PGL paraganglioma; RCC, renal cell carcinoma; s-d, surveillance-detected.
[a]$P < .01$.
[b]$P < .05$.

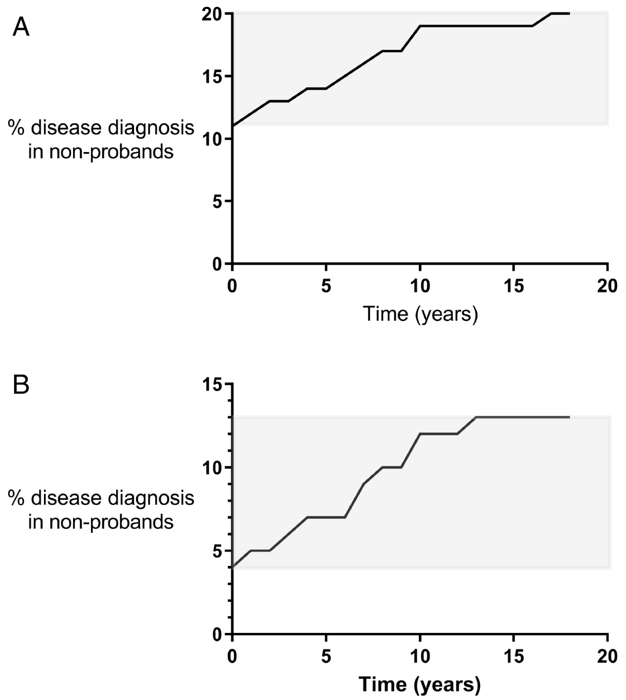

Figure 1. (A) Cumulative frequency of disease diagnosis in nonprobands. The prevalence of disease at the initial surveillance episode was 11% (n = 17) and 12 new cases were detected during surveillance. In total, 29 of 148 nonprobands were diagnosed with disease. The risk of disease in nonprobands was 20%. (B) Cumulative frequency of disease diagnosis made following predictive genetic diagnosis of *SDHB* PV. The prevalence of disease at the initial surveillance episode was 4% (n = 6) and 11 new cases were detected during surveillance. In total, 17 of 136 nonprobands were diagnosed with disease following the genetic diagnosis. The risk of disease in nonprobands following the genetic diagnosis was 13%.

recurrence or multifocal disease including gender, loss of function genotype, history of smoking, typical symptoms, hypertension, age, tumor size, and Ki-67.

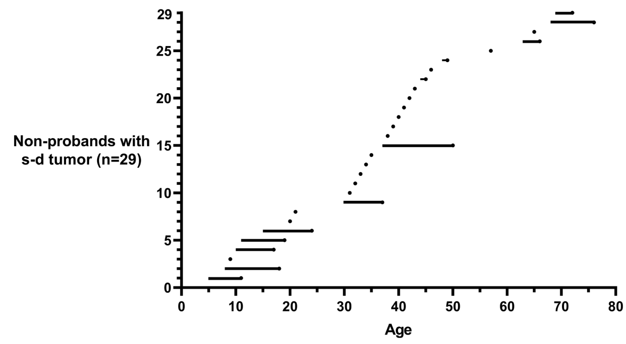

Figure 2. Nonprobands diagnosed with s-d tumors (n = 29). The age of case detection of each participant is represented by the black circle. Nonprobands who were not diagnosed with disease at the initial screen have a line to represent the age from the start of surveillance until case detection.

Metastatic disease

Patients with s-d tumors were less likely to be associated with metastatic than probands. Three (10%) of 29 patients with s-d tumors developed metastatic disease at most recent follow-up compared with 10 (31%) of 32 probands ($P = .04$). Overall, 13 (21%) of 62 patients with disease showed evidence of metastatic progression at a median of 66 months (range 0-127 months) with no difference in synchronous metastases between probands and nonprobands ($P = .061$). Two probands had synchronous metastases. Probands had increased risk of metastatic progression compared with nonprobands with s-d tumors (HR 4.13, 95% CI 1.15-14.9, $P = .03$). Estimated 5- and 10-year metastasis-free survival was 82% and 66% for probands and 100% and 84% for nonprobands ($P = .027$, Fig. 3A). Median time between initial diagnosis of any *SDHB*-associated tumor and diagnosis of metastatic disease was 68 months (range 0-193 months).

The risk of metastatic disease in HNPGL was 10% (2/20). In comparison, 10 (25%) of the 40 patients with thoraco-abdominal PPGL had evidence of metastatic progression at median 61 months (range 3-127 months) with higher risk of metastatic progression in probands than in nonprobands (OR 11.35, 95% CI 1.51-128.2, $P = .013$). One proband with thoraco-abdominal PPGL had synchronous metastases. Estimated 5- and 10-year metastasis-free survival for patients with thoraco-abdominal PPGL was 77% and 57% for probands and 100% and 91% for nonprobands ($P = .023$, Fig. 3B). Median time between initial diagnosis of thoraco-abdominal PPGL and diagnosis of metastatic disease was 60 months (range 3-127 months).

Tumor size was the only statistically significant predictor of metastatic disease on a multivariate generalized linear model with binary logistic regression (OR 1.08, 95% CI 1.03-1.14, $P = .004$, Table 3). S-d tumors were smaller than those in probands (median 27 mm (range 8-85 mm) vs 45 mm (range 13-190 mm) respectively, $P = .001$). Tumor size of 40 mm or higher was associated with progression to metastatic disease for all *SDHB*-associated tumors (OR 16.9, 95% CI 2.3-187.9, $P = .001$) and for patients with thoraco-abdominal PPGL (OR 11.5, 95% CI 1.57-133.1, $P = .02$). ROC curve analysis determined that size of the primary tumor >39 mm was the optimal cut-off value associated with increased risk of metastatic disease for all *SDHB*-associated tumors (area under the curve = 0.86, sensitivity = 58.5% [95% CI 42.1-73.7], specificity = 100% [95% CI 75.3-100], Youden's index = 0.59,

Table 3. Generalized linear model with binary logistic regression of predictors of disease diagnosis (n = 181), metastatic disease (n = 62), and death from disease (n = 62) for *SDHB* PV carriers

Predictors of disease diagnosis	*P* value	OR (95% CI)
Male	.007[a]	3.05 (1.36-6.89)
Loss of function PV	.43	0.72 (0.32-1.62)
History of smoking	.33	1.75 (0.56-5.44)
Classic symptoms (headaches, palpitations or sweats)	.000[a]	4.90 (2.03-11.84)
Hypertension (BP ≥ 140/90)	.000[a]	0.10 (0.03-0.29)
Age at first surveillance episode	.003[a]	0.97 (0.94-0.99)
Test	***P* value**	**Σ²**
Overall model likelihood ratio test (omnibus test)	.000[a]	38.79
Predictors of metastatic disease		
Tumor size	.004[a]	1.08 (1.03-1.14)
Male	.36	0.29 (0.02-4.19)
Loss of function PV	.39	0.29 (0.18-4.81)
History of smoking	.81	1.21 (0.25-5.91)
Classic symptoms (headaches, palpitations or sweats)	.48	3.35 (0.11-102.89)
Hypertension (BP ≥ 140/90)	.63	2.17 (0.09-50.49)
Age at first surveillance episode	.14	1.07 (0.98-1.16)
Multifocal tumors	.13	8.05 (0.55-117.43)
Tumor location	.09	5.99 (0.78-45.90)
Tumor functioning status	.47	2.09 (0.28-15.68)
Ki-67	.84	0.72 (0.02-8.44)
Test	***P* value**	**Σ²**
Overall model likelihood ratio test (omnibus test)	.009[b]	29.41
Predictor of death from disease		
Tumor size	.09	0.97 (0.94-1.01)
Synchronous metastases	.99	No value[c]
Male	.38	0.28 (0.02-4.93)
Loss of function PV	.77	1.71 (0.05-59.16)
History of smoking	.20	6.58 (0.67-62.06)
Typical symptoms (headaches, palpitations or sweats)	.47	3.93 (0.10-157.93)
Hypertension (BP ≥ 140/90)	.63	1.97 (0.13-29.97)
Age at first surveillance episode	.74	0.98 (0.89-1.09)
Multifocal tumors	.33	3.79 (0.25-56.57)
Tumor location	.99	0.00 (no value)
Tumor functioning status	.99	0.00 (no value)
Ki-67	.99	0.00 (no value)
Test	***P* value**	**Σ²**
Overall model likelihood ratio test (omnibus test)	.19	18.47

loss of function defined as nonsense, splicing, deletion, or frameshift PV.
Abbreviations: CI, confidence interval; PV, pathogenic variant.
[a]$P < .01$;
[b]$P < .05$.
[c]No value due to a quasi-complete separation in the data.

$P = .0001$, Fig. 4A) and for patients with thoraco-abdominal PPGL (area under the curve = 0.87, sensitivity = 56.0% [95% CI 34.9-75.6], specificity = 100% [95% CI 69.2-100], Youden's index = 0.56, $P = .0007$, Fig. 4B). Cox proportional hazards regression could not determine the hazard ratio of metastatic disease associated with tumors 40 mm or larger due to a quasi-separation in the data, whereby there were 0 cases of metastatic progression with tumor size below 40 mm. Similarly, a multivariate generalized linear model with binary logistic regression for predictors of metastatic disease in patients with thoraco-abdominal PPGL was unable to be obtained due to a quasi-complete separation in the data.

Mortality

Nine patients (14.5% of patients with disease) died in this cohort, 1 from a non-*SDHB*–related cause (metastatic colorectal cancer on a background of ulcerative colitis). One nonproband and 7 probands died from disease: 1 from bilateral RCC, 3 from APGLs, 1 from metachronous APGL (including 1 bladder PGL), 1 from metachronous APGL/PC, and 2 from metachronous APGL/HNPGL. Of patients who died of disease, primary tumor size was a median 68 mm (range 40-105 mm), age at initial disease diagnosis was median 38 years (range 27-60 years), and 2 patients had synchronous metastases at initial diagnosis. For the remaining patients who did not have synchronous metastases, time to metastatic disease following initial diagnosis was median 71 months (range 4-193 months). Duration of survival following the diagnosis of metastatic disease was median 61 months (range 27-311 months). The age at death was median 47 years (range 29-84 years).

Patients with s-d tumors had lower mortality than probands. Risk of death from disease was 21.9% for probands and 3.4% for nonprobands (OR 7.8, 95% CI 1.2-90.1, $P = .054$). Estimated 5- and 10-year overall survival was 89% and 79% for probands and 100% for nonprobands ($P = .029$, Fig. 3C). There was no difference in overall survival for patients with thoraco-abdominal PPGL between probands and nonprobands ($P = .179$, Fig. 3D).

In nonprobands diagnosed with disease, the risk of death was 8.3% and 0% for those with clinical and genetic s-d tumors respectively ($P = .433$). Results from the multivariate generalized linear model with binary logistic regression showed that no factors were able to predict death from disease (Table 3).

Discussion

Our study is 1 of the largest to report surveillance practice, and describe s-d tumors and disease-specific outcomes for *SDHB* PV carriers in a surveillance program. We found surveillance successfully detected *SDHB*-associated tumors prior to development of metastatic disease. We found that a tumor size of 40 mm or higher was associated with increased risk of metastatic progression. Ours is the first study to show surveillance is associated with lower mortality in an *SDHB*-specific population.

Median age of disease diagnosis in our cohort was 37 years in the 62 patients with disease diagnoses. Several studies have noted *SDHB* PV carriers are more likely to be diagnosed with disease at a young age (10, 16-19). Median age at diagnosis was younger for females than males (34 vs 41 years respectively) although male gender was associated with a higher risk of disease. Jochmanova et al found that the median age at diagnosis was younger for male *SDHB* PV carriers, but also observed a higher likelihood of disease in male than in

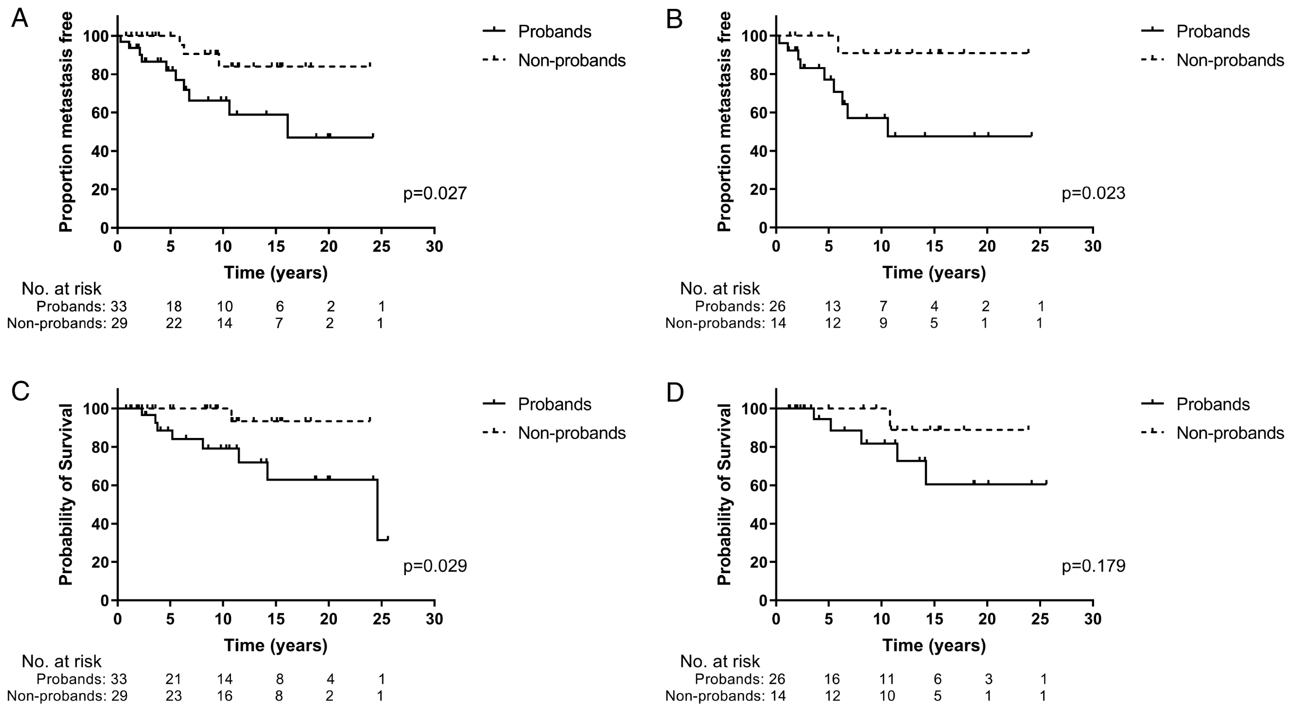

Figure 3. Kaplan–Meier curves of (A) metastasis-free survival for all patients with *SDHB*-associated tumors. (B) Metastasis-free survival for patients with thoraco-abdominal PPGL. (C) Overall survival for all patients with *SDHB*-associated tumors. (D) Overall survival for patients with thoraco-abdominal PPGL.

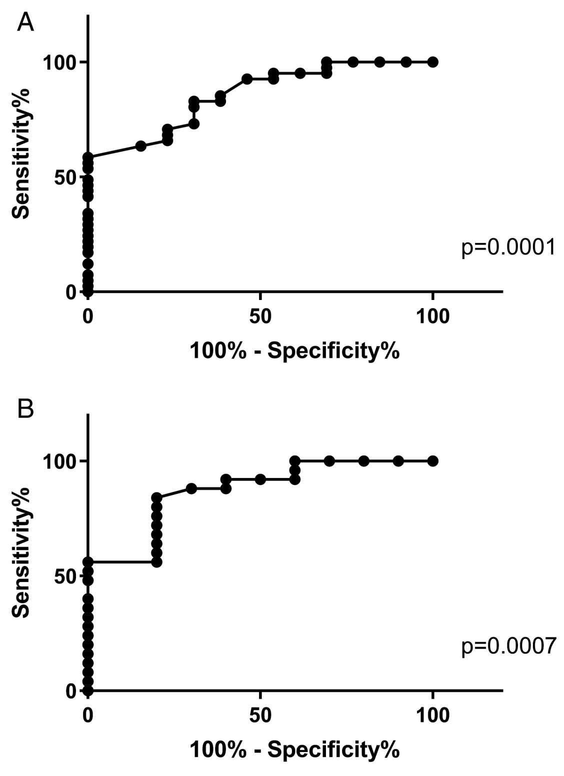

Figure 4. (A) ROC curve analysis of tumor size for prediction of metastatic disease in all patients with *SDHB*-associated tumors. (B) ROC curve analysis of tumor size for prediction of metastatic disease in patients with thoraco-abdominal PPGL.

female carriers (18). Andrews et al performed a retrospective multicenter study of 584 *SDHB* PV carriers with statistical adjustment for ascertainment bias, and similarly found a higher age-specific penetrance of HNPGL and PPGL in males (20).

The median age of first surveillance in nonprobands was 33 years (range 1-81 years). The youngest age of a s-d tumor (n = 29) was a bladder paraganglioma diagnosed at age 9 years; in the literature, APGL has been described in a proband as young as 6 years (21). The oldest age of a s-d tumor was an asymptomatic gastric GIST detected at age 76 years.

Blood pressure measurement is routinely recommended for asymptomatic *SDHB* carriers (4), but paradoxically in our cohort pretreatment hypertension was more prevalent in those without disease than those with disease. Our study may have found normotension was associated with disease due to interaction between young age and normotension, whereby young age was a risk factor for disease detection and essential hypertension is more common with advanced age (22).

Our study is the first to show the presence of "classic" symptoms of headaches, palpitations or sweats is associated with disease detection (OR 4.9), and highlights the importance of clinical history during surveillance of these patients. While association between classic symptoms and disease may have been due to ascertainment bias, we felt this was unlikely in this cohort where patients were routinely asked about symptoms at surveillance visits. Our observation that a subset of patients with apparently nonfunctioning tumors had symptoms has been noted by others (23). In a case series of 4 patients with biochemically silent *SDHB*-associated tumors, 1 patient reportedly presented with palpitations, headaches, and sweats (23). Absence of classic symptoms did not exclude disease, and indeed 10 of 29 (34%) s-d tumors were asymptomatic.

Regarding surveillance imaging modalities, MRI base of skull to coccyx formed the basis of surveillance in keeping with international guidelines (4). CT was used for anatomical imaging surveillance at all centers, but less commonly than MRI, to minimize radiation exposure per international recommendations (4). For example, as shown in the supplemental tables, 33 CT head and neck/base of skull scans were performed on the entire cohort in comparison with 189 MRI head and neck/base of skull scans. International guidelines suggest functional imaging could be considered for surveillance in adults (4). In our cohort, adults at RNSH and PoWH undertook ^{68}Ga-DOTATATE PET/CT or ^{18}F-FDG-PET/CT 5-yearly and at RHH adults had ^{18}F-FDG-PET/CT 4 yearly. At RHH, functional imaging with ^{18}F-FDG-PET/CT was undertaken as a key component of imaging surveillance and was noted to have high sensitivity and specificity for the detection of sympathetic PPGL with the advantage of providing both anatomical and functional detail. Functional imaging with ^{68}Ga-DOTATATE PET/CT had high sensitivity and specificity for sympathetic PPGL and HNPGL. PET imaging was 4 to 5 yearly rather than biennially to reduce radiation exposure, despite being sensitive and specific. Ultrasound surveillance 4 yearly at RHH was also noted to have high specificity for the detection of sympathetic PPGL and HNPGL. ^{123}I-MIBG imaging was rarely utilized after the advent of PET imaging. Future research could assess cost-effectiveness of different imaging modalities for surveillance of *SDHB* PV carriers.

We observed that probands had higher numbers of APGLs and were more likely to have multifocal disease over time (OR 3.3). Given probands did not have a higher risk of multifocal disease at baseline, we speculate this finding is due to delayed diagnosis and therefore a longer period of "tumor incubation" in probands than in nonprobands. Tufton et al reported that 10 (11%) of *SDHB* PV carriers in their cohort had multifocal disease and all were index cases (3). Bausch et al found that of 6 of 25 (24%) probands with *SDHB* PV developed a second PPGL (24) and Daniel et al reported 2 of 9 (22%) probands developed a second tumor (25). More research is needed with longer follow-up to determine whether proband/index cases with *SDHB* PV have intrinsically increased risk of multiple tumors.

We found the risk of metastatic disease in *SDHB* carriers with s-d tumors was 10% and in the overall cohort (including probands) was 21%. A systematic review and meta-analysis found the risk of metastatic disease for *SDHB* PV carriers was 17% for a cohort including probands and asymptomatic carriers (26). A more recent review found overall risk of metastatic disease was 27.6% and like our study found the risk of metastatic disease was higher in thoraco-abdominal PPGLs than HNPGLs (10). Metastases were synchronous in 2 of 13 (15%) patients, which is slightly lower than reported rates in the literature ranging from 19% to 44% of *SDHB* PV carriers with metastatic disease (3, 7, 27-30). Schovanek et al noted synchronous metastases were associated with a median tumor size of 75 mm in *SDHB* PV carriers (7), whereas our patients with synchronous metastases had primary tumor sizes of 40 mm. In patients with disease (n = 63), median time to development of metastases was 68 months (5.7 years), which was comparable with the literature where median time to metastases was 4 to 5 years (8, 31, 32).

To our knowledge, ours is the first study to compare metastasis and overall 5- and 10- year survival of *SDHB* carriers between patients with s-d tumors and probands. In total, 14.5% of patients with disease died in this study. In other studies mortality of *SDHB* PV carriers with disease was between 2.1% and 13.0% (3, 8, 9, 28, 33-36). In studies that only examined patients with metastatic PPGL, mortality rates were understandably higher from 18.5% to 60.9% (2, 32, 37, 38).

Our study has implications for surveillance recommendations for *SDHB* PV carriers. Patients with s-d tumors of 40 mm or larger and those with abdominal or pelvic PPGLs should be followed more frequently to detect metastatic progression. Probands/index cases may also be at higher risk of metastatic progression and/or multiple tumors and surveillance should be increased accordingly. Given that the age of tumor detection is broad as shown in Fig. 2, we concur lifelong surveillance should start at a young age, in keeping with the current consensus guidelines (4). The value of surveillance programs has also been reported by others (3, 39-41). Buffet et al examined patients with PPGL who carried a germline PVs of whom 95 had a confirmed *SDHB* PV; they compared overall survival between those who received germline testing within 12 months after diagnosis of their first PPGL (the genetic group) and within 7 years of the PPGL (the historic group) (39). Overall survival was 100% in the genetic group and 50% in the historic group, and the authors postulated that patients with a knowledge of their genetic risk may have experienced a higher quality of surveillance (39). Greenberg et al described surveillance of 188 *SDHB* PV carriers and observed 15% developed tumors over mean follow-up duration 1.81 ± 2.75 years (40). A multicenter study of asymptomatic *SDHx* mutation carriers (n = 171 *SDHB* carriers) noted 22 of 171 (13%) asymptomatic *SDHB* carriers had a tumor detected during surveillance and 14 of 22 (64%) tumors were detected at the initial surveillance episode (41). Tufton et al reported that 10 of 15 (67%) tumors detected during surveillance were identified at the first surveillance episode (3) and we also noted that 59% (17/29) of all s-d tumors were detected at the initial surveillance episode. The optimal frequency of surveillance following an initial negative surveillance episode deserves further research.

There were several limitations to our study. Being a medical chart review, some historic data on primary tumor characteristics and initial presentation were not available. Similarly, we were only able to observe the data available and the form in which it was entered, which varied across the centers. Extraction of data by 1 researcher ensured consistency with respect to the interpretation of chart notation where required. Although the study was multicenter it was not a nationwide Australian cohort. The association of classic symptoms with disease may have been due to ascertainment bias. More studies are needed to further evaluate the sensitivity and specificity of diagnostic modalities for *SDHB*-associated tumors. However, strengths of this study were the long duration of follow-up, small rate of loss to follow-up (42), large patient numbers, and good adherence to standardized surveillance for *SDHB* PV carriers in our genetics clinics.

Conclusion

This is one of the largest studies to describe s-d tumors and outcomes for *SDHB* PV carriers in a surveillance program. Patients with s-d tumors had smaller tumors, reduced risk of metastatic disease and lower mortality than probands. Tumor size ≥40 mm was associated with increased risk of metastatic progression. Our results suggest that *SDHB* PV carriers should undertake surveillance to improve clinical outcomes.

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Author Contributions

D.F.D. designed the study, acquired the data, performed data analyses, and drafted/approved all versions of the manuscript. R.J.C.B. and R.D.A.L. designed the study, aided with interpretation of data, and edited/approved the manuscript. D.E.B., M.F., A.C., B.G.R, K.T., and J.R.B. aided with interpretation of data and edited/approved the manuscript.

Conflicts of Interest

The authors have nothing to declare.

Data Availability

Some or all datasets generated during and/or analyzed during the current study are available from the corresponding author on request.

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THEMATIC RESEARCH

Distortion in transmission of pathogenic *SDHB*- and *SDHD*-mutated alleles from parent to offspring

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Abstract

Phaeochromocytoma and paraganglioma are highly heritable tumours; half of those associated with a germline mutation are caused by mutations in genes for Krebs's cycle enzymes, including *succinate dehydrogenase* (*SDH*). Inheritance of *SDH* alleles is assumed to be Mendelian (probability of 50% from each parent). The departure from transmission of parental alleles in a ratio of 1:1 is termed transmission ratio distortion (TRD). We sought to assess whether TRD occurs in the transmission of *SDHB* pathogenic variants (PVs). This study was conducted with 41 families of a discovery cohort from Royal North Shore Hospital, Australia, and 41 families from a validation cohort from St. Bartholomew's Hospital, United Kingdom (UK). Inclusion criteria were a clinically diagnosed *SDHB* PV and a pedigree available for at least two generations. TRD was assessed in 575 participants with the exact binomial test. The transmission ratio for *SDHB* PV was 0.59 ($P = 0.005$) in the discovery cohort, 0.67 ($P < 0.001$) in the validation cohort, and 0.63 ($P < 0.001$) in the combined cohort. No parent-of-origin effect was observed. TRD remained significant after adjusting for potential confounders: 0.67 ($P < 0.001$) excluding families with incomplete family size data; 0.58 ($P < 0.001$) when probands were excluded. TRD was also evident for *SDHD* PVs in a cohort of 81 patients from 13 families from the UK. The reason for TRD of *SDHB* and *SDHD* PVs is unknown, but we hypothesize a survival advantage selected during early embryogenesis. The existence of TRD for *SDHB* and *SDHD* has implications for reproductive counselling, and further research into the heterozygote state.

Key Words

- phaeochromocytoma
- molecular genetics

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Introduction

The phaeochromocytoma and paraganglioma (PPGL) tumour group is the most heritable of tumours, with at least 40% of cases arising from a pathogenic germline mutation (Dahia 2014). Of these, around half are caused by pathogenic variants (PVs) in genes encoding critical enzymes of the tricarboxylic acid cycle, including *succinate dehydrogenase (SDH)*. PVs in *SDH* subunits result in loss of function of the SDH protein complex; *SDH*-deficient tumours are in the cluster of PPGL with a pseudo-hypoxic cellular response and the greatest potential for metastatic disease (Nölting *et al.* 2022), and the metastatic tendency is particularly apparent with PVs in the *SDHB* subunit. Inheritance of *SDH* alleles is assumed to follow Mendelian laws of segregation, with a probability of 50% from each parent, but confirmation of this in clinical practice is made difficult by the highly variable penetrance across the subunits *SDH-A* to *-D* (Tufton *et al.* 2019) and the rarity of *SDH*-deficient tumours in general.

There exist monogenic familial diseases which are not necessarily transmitted according to Mendelian laws of inheritance, including Factor V Leiden deficiency (Infante-Rivard & Weinberg 2005), Long QT syndrome (Imboden *et al.* 2006), and some of the spinocerebellar ataxias (Riess *et al.* 1997, Bettencourt *et al.* 2008) (Table 1). The departure from a transmission of parental alleles in a ratio of 1:1 is termed transmission ratio distortion (TRD) (Pardo-Manuel de Villena *et al.* 2000). There are five key timepoints at which TRD can occur (Huang *et al.* 2013): (i) germline selection (e.g. mutation, recombination, non-allelic gene conversion) during mitosis; (ii) mechanisms that occur in meiosis and prior to fertilization known as meiotic drive, where the structural characteristics of a certain chromatid result in increased transmission during oogenesis (maternal germline) or spermatogenesis (paternal germline); (iii) gametic competition (by sperm) prior to fertilization, resulting in gamete selection; (iv) imprint resetting at the post-implantation stage, when parental imprints are erased and re-established; and (v)

Table 1 Genes known to demonstrate transmission ratio distortion.

Gene	Function
	Relates to tumourigenesis
CDKN1C	Tumour suppressor
HRAS1	Oncogene
IGF2	Intestinal adenoma
RB-1	Retinoblastoma tumour suppressor
SIRT3	Node-positive breast cancer
TNFa and *TNFb*	Tumour necrosis
	Relates to neurological development
ARX	Non-syndromic intellectual disability and brain malformations
CTDP1	Congenital cataract, facial dysmorphism, peripheral neuropathy
DMPK	Muscular dystrophy
HASH2 (ASCL2)	Neuronal precursor for central and peripheral nervous systems
SCA1, SCA3 (ATXN3)	Spinocerebellar ataxia types 1 and 3 (respectively)
SMN1	Spinal muscular atrophy
TH	Neuropathology
	Overlap of roles in tumourigenesis and early neurological development
DBC1, CDK5RAP2, MEGF9	Neuronal differentiation; bladder cancer
MTHFR	Acute leukaemia; colon cancer; neural tube defects
NBPF8 and *HFE2*	Neuroblastoma tumour suppressor, cognitive development; iron metabolism
	Other
ATG16L1, DLG5	Inflammatory bowel disease
BHLHA9	Split-hand/foot malformation +/− Long bone deficiency
CLC1, IGFR2 (FCGR2B)	Autoimmunity
F2 (Factor II / thrombin)	Thrombosis
F5 (Factor V Leiden)	Thrombophilia
HSP70.1	Graft vs host disease
INS	Hyperinsulinism
KCNQ1, KCNH2	Long QT syndrome
STX16-GNAS	Autosomal dominant pseudohypoparathyroidism type 1b
SUPT3H-MIRN586-RUNX2	Cleft palate; skeletal morphogenesis; haematological neoplasia
TGFB1	Cystic fibrosis severity and endophenotype

Data from Huang *et al.* (2013). See references for further details.

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post-fertilization mechanisms of embryonic or neonatal lethality from the inherited allele, resulting in differential survival of offspring. We suggest that at this time point, advantageous selection may also occur (Fig. 1).

Our interest in whether *SDH* PVs are inherited according to Mendelian laws of segregation or in an imbalanced, distorted way arose anecdotally: an *SDHB* PV carrier underwent pre-implantation genetic testing and reported that high numbers of embryos harboured the affected allele. We sought to assess whether TRD occurs in the transmission of *SDHB* PVs and posit that a post-fertilization survival advantage is the cause.

Materials and methods

This study has been conducted with 41 families of a discovery cohort in Australia, from Royal North Shore Hospital (RNSH), and a validation cohort in the United Kingdom, from St. Bartholomew's Hospital (SBH), together representing a range of different PVs in the *SDHB* gene. Inclusion criteria were a confirmed *SDHB* PV and a pedigree available for at least two generations (such that data on transmission could be analysed from the second generation onwards). Probands were defined as the first individual in a family to be diagnosed with an *SDHB* PV after presenting with a PPGL. PVs were classified as loss of function (nonsense, splicing, deletion, or frameshift) or missense. PVs were defined as being in the proximal region of the *SDHB* gene if they occurred in exons 1–3 or intronic regions up to IVS3. Ethics approval was obtained from the Northern Sydney Local Health District Ethics Committee for the discovery cohort (Ref: 2022/ETH01880), including waiver of consent, and Cambridge East Medical Research Ethics Committee for the validation cohort (Ref: 06/Q0104/133). Patients provided consent after a full explanation of the purpose of the study.

Statistical analysis was performed using IBM SPSS version 28. The forest plot figure was produced using GraphPad Prism version 9. Categorical data were tested with the binomial test to obtain a true estimate, with 95% confidence intervals using the Clopper–Pearson method. Continuous data were assessed with the exact Mann–Whitney test for non-parametric data. A P-value ≤ 0.05 (two-tailed) was considered statistically significant. Results that were not significant were assessed for heterogeneity with Levene's test. Several sensitivity analyses were undertaken in this study by (i) excluding probands, (ii) excluding families with incomplete family pedigree data, and (iii) excluding untested participants younger than 20 years of age. Potential predictors of TRD were assessed in the cohort that underwent genetic testing and the cohort with complete family data, using a generalized linear model with binary logistic regression to perform a multivariate analysis. Explanatory variables included in this model were sex, genotype, parent of origin, birth order, and family size.

Results

A total of 575 participants from 82 families from RNSH and SBH were assessed. There was a difference between centres in the proportion that underwent genetic testing and the number of generations assessed in each family (Table 2). Of the 575 participants assessed, 503 underwent genetic testing, with 316 found to harbour an *SDHB* PV. Thirty-six different *SDHB* PVs were represented in the combined cohort: 12 missense PVs were present in 29 families, and a further 24 PVs were loss of function mutations (Supplementary Table 1, see section on supplementary

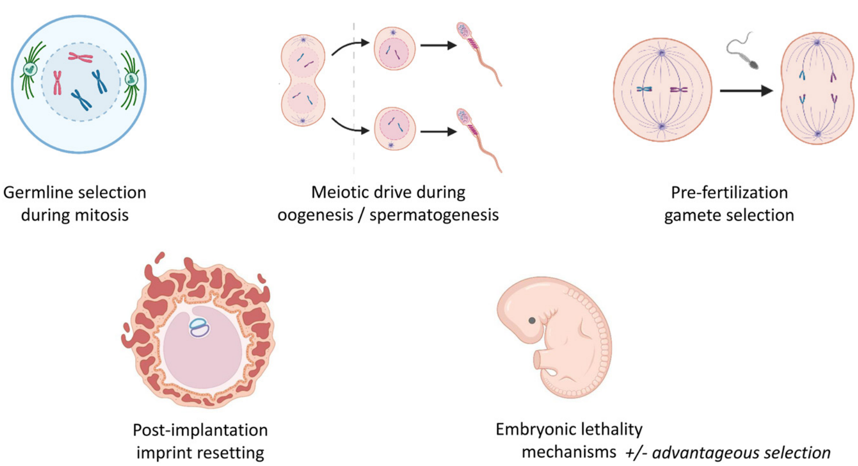

Figure 1
Five key timepoints at which transmission ratio distortion may occur. Created with https://www.biorender.com/.

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Table 2 Baseline characteristics of *SDHB* cohorts.

	RNSH – discovery cohort ($n = 279$)	SBH – validation cohort ($n = 296$)	RNSH and SBH – combined cohort ($n = 575$)	*P*-value
Male, *n* (%)	133 (48)	149 (50)	282 (49)	0.48
Probands, *n* (%)	32 (11)	25 (8)	57 (10)	0.23
Genetic testing, *n* (%)	260 (93)	243 (82)	503 (88)	<0.001[b]
Loss of function pathogenic variant, *n* (%)	172 (62)	179 (60)	351 (61)	0.77
Birth order				
First, *n* (%)	49 (18)	120 (41)	169 (29)	0.43
Second, *n* (%)	47 (17)	96 (32)	143 (25)	
Third or later, *n* (%)	39 (14)	64 (22)	103 (18)	
Not available, *n* (%)	144 (52)	16 (5)	160 (28)	
Family size: children				
One, *n* (%)	15 (5)	20 (7)	35 (6)	0.08
Two, *n* (%)	71 (25)	120 (41)	190 (33)	
Three or more, *n* (%)	188 (67)	156 (53)	345 (60)	
Not available, *n* (%)	5 (2)	0	5 (1)	
Family size: generations				
Two; *n* families (%)	18 (44)	11 (27)	29 (35)	0.02[a]
Three; *n* families (%)	23 (56)	25 (61)	48 (59)	
Four; *n* families (%)	0	5 (12)	5 (6)	

[a]*P*-value < 0.05; [b]*P*-value < 0.01.

materials given at the end of this article). Disease had manifested in approximately 19% of *SDHB* participants at any timepoint, which is similar to reported disease penetrance in the literature (Benn *et al.* 2018, Rijken *et al.* 2018). In the validation cohort, most families (61%) were represented by three generations and most nuclear families had two or three offspring (biological children.)

The transmission ratio for *SDHB* PV was 0.59 ($P = 0.005$) in the discovery cohort (Table 3), 0.67 ($P < 0.001$) in the validation cohort, and 0.63 ($P < 0.001$) in the combined

Table 3 Transmission ratio in *SDHB* families in the discovery cohort.

	Actual (95% CI)	Expected	*P*-value
Cohort that underwent genetic testing ($n = 260$)	0.59 (0.53–0.65)	0.50	0.005[b]
Probands excluded ($n = 228$)	0.53 (0.46–0.60)	0.50	0.40
Cohort with complete family size data ($n = 30$)	0.63 (0.44–0.80)	0.50	0.20
Cohort excluding those <20 years of age without genetic test ($n = 269$)	0.57 (0.51–0.63)	0.50	0.03[a]
Paternal inheritance ($n = 123$)	0.62 (0.53–0.70)	0.50	0.01[a]
Maternal inheritance ($n = 124$)	0.57 (0.47–0.65)	0.50	0.18
Loss of function pathogenic variant ($n = 157$)	0.59 (0.51–0.67)	0.50	0.03[a]
Missense pathogenic variant ($n = 103$)	0.58 (0.48–0.68)	0.50	0.12
Pathogenic variant in exons 1–3 or intronic region up to IVS3 ($n = 193$)	0.59 (0.52–0.66)	0.50	0.01[a]
Pathogenic variant in exons 4–8 or intronic region from IVS3 to IVS6 ($n = 60$)	0.57 (0.43–0.69)	0.50	0.37
Male sex ($n = 125$)	0.59 (0.51–0.68)	0.50	0.04[a]
Female sex ($n = 135$)	0.58 (0.49–0.67)	0.50	0.06
Second generation ($n = 156$)	0.64 (0.56–0.72)	0.50	<0.001[b]
Third generation ($n = 104$)	0.51 (0.41–0.61)	0.50	0.92
Birth order first[c] ($n = 13$)	0.69 (0.39–0.91)	0.50	0.27
Birth order second[c] ($n = 10$)	0.60 (0.26–0.89)	0.50	0.75
Birth order third or later[c] ($n = 6$)	0.50 (0.12–0.88)	0.50	1.0
Family size one child[c] ($n = 1$)	1.0 (0.25–1.0)	0.50	1.0
Family size two children[c] ($n = 12$)	0.58 (0.28–0.85)	0.50	0.77
Family size three or more children[c] ($n = 17$)	0.65 (0.38–0.86)	0.50	0.33

[a]*P*-value < 0.05; [b]*P*-value < 0.01; [c]Analysis in families with complete family size data.

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Table 4 Transmission ratio in *SDHB* families in the combined cohort.

	Actual (95% CI)	**Expected**	***P*-value**
Cohort that underwent genetic testing (*n* = 503)	0.63 (0.58–0.67)	0.50	<0.001[b]
Probands excluded (*n* = 446)	0.58 (0.53–0.63)	0.50	<0.001[b]
Cohort with complete family size data (*n* = 273)	0.67 (0.61–0.72)	0.50	<0.001[b]
Cohort excluding those < 20 years of age without genetic test (*n* = 539)	0.59 (0.54–0.63)	0.50	<0.001[b]
Paternal inheritance (*n* = 234)	0.64 (0.57–0.70)	0.50	<0.001[b]
Maternal inheritance (*n* = 252)	0.64 (0.57–0.69)	0.50	<0.001[b]
Loss of function pathogenic variant (*n* = 297)	0.66 (0.60–0.71)	0.50	<0.001[b]
Missense pathogenic variant (*n* = 206)	0.59 (0.52–0.66)	0.50	0.02[a]
Pathogenic variant in exons 1–3 or intronic region up to IVS3 (*n* = 344)	0.63 (0.58–0.68)	0.50	<0.001[b]
Pathogenic variant in exons 4–8 or intronic region from IVS3 to IVS6 (*n* = 152)	0.62 (0.54–0.70)	0.50	0.005[b]
Male sex (*n* = 236)	0.66 (0.59–0.72)	0.50	<0.001[b]
Female sex (*n* = 264)	0.60 (0.54–0.66)	0.50	0.001[b]
Second generation (*n* = 294)	0.66 (0.60–0.71)	0.50	<0.001[b]
Third generation (*n* = 194)	0.60 (0.53–0.67)	0.50	0.005[b]
Birth order first[c] (*n* = 110)	0.75 (0.65–0.82)	0.50	<0.001[b]
Birth order second[c] (*n* = 90)	0.67 (0.60–0.76)	0.50	0.002b
Birth order third or later[c] (*n* = 56)	0.55 (0.42–0.69)	0.50	0.50
Family size one child[c] (*n* = 15)	1.00 (0.78–1.00)	0.50	<0.001[b]
Family size two children[c] (*n* = 112)	0.72 (0.63–0.80)	0.50	<0.001[b]
Family size three or more children[c] (*n* = 146)	0.59 (0.51–0.67)	0.50	0.004[b]

[a]*P*-value < 0.05; [b]*P*-value < 0.01; [c]Analysis in families with complete family size data.

cohort (Table 4 and Fig. 2). For the discovery cohort, TRD was apparent when analysing for each of paternal inheritance, loss of function PV, mutation within the proximal region of the gene (exons 1–3 and up to IVS3), male sex, and the second generation from the proband (Table 3). No parent-of-origin effect was observed in the combined cohort. TRD remained significant after adjusting for potential confounders: 0.67 ($P < 0.001$) if families with incomplete family size data were excluded and 0.58 ($P < 0.001$) if probands were excluded. Of the

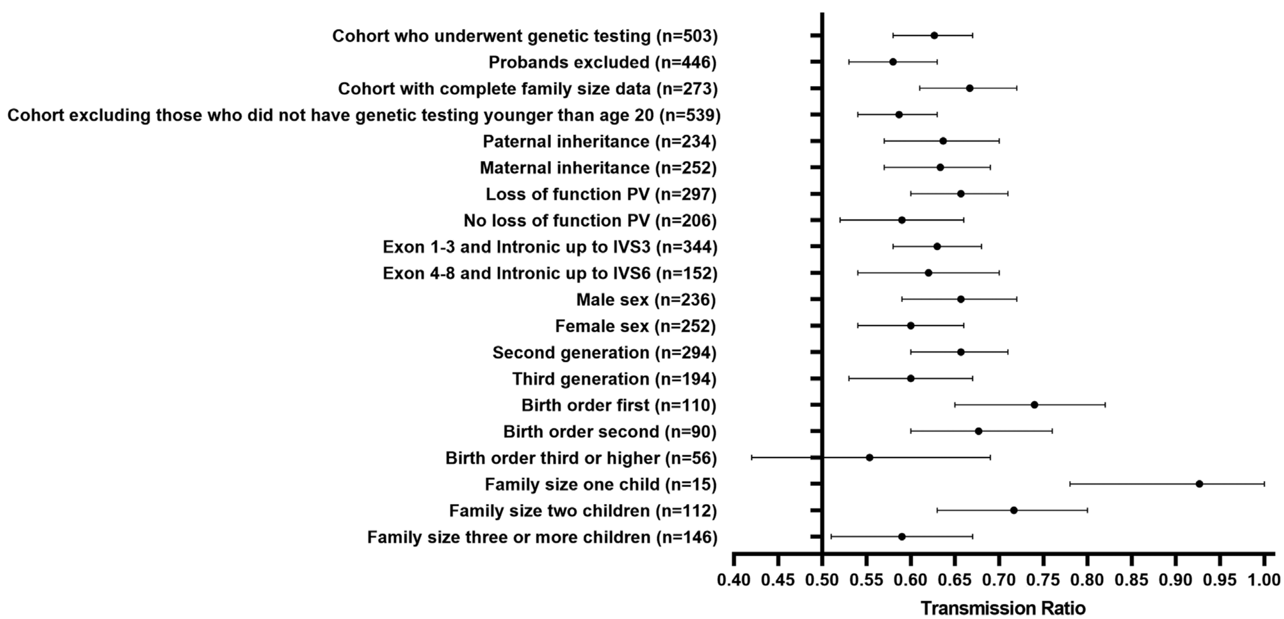

Figure 2
Forest plot of transmission ratio in *SDHB* families in the combined cohort.

Table 5 Generalized linear model with binary logistic regression of predictors of TRD in the combined cohort of participants that underwent genetic testing ($n = 503$).

Predictors of TRD	*P*-value	OR (95% CI)
Male	0.41	0.85 (0.69–1.24)
Loss of function pathogenic variant	0.21	1.29 (0.87–1.91)
PV in exons 1–3 or intronic region up to IVS3	0.79	0.94 (0.62–1.44)
Paternal inheritance	0.96	0.99 (0.68–1.44)
Test	***P*-value**	χ^2
Overall model likelihood ratio test (omnibus test)	0.99	2.39

72 individuals who did not have genetic testing, 36 were younger than 20 years of age; after excluding these participants, the transmission ratio was 0.59 ($P < 0.001$). No factors predicted TRD on a generalized linear model with binary logistic regression (Tables 5 and 6).

Transmission ratio analysis was replicated for the *SDHD* cohort at St Bartholomew's Hospital: 81 patients from 13 families, most commonly of 3 generations (range 2–4) and with 2 children per nuclear family (range 1–5) (Table 7). Phenotype expression was dependent on paternal inheritance, as expected. Of the 61 participants with confirmed germline testing, 43 harboured an *SDHD* PV (Supplementary Table 2), which represents a significant distortion in transmission ratio: 0.70 ($P = 0.0019$). TRD in *SDHD* was upheld even when assuming that a Mendelian 50% of those with unknown genotypes were carriers (0.65, $P = 0.0073$). Neither clinical centre had *SDHA* or *SDHC* cohorts of sufficient size for analysis.

Discussion

A TRD of 60% in favour of the *SDHB* PV being transmitted was evident in our cohort. In the discovery cohort, it appeared that TRD was associated with particular variables, but the analysis was limited by incomplete family data and insufficient power. In the combined cohort, TRD was observed irrespective of sex, parent of origin, loss of function PV, or location of the mutation within the proximal or distal region of the gene. Given that rates of genetic testing differed between centres, we assessed complete family data. When families with incomplete family size data were excluded, TRD was still noted. When probands were excluded, TRD still occurred, suggesting TRD was not due to oversampling of cases (Gemechu *et al.* 2020). We considered the possibility of bias in young individuals not undergoing genetic testing due to being asymptomatic since the median age of disease diagnosis is 37 years (Davidoff *et al.* 2022), but after the exclusion of participants younger than age 20 years without a genetic test, TRD was still observed. Birth order third or later was not associated with TRD, likely due to insufficient power ($n = 56$), given that heterogeneity was absent on Levene's test. As TRD was consistently observed across different variables, the finding that no particular factors predicted TRD on the generalized linear model was unsurprising.

The 60% distortion in transmission of pathogenic *SDHB* alleles is consistent with the magnitude of other examples of TRD: PVs of the tumour suppressor gene for retinoblastoma, *RB-1,* were found to have 63% transmission from affected males to sons (Naumova & Sapienza 1994); *STX16-GNAS* mutations in autosomal dominant pseudohypoparathyroidism type Ib were transmitted to 63% of offspring (Kiuchi *et al.* 2021); mutated alleles in long-QT syndrome conferred 55% transmission to female offspring (Imboden *et al.* 2006); and a study of embryos

Table 6 Generalized linear model with binary logistic regression of potential predictors of TRD in the combined cohort of families with complete family size data ($n = 273$).

Predictors of TRD	*P*-value	OR (95% CI)
Male	0.23	0.71 (0.41–1.24)
Loss of function pathogenic variant	0.06	1.73 (0.97–3.11)
PV in exons 1–3 or intronic region up to IVS3	0.83	0.93 (0.49–1.78)
Paternal inheritance	0.99	1.00 (0.56–1.80)
Birth order	0.67	1.06 (0.80–1.41)
Family size (number of children)	0.37	1.10 (0.88–1.41)
Test	***P*-value**	χ^2
Overall model likelihood ratio test (omnibus test)	0.12	10.05

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Table 7 Baseline characteristics of the *SDHD* cohort.

SDHD* cohort, *n	**81**
Male, *n* (%)	45 (57)
Probands, *n* (%	13 (16)
Genetic testing, *n* (%)	61 (75)
Loss of function pathogenic variant, *n* (%)	18 (22)
Birth order	
First, *n* (%)	39 (48)
Second, *n* (%)	25 (31)
Third or later, *n* (%)	17 (21)
Family size: children	
One, *n* (%)	10 (12)
Two, *n* (%)	14 (17)
Three or more, *n* (%)	9 (11)
Family size: generations analysed	
Two, *n* (%)	41 (51) from 8 families
Three, *n* (%)	25 (31) from 3 families
Four, *n* (%)	15 (18) from 2 families

from preimplantation genetic testing for myotonic dystrophy type 1 found that 59% harboured the CTG nucleotide repeat expansion (Dean *et al.* 2006).

It has been suggested that the phenomenon of anticipation is apparent in *SDH*-deficient disease (Antonio *et al.* 2020), albeit without a trinucleotide repeat expansion to facilitate this in a classical way. Whilst an earlier age of tumour diagnosis was documented in some subsequent generations, this was attributed to an early age of screening and surveillance; furthermore, this phenomenon was not borne out across our 82 families to suggest a genuine pattern of an underlying biological change in disease penetrance across the generations.

Limitations to this study included some uncollected data that could hypothetically influence the interpretation of inheritance patterns, such as age of parenthood, miscarriage rate, and birth order. The sample size was robust relative to accessible cohorts of rare disease but may limit extrapolation from statistical significance to biological significance, such as with the question of a parent-of-origin effect on transmission. However, to counter potential sources of bias, we tested the sensitivity of our results by excluding, in turn, possible confounders: families with incomplete family size data, probands, and untested participants less than 20 years old. None of these analyses significantly altered the main finding of TRD in favour of *SDHB* PVs.

The reason for a TRD in *SDH-B* and *-D* is unknown. We hypothesize the mechanism could occur at the post-fertilization stage and arise as a selective advantage, perhaps for adapting to hypoxia; however, assessing the timing and mechanisms for TRD was beyond the scope of the present study. It is fascinating to consider how an embryonic survival advantage for hypoxia/pseudo-hypoxia might then be accompanied by variably penetrant tumour risk in postnatal life. Intriguingly, several tumour suppressor genes and oncogenes have also been demonstrated to manifest TRD in favour of the mutant allele (Huang *et al.* 2013), including *CDKN1C* (Sazhenova & Lebedev 2008), *HRAS1*, and *SIRT3* (De Rango *et al.* 2008). Moreover, *CDKN1C* has been implicated in the pathogenicity of *SDHAF2* and *SDHD* mutations when arising from loss of maternal chromosome 11 (Hoekstra *et al.* 2017), whereas HRAS and sirtuin-3 both regulate mitochondrial function (Dard *et al.* 2022, Papa & Germain 2014).

The clinical relevance of distortion in the transmission of *SDH-B* and *-D* mutations is immediately apparent: a TRD that favours the potential for tumourigenesis has significant implications for genetic counselling of all carriers. The role of pre-implantation genetic diagnosis is arguably stronger when the odds are against the likelihood of healthy offspring. We encourage other centres to analyse their cohorts similarly to validate our findings, with a view to updating guidelines on genetic counselling. An understanding of the mechanism behind TRD in *SDH*, where the heterozygous state may have an advantage, might lead to insights that later allow interventions in carriers to decrease the risk of tumour development.

Supplementary materials

This is linked to the online version of the paper at https://doi.org/10.1530/ERC-22-0233.

Declaration of interest

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Author contribution statement

S A Akker and R J Clifton-Bligh authors are Senior co-authors. Conceptualization: RCB; Data curation: DFD, ESL, DEB, YS, ED, JRB; Formal analysis: DFD, ESL, ED; Writing – original draft: DFD, ESL; Writing – review & editing: RCB, SAA

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Meta-Analysis

Outcomes of *SDHB* Pathogenic Variant Carriers

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Abstract

Context: Carriers of germline pathogenic variants (PVs) in succinate dehydrogenase type B (SDHB) are at increased risk of developing pheochromocytomas and paragangliomas (PPGLs). Understanding their outcomes can guide recommendations for risk assessment and early detection.

Objective: We performed a systematic review and meta-analysis of the following outcomes in SDHB PV carriers: age-specific risk of developing tumors, metastatic progression, second primary tumor development, and mortality.

Methods: PubMed, MEDLINE, and EMBASE were searched. Sixteen studies met the inclusion criteria and were sorted into 4 outcome categories: age-specific penetrance, metastatic disease, risk of second tumor, and mortality. We assessed heterogeneity and performed a meta-analysis across studies using a random-effects model with the DerSimonian and Laird method.

Results: Penetrance of PPGLs for nonproband/nonindex SDHB PV carriers by age 20 was 4% (95% CI, 3%-6%), 11% (95% CI, 8%-15%) by age 40, 24% (95% CI, 19%-31%) by age 60%, and 35% (95% CI, 25%-47%) by age 80. The overall risk of metastatic disease for nonproband/nonindex carriers with PPGLs was 9% (95%, CI 5%-16%) per lifetime. In all affected cases (combining both proband/index and nonproband/nonindex carriers with tumors), the risk of a second tumor was 24% (95% CI, 18%-31%) and all-cause 5-year mortality was 18% (95% CI, 6%-40%).

Conclusion: Penetrance for PPGLs in SDHB PV carriers increases linearly with age. Affected carriers are at risk of developing and dying of metastatic disease, or of developing second tumors. Lifelong surveillance is appropriate.

Key Words: SDHB, succinate dehydrogenase, pheochromocytoma, paraganglioma

Abbreviations: HNPGL, head or neck paraganglioma; PV, pathogenic variant; PPGL, pheochromocytomas and paraganglioma; SDHB, succinate dehydrogenase type B; TAPPGL, thoracoabdominal paragangliomas and pheochromocytoma; VUS, variants of uncertain significance.

Carriers of germline pathogenic variants (PVs) in succinate dehydrogenase type B (*SDHB*) are at increased risk of developing pheochromocytomas and paragangliomas (PPGLs) (1). Pheochromocytomas arise from chromaffin cells in the adrenal medulla, and paragangliomas arise from chromaffin cells in sympathetic, or chief cells in parasympathetic, ganglia (2). Overall, around 20% of all PPGL cases are attributed to germline PVs in genes encoding *SDH* subunits (*SDHx*), with the most frequent being *SDHB* (3). *SDHB* PV carriers are typically identified after a proband/index patient presents with PPGL and undergoes genetic testing, followed by family risk notification and predictive testing.

SDHB PV carriers have a significantly increased risk of metastatic progression and mortality. A recent analysis of 448 proband cases from a registry data set noted that metastatic disease affected 27% of *SDHB* PV carriers (4). In another paper, proband patients were 4 times more likely to develop metastatic disease compared with nonproband cases (5). A recent analysis of PPGLs from the Cancer Genome Atlas found that patients with PPGLs associated with a germline *SDHB* PV had significantly higher 15-year mortality (HR = 4.7), compared to non-*SDHB* cases (6).

Understanding the outcomes facing this group is clinically important, as it can help to inform patients, their families, and health-care providers regarding risk assessment, early detection, and intervention strategies. Previous systematic reviews and meta-analyses (7-9) have not focused on outcomes facing nonproband/nonindex *SDHB* PV carriers. The risk of metastatic progression for nonindex *SDHB* PV carriers was last assessed in a systematic review and meta-analysis more than a decade ago (10), and in light of subsequent studies deserves an update. The aim of this study was to assess the following outcomes in *SDHB* PV carriers by meta-analysis of published studies in the field: age-specific risk of developing tumors, risk of metastatic progression, risk of developing a second primary tumor, and risk of death. An understanding of these risks is crucial

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for optimizing patient management and improving clinical outcomes, because these 4 risks are key factors in driving the high burden of disease facing *SDHB* PV carriers and health-care utilization. Tumor development is associated with symptoms of catecholamine excess and/or mass-effect symptoms to surrounding structures (11); each subsequent tumor increases the likelihood of morbidity and mortality; metastatic progression is associated with morbidity from tumor symptoms (11) and is a risk factor for mortality (12, 13).

Materials and Methods

Eligibility Criteria

We searched for original retrospective and prospective observational studies reporting on outcomes for *SDHB* PV carriers whose carrier status was confirmed on genetic testing. Studies were included if they reported on any of the following outcome measures: (1) age-specific penetrance (age 20, 40, 60, and/or 80 years), (2) number or proportion of *SDHB* PV carriers who developed metastatic disease, (3) number or proportion of *SDHB* PV carriers who developed second tumor(s), and (4) mortality of *SDHB* PV carriers with metastatic disease. Penetrance was defined as the proportion of *SDHB* PV carriers who developed PPGL. Metastasis was defined by World Health Organization classification as the presence of chromaffin tissue in nonchromaffin organs such as lymph nodes, liver, lungs, and bone (2). In the event a study reported metastasis on imaging without a biopsy diagnosis, we categorized this as metastasis and not as a second tumor. We classified second tumor only when a study used the phrases "second tumor" or "multifocal" or provided individual patient data on the particular second tumor diagnoses (the type of head or neck paraganglioma [HNPGL] or thoracoabdominal paragangliomas and pheochromocytomas [TAPPGLs]). Mortality was defined as the number of patients who died of the disease. For mortality estimates we included studies that reported 5-year mortality of *SDHB* PV carriers with metastatic disease to minimize bias from studies with unclear or short duration of follow-up. We did not exclude studies that reported on *SDHB* PV carriers who presented with symptomatic disease. We also did not exclude studies with probands, defined as the first individual in a family to be diagnosed with an *SDHB* PV after presenting with a tumor, nor did we exclude studies with index cases, defined as the first identified case of *SDHB* PV. However, if data on nonproband/nonindex *SDHB* PV carriers were available, we assessed these results preferentially to data on probands/index carriers. We only included results for nonproband/nonindex cases for the outcomes of age-specific penetrance and risk of metastatic progression. However, for risk of second tumor, we found only one study that reported nonproband/nonindex carrier data, so we could not assess nonproband/nonindex carrier data preferentially. Similarly for mortality risk, we found only one study that reported nonproband/nonindex carrier 5-year mortality data, so we could not assess nonproband/nonindex carrier data preferentially.

As a systematic review and meta-analysis has been performed looking at the risk of metastatic disease for the combined group of proband/index and nonproband/nonindex *SDHB* PV carriers (9), we performed a meta-analysis of the risk of metastatic progression for nonproband/nonindex *SDHB* PV carriers with disease only. Studies with fewer than 10 *SDHB* PV carriers were excluded. Where studies reported on the same cohort of patients, we assessed the study with the larger number of *SDHB* PV carriers addressing the outcome of interest.

Search Strategy

In March 2022 the databases PubMed, Ovid MEDLINE, and Ovid EMBASE were searched by D.F.D. Studies were limited to those in humans but there was no limit on language. Additional records were identified through primary article references. The PubMed search was as follows: ((paraganglioma or pheochromocytoma) and (succinate or SDHB or SDH)) limited to humans. The Ovid MEDLINE search was as follows: (succinates/or succinate.mp. or SDHB.mp.) and (exp paraganglioma/or paraganglioma.mp.) limited to humans. The Ovid EMBASE search was as follows: (exp succinate dehydrogenase/or succinate.mp. or sdhb.mp. or sdh.mp) and (exp paraganglioma/or exp pheochromocytoma/or paraganglioma.mp or pheochromocytoma) limited to humans.

Data Selection

Studies from the search were entered into the reference management software (Endnote X9). Duplicates were removed and articles were screened for eligibility based on title and abstract by D.F.D. Basic science reports, case reports, review articles, editorials, conference abstracts with insufficient data, nonoriginal research, and unrelated articles (those that did not address the research questions or meet the inclusion criteria) were removed. Once relevant full-text articles were obtained, a full-text review of screened articles was conducted by authors D.F.D., D.E.B., V.H.M.T., and R.C.B. with disagreements determined by group consensus with R.D.A.L. We performed an analysis of the penetrance data from our cohort (5) (Appendix 1 (14)) and included the data in the pooled penetrance assessment.

Articles were separated into 4 categories according to penetrance for nonproband/nonindex *SDHB* PV carriers, risk of metastatic disease for *SDHB* PV carriers with disease, risk of a second tumor for *SDHB* PV carriers with disease, and 5-year mortality for *SDHB* PV carriers with metastatic disease.

Data Extraction

The following data were extracted from eligible articles: first author, year of publication, observational study design, country, data collection, duration of follow-up, number of participants who were nonproband/nonindex *SDHB* PV carriers, number of nonproband/nonindex *SDHB* PV carriers who developed disease by age 20, 40, 60, and/or 80 years based on reported age-specific penetrance, total number of *SDHB* PV carriers who developed disease, number of *SDHB* PV carriers who developed metastatic disease, number of *SDHB* PV carriers who developed a second tumor, and number of *SDHB* PV carriers with metastatic disease who died within 5 years. Studies were classified as either cohort studies (with follow-up of a cohort of *SDHB* PV carriers to observe who developed outcomes of interest (15)) or cross-sectional; as retrospective or prospective; and as single-center or multicenter.

Risk of Bias Assessment

The quality of the observational studies was assessed independently by authors D.F.D., D.E.B., and R.C.B. using a modified Newcastle-Ottawa tool described by Hamidi et al

(8) and further adapted to this study. The following were described: (1) how the sample represented the population of interest, (2) how genetic information was assessed, (3) how the outcome measures were assessed, (4) sufficient duration of follow-up and, (5) adequacy of follow-up. A detailed description of the tool is listed in the supplementary material (14). The tool used by Hamidi et al (8) was adapted by replacing their question "how the data on metastatic PPGL was collected" with "how genetic information was assessed" because we assessed *SDHB* PV carriers only and we assessed 4 outcomes rather than the 1 outcome of metastatic disease risk.

Statistical Analyses

Penetrance in our cohort of *SDHB* PV carriers (5) was performed using the Kaplan-Meier method. The outcome of the meta-analysis was the pooled penetrance of PPGL in *SDHB* PV carriers. The proportion of patients with PPGLs by age 20, 40, 60, and 80 years was calculated as the number of patients with PPGLs at those ages divided by the total number of *SDHB* PV carriers. Probands/Index cases were excluded to reduce ascertainment bias (16). For all 4 outcomes of interest, a meta-analysis of proportions was performed by using a random-effects generalized linear mixed model due to expected differences in the populations from which data was pooled. The random-effects model was determined using the DerSimonian and Laird method (17), with the estimate of heterogeneity taken from the inverse variance method. CIs were obtained using the Jackson method. Prediction intervals were calculated to denote the penetrance that may be observed in future studies. Publication bias assessment was attempted but ultimately could not be assessed with Egger's regression test, funnel plot asymmetry or the meta-regression "weightr" package in R, because there were too few studies and insufficient data to run these tests, defined as fewer than 10 studies for each pooled assessment (18). Analyses were performed with R version 4.2.2.

Results

Study Selection

The search of publications produced 4035 references. Duplicates were removed and abstracts reviewed, leaving 241 publications for assessment. The manuscripts were reviewed and a further 224 publications were removed: A total of 12 publications were eliminated due to being review articles, 9 publications were conference abstracts, 29 studies were case reports, 13 studies were case reports on *SDHB* PV carriers, 2 were pathology studies, 132 publications were unrelated, and 19 were basic science publications. One study was an identical cohort presented in another publication by the same authors. Two studies reported risk of metastatic disease but were not included in the assessment because there were fewer than 10 nonproband/nonindex *SDHB* PV carriers with disease. Five studies that reported risk of metastatic disease were excluded because there were insufficient data on nonproband/nonindex *SDHB* PV carriers. One study reported 5-year mortality but did not report the number of *SDHB* PV carriers with metastatic disease. Altogether, 16 studies met the inclusion criteria (5,12, 13, 16, 19-30). The study inclusion flow diagram is shown in Fig. 1.

Study Characteristics

Of the 16 studies, 10 were cohort studies (5, 12, 13, 19-21, 23, 26, 28, 30), and 6 were cross-sectional studies (16, 22, 24, 25, 27, 29) (Table 1). The follow-up duration of cohort studies was variable and median follow-up ranged between 1 year and 5.9 years. Twelve studies were retrospective, 3 were prospective, and 1 study was retrospective with ongoing prospective follow-up. Nine were single-center and 7 were multicenter. Seven studies were suitable for the penetrance assessment, 5 for assessing risk of metastatic disease assessment, 5 for assessing risk of a second tumor, and 6 for the 5-year mortality of *SDHB* PV carriers with metastatic disease assessment. The number of *SDHB* PV carriers in each study ranged from 11 to 317.

Risk of Bias Assessment

Risk of bias assessments were performed for each study, and results are summarized in Fig. 2. Patient selection assessment was assessed as having a low risk of bias for 10 studies and a high risk of bias for 6 studies. Genetic diagnosis (14/16) and clinical outcomes (15/16) were assessed by our authors as having a low risk of bias. Follow-up duration (12/16 high or unclear risk) and follow-up completeness (15/16 high or unclear risk) were assessed as having high risk of bias.

Penetrance for Nonproband/Nonindex *SDHB* Pathogenic Variant Carriers: Meta-Analysis

Results for age-specific penetrance for nonproband/nonindex *SDHB* PV carriers are shown in Fig. 3. The pooled penetrance of PPGL by age 20 was 4% (95% CI, 3%-6%; prediction interval, 2%-7%; n = 761, 5 studies) with I^2 of 0%. By age 40 the pooled penetrance was 11% (95% CI, 8%-15%; prediction interval, 5%-25%; n = 831, 6 studies). There was moderate variability between studies with I^2 of 52%. By age 60 pooled penetrance was 24% (95% CI, 19%-31%; prediction interval, 9%-50%; n = 1202, 7 studies). There was high variability between studies with I^2 of 83%. By age 80 pooled penetrance was 35% (95% CI, 25%-47%; prediction interval, 5%-84%; n = 889, 4 studies). There was high variability between studies with I^2 of 91%. The overall pooled penetrance is summarized in Fig. 4. Excluding the penetrance data from our cohort (5) produced similar results for pooled penetrance and heterogeneity at age 20, 40, and 60 years but gave a higher penetrance for age 80 years (Appendices 1 and 2) (14).

Risk of Metastatic Disease for Nonproband/Nonindex *SDHB* Pathogenic Variant Carriers With Disease: Meta-Analysis

Results for the risk of metastatic disease for *SDHB* PV carriers with disease, excluding proband/index cases, are shown in Fig. 5. The pooled risk of metastatic disease for nonproband/nonindex *SDHB* PV carriers with tumors was 9% (95% CI, 5%-16%; prediction interval 2%-34%; n = 251, 5 studies). There was mild variability between studies with I^2 of 33%. The subgroup analysis of risk of metastatic disease in nonproband/nonindex carriers with HNPGLs and TAPPGLs are provided in Supplementary Fig. S1 (14).

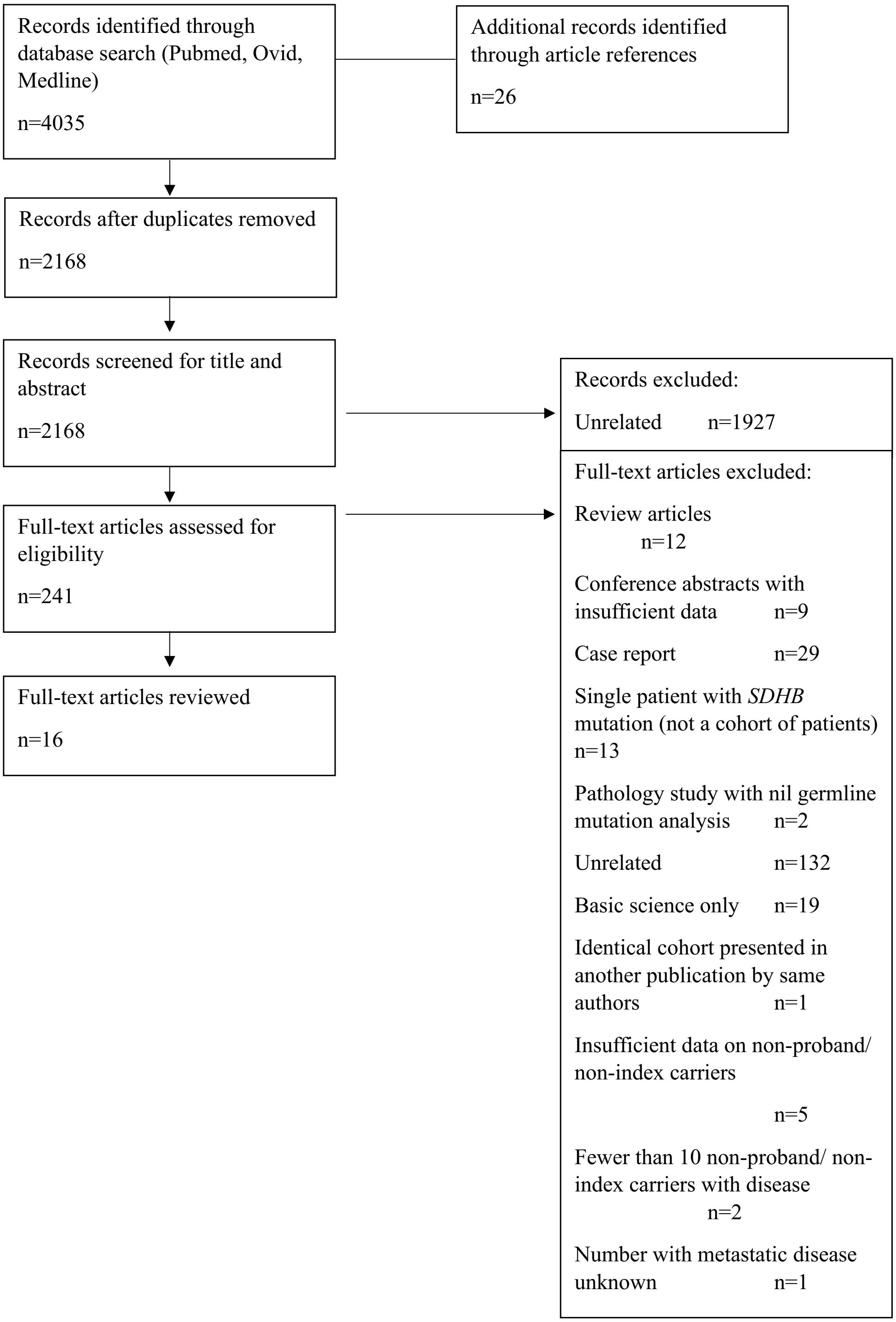

Figure 1. Study flow diagram.

Risk of a Second Tumor for *SDHB* Pathogenic Variant Carriers With Disease: Meta-Analysis

The risk of developing a second primary tumor could not be determined for nonindex carriers alone due to insufficient data in the literature. Considering outcomes for probands and nonprobands/nonindex cases with disease combined, the risk of a second primary tumor is shown in Fig. 6. The pooled risk of a second tumor was 24% (95% CI, 18%-31%; prediction interval 15%-37%; n = 156, 5 studies) with I^2 of 0%.

Five-Year Mortality for *SDHB* Pathogenic Variant Carriers With Metastatic Disease: Meta-Analysis

Five-year mortality could not be determined for nonindex carriers alone due to insufficient data in the literature. Combining outcomes for probands and nonprobands/nonindex cases with metastatic disease is shown in Fig. 7. Five-year mortality of *SDHB* PV carriers with metastatic disease was 18% (95% CI, 6%-40%; prediction interval 1%-90%; n = 254, 6 studies). There was high variability between studies with I^2 of 86%.

Table 1. Characteristics of studies

Author	Year	Country	Study design	Population studied	Data collection	Follow-up	Outcome category of this meta-analysis	No. of nonproband/nonindex *SDHB* PV carriers	Penetrance of *SDHB* PV carriers with PPGL and/or HNPGL (probands/index cases excluded where known)	Total No. of *SDHB* PV carriers with PPGL and/or HNPGL	No. of *SDHB* PV carriers with multifocal disease or second tumor (excluding metastatic disease)	Total No. of *SDHB* PV carriers with metastatic disease	5-y survival of *SDHB* PV carriers with metastatic disease
Amar et al	2007	France	Retrospective multicenter cohort study	Patients with metastatic PPGL	NA-2005	For *SDHB* carrier, median follow-up was 28 mo	Mortality	NA	NA	23	NA	23	36%
Andrews et al	2018	UK	Retrospective multicenter cross-sectional study	*SDHB/SDHC/SDHD* carriers	NA	NA	Penetrance, metastatic disease	371	Age 60: 83; age 80: 145[a]	NA	NA	9	NA
Bausch et al	2014	Germany, Italy, Poland, France, UK, Hungary, Ukraine, Latvia, Argentina, USA	Prospective multicenter cohort study	Patients with symptomatic paraganglial tumors	NA-2013	10 y (range, 1-42 y) for *SDHB* carriers	Second tumor	NA	NA	25	6	2	NA
Daniel et al	2016	UK	Retrospective single-center cohort study	*SDHx* PV carriers	2005-2015	6.4 y (range, 3.1-10.0 y)	Second tumor	27	NA	11	2	1	NA
Davidoff et al	2022 (this study for penetrance assessment)	Australia	Retrospective and ongoing prospective multicenter cohort study	*SDHB* PV carriers	1994-2021	Median 5.9 y (range, 1 mo-23.9 y)	Penetrance, metastatic disease, second tumor, Mortality	148	Age 20: 7; age 40: 17; age 60: 25; age 80: 29[b]	62	18	3	69%
Eijkelenkamp et al	2017	Netherlands	Retrospective single-center cohort study	*SDHB* PV carriers	2008-2015	Median 3.3 y (IQR 2.2-4.5)	Penetrance	70	Age 40: 1; age 60: 8[c]	NA	NA	8	NA
Jafri et al	2013	UK	Prospective multicenter cross-sectional study	*SDHB* probands who presented with PPGL and/or HNPGL and nonproband carriers	2001-2011	NA	Penetrance	187	Age 20: 9; age 40: 30; age 60: 75[d]	NA	NA	NA	NA
Jochmanova et al	2017	USA	Retrospective single-center cohort study	Family members of *SDHB* index cases who presented with PPGL	2004-2016	Median 1 y (range 0-14 y)	Penetrance, metastatic disease	241	Age 20: 8; age 40: 30; age 60: 64; age 80: 118[e]	143	NA	7	NA
Jochmanova et al	2020	USA	Retrospective single-center cross-sectional study	*SDHB* PV carriers with PPGL	2000-2019	NA	Mortality	NA	NA	64	NA	45	100%
King et al	2011	USA	Retrospective single-center cross-sectional study	Patients with metastatic PPGL	2000-2010	NA	Mortality	NA	NA	23	NA	23	96%
Niemeijer et al	2017	Netherlands	Retrospective multicenter cohort study	*SDHB* carriers	Prior to 2014	Median 2.6 y (range, 0-36 y)	Penetrance, metastatic disease	129	Age 20: 3; age 40: 13; age 60: 32; age 80: 43[f]	83	NA	15	NA

(*continued*)

Table 1. Continued

Author	Year	Country	Study design	Population studied	Data collection	Follow-up	Outcome category of this meta-analysis	No. of nonproband/nonindex *SDHB* PV carriers	Penetrance of *SDHB* PV carriers with PPGL and/or HNPGL (probands/index cases excluded where known)	Total No. of *SDHB* PV carriers with PPGL and/or HNPGL	No. of *SDHB* PV carriers with multifocal disease or second tumor (excluding metastatic disease)	Total No. of *SDHB* PV carriers with metastatic disease	5-y survival of *SDHB* PV carriers with metastatic disease
Schovanek et al	2014	USA	Retrospective single-center cross-sectional study	Patients with *SDHB*-related PPGL	NA	NA	Mortality	NA	NA	NA	NA	77	76%
Srirangalingam et al	2008	UK	Retrospective multicenter cohort study	*SDHB* PV carriers	NA	Mean follow-up of 5.8 y (SD 7.4, range 0-31 y)	Second tumor	NA	NA	16	3	NA	NA
Tufton et al	2017	UK	Prospective single-center cohort study	*SDHB* PV carriers	1975-2015	Mean follow-up 5.7 y (range, 0-14 y)	Second tumor, metastatic disease	65	NA	40	8	8	NA
Turkova et al	2016	USA	Retrospective single-center cross-sectional study	Patients with metastatic PPGL	2000-2015	NA	Mortality	NA	NA	73	NA	73	92%
White et al	2022	UK	Retrospective single-center cohort study	*SDHx* carriers who presented with PPGL and first-degree relatives with *SDHx* PV	2000-2020	Median 3 y	Penetrance	56	Age 20: 1; age 40: 3; age 60: 16[g]	NA	NA	NA	NA

Abbreviations: HNPGL, head and neck paraganglioma; IQR, interquartile range; NA, not applicable; na, not available; PPGL, pheochromocytoma and paraganglioma; PV, pathogenic variant; *SDHB*, succinate dehydrogenase type B; *SDHx*, succinate dehydrogenase; UK, United Kingdom; USA, United States of America.

[a–g]Penetrance figures are based on the assumption that all nonproband/nonindex *SDHB* PV carriers were included in the age-specific penetrance proportions reported for nonproband/nonindex carriers in each study.

[a]Andrews et al (2013): age 60: 22.5%; age 80: 39%.

[b]Davidoff et al (this study): age 20: 5%; age 40: 11%; age 60: 17%; age 80: 20%.

[c]Eijkelenkamp et al (2017): age 40: 2%; age 60: 12%.

[d]Jafri et al (2013): age 20: 5%; age 40: 16%; age 60: 40%.

[e]Jochmanova et al (2017): age 20: 3.3%; age 40: 12.4%; age 60: 26.4%; age 80: 48.8%.

[f]Niemeijer et al (2017): age 20: 2%; age 40: 10%; age 60: 25%; age 80: 33%.

[g]White et al (2022): age 20: 2.5%; age 40: 5%; age 60: 28.7%.

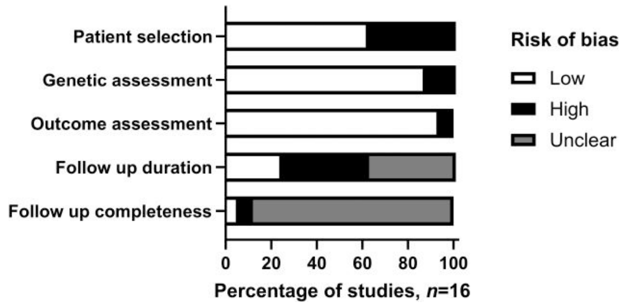

Figure 2. Risk of bias assessment.

Discussion

In this systematic review and meta-analysis, we aimed to assess 4 clinically relevant outcomes facing *SDHB* PV carriers, namely, penetrance of disease, risk of metastatic progression, risk of developing a second tumor, and mortality following a diagnosis of metastatic disease. These outcomes were selected due to their relevance to patients, families, and health-care providers conducting surveillance and treatment.

To our knowledge, this is the first systematic review and meta-analysis to examine penetrance for *SDHB* PV carriers excluding proband/index cases, to assess the risk of developing second tumor, and to report on 5-year mortality of *SDHB* PV carriers with metastatic disease altogether.

We found penetrance of nonproband/nonindex *SDHB* PV carriers rose from 4% at age 20 to 24% by age 60 and to 35% by age 80. For nonproband/nonindex *SDHB* PV carriers who had developed PPGL, overall risk of metastatic progression was 9% per lifetime. Carriers with a history of tumors (proband/index carriers or nonproband/nonindex carriers) had a 24% risk of a second tumor. Finally, all cause 5-year mortality was 18% for both proband/index and nonproband/nonindex carriers with metastatic disease.

Our meta-analysis updates the risk of metastatic progression for *SDHB* PV carriers excluding proband/index cases. The most recent systematic review and meta-analysis of metastatic disease risk in *SDHx* PV carriers by Lee et al (9) differed from our assessment, as they included symptomatic and proband/index cases whereas we excluded proband/index cases in the analysis of risk of metastatic progression for *SDHB* PV carriers who had developed PPGL. Lee et al (9) found a higher risk of 23% of metastatic progression compared to our estimate of 9% in nonproband/nonindex carriers with tumors. We speculate finding a higher risk of metastatic progression when including proband/index cases is likely due to delayed diagnosis and therefore a longer period of "tumor incubation." In addition, symptomatic patients may have larger tumors, and tumor size is a risk factor for metastatic progression (5, 24, 31).

More than a decade ago, van Hulsteijn et al (10) reported on the risk of malignant (metastatic) paraganglioma in *SDHB* PV carriers and found the pooled risk in nonindex carriers to be 13%, broadly in keeping with our risk estimate of 9%. In their systematic review and meta-analysis, the heterogeneity of studies was not stated and the investigators cautioned the generalizability of applying the findings, citing the paucity of cohort studies and high risk of bias (10). Our meta-analysis was strengthened by finding only mild variability between studies reporting metastatic progression, with an I^2 of 33%; and 4 of the 5 studies in our assessment were cohort studies, compared to 2 of 12 studies in that by van Hulsteijn et al (10).

Hamidi et al (8) performed a systematic review and meta-analysis of overall mortality following a diagnosis of metastatic disease with subgroup analysis of 2 studies reporting on *SDHB* PV carriers. In our updated analysis we assessed 5-year mortality in 3 times more participants than this previous study (8). We found a lower pooled mortality of 18%, compared to their mortality estimate of 35% to 55% (8). Changes in surveillance practices and treatment options for patients with advanced disease might account for the decrease in mortality estimates.

There were several limitations of the evidence in the pooled analysis. The I^2 result of 0% for the studies on pooled penetrance at age 20 years and risk of developing a second tumor suggests there was a potential sampling error (32). While some studies reported their surveillance protocols (5, 13, 20, 21, 24, 26, 28, 30), others did not and differences in follow-up across centers may have modified outcomes reported for *SDHB* PV carriers. Some of the variants in earlier studies have subsequently been reclassified as variants of uncertain significance (VUS) or likely benign. Of 344 *SDHB* PV carriers studied by Jochmanova et al (23), 2 index cases in total might have been VUS and 1 index case and 1 nonindex carrier without disease might have been likely benign variants. Of 16 *SDHB* PV carriers with disease studied by Srirangalingam et al VUS, 1 might have been (28). There was high heterogeneity in the pooled penetrance at age 80 years. There was also high heterogeneity in the 5-year mortality rate of *SDHB* PV carriers with metastatic disease, and given many of the studies did not describe treatment protocols for carriers with metastatic disease (5, 23, 25, 27, 29), potential treatment differences may have been a factor in variable mortality outcomes. The quality of individual studies included in the systematic review and meta-analysis varied, as shown in the risk of bias assessment. For example, in some studies follow-up duration or completeness was unclear at an individual patient level. Therefore in the pooled analysis of age-specific penetrance, we assumed all nonproband/nonindex *SDHB* PV carriers were included in the age-specific penetrance proportions reported in each study. Similarly in the pooled analysis of 5-year mortality, we assumed all patients with metastatic disease were included in the 5-year mortality assessment for each study. While these assumptions would not have affected the penetrance or 5-year mortality proportions, the combined weighting of the studies may have been different. We tried to assess studies that reported on nonproband/nonindex carriers, but there were limited eligible studies to assess risk of developing a second tumor and 5-year mortality for nonproband/nonindex carriers alone. Six studies included highly selected populations such as *SDHB* PV carriers with advanced or metastatic disease whereas a typical group of *SDHB* PV carriers undergoing surveillance often included asymptomatic or nonproband/index carriers. Therefore, while this was the first meta-analysis to assess risk of developing a second tumor and 5-year mortality, we acknowledge a risk of ascertainment bias as we included proband/index cases in these analyses.

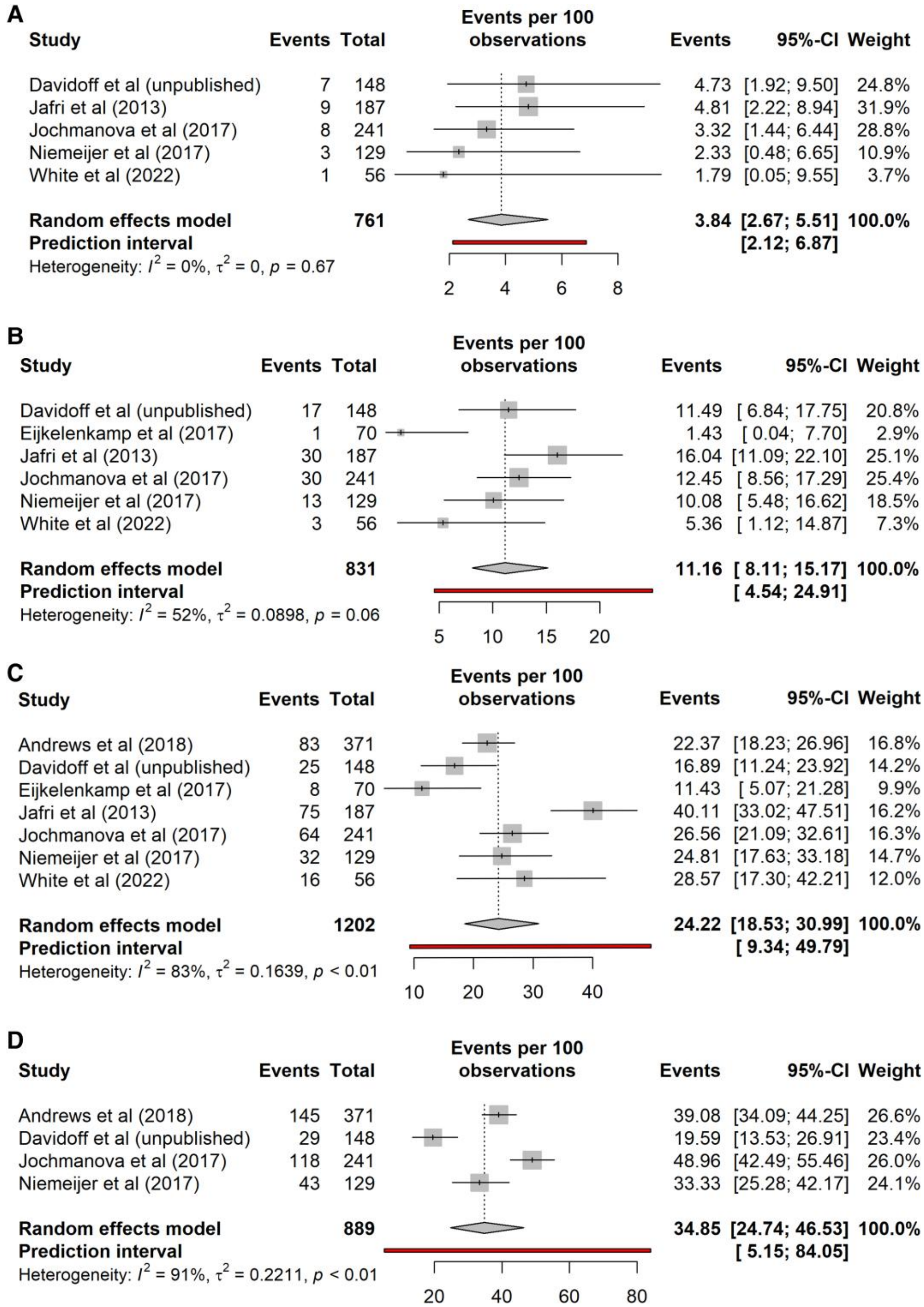

Figure 3. Penetrance of pheochromocytomas and paragangliomas for nonproband/nonindex *SDHB* pathogenic variant carriers. A, Age 20 years. B, Age 40 years. C, Age 60 years. D, Age 80 years.

Limitations to this study included the potential that search terms missed articles that were not indexed under those terms or if they were published in sources not included in the search. To counter this possibility, we also included articles identified in references. Similarly, there was the possibility of language bias; while our search was not limited to the English language, it transpired the articles selected for eligibility were all in English. Inclusion and exclusion criteria used in the review were narrow, but we felt this was appropriate given we were interested in outcomes of this specific cohort.

Surveillance for *SDHB* PV carriers aims to detect PPGLs before metastasis has occurred, to facilitate surgical cure (3). Our meta-analysis has potential implications for surveillance

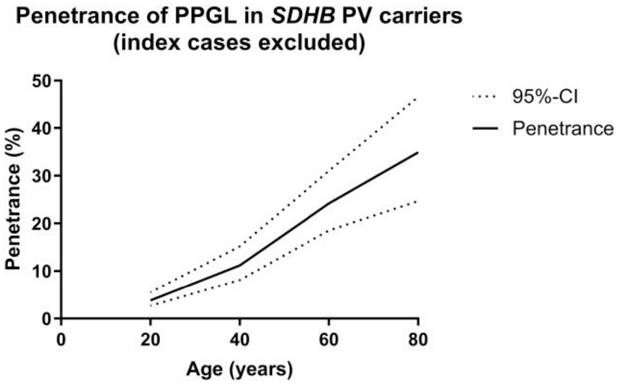

Figure 4. Overall pooled penetrance of pheochromocytomas and paragangliomas (PPGL) for *SDHB* pathogenic variant (PV) carriers, excluding proband/index cases.

approaches among *SDHB* PV carriers. We found that penetrance appears to increase linearly across the lifespan up to and including age 80 years, suggesting that there is utility in ongoing screening up to age 80 years. Current guidelines recommend less screening after age 70 years (3). *SDHB* PV carriers with a history of tumors (either proband/index or nonproband/nonindex carriers) have a 24% risk of a second tumor, so surveillance must continue even following surgical excision of the primary tumor. The frequency of such surveillance requires further research. Moreover, the risk of metastatic progression for nonproband/nonindex carriers is 9%, which although lower than previously estimated for index cases (4) still highlights the need for careful follow-up of these cases. Apart from tumor size (5, 24, 27, 31), risk factors for metastatic progression are not currently well known. As risk factors for metastatic progression are identified in the future, health-care

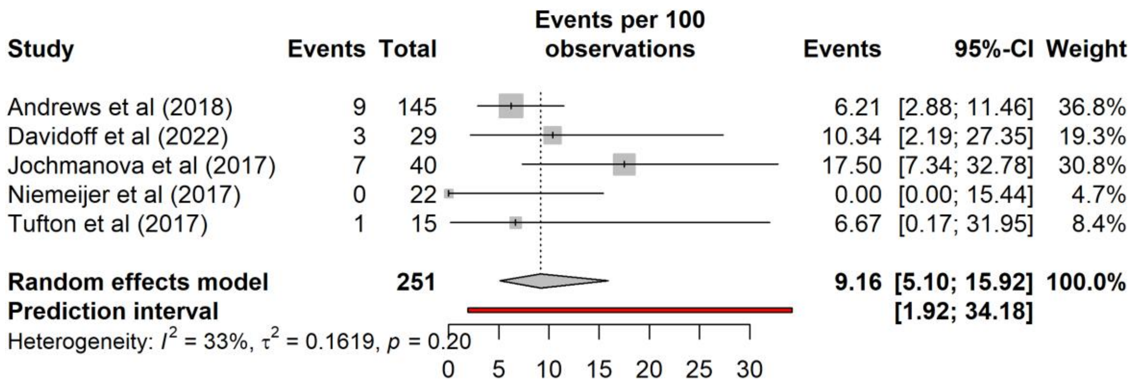

Figure 5. Risk of metastatic disease for *SDHB* pathogenic variant carriers with disease, excluding proband/index cases.

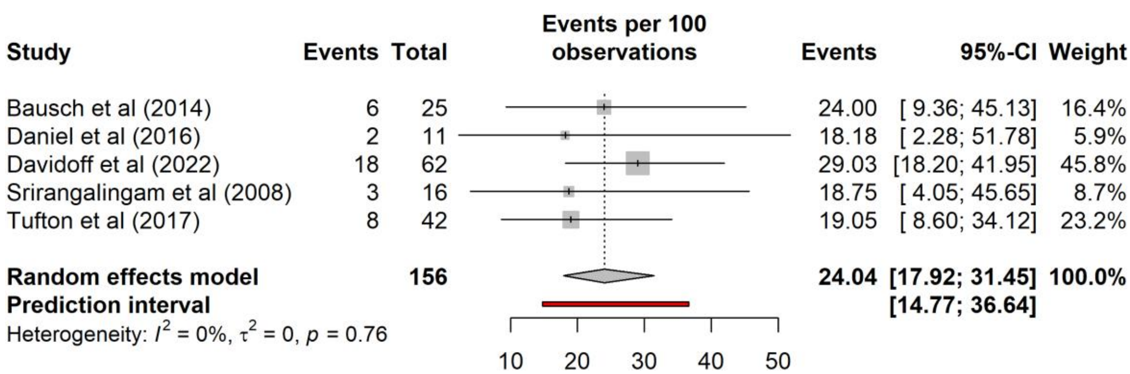

Figure 6. Risk of a second tumor for *SDHB* pathogenic variant carriers with disease.

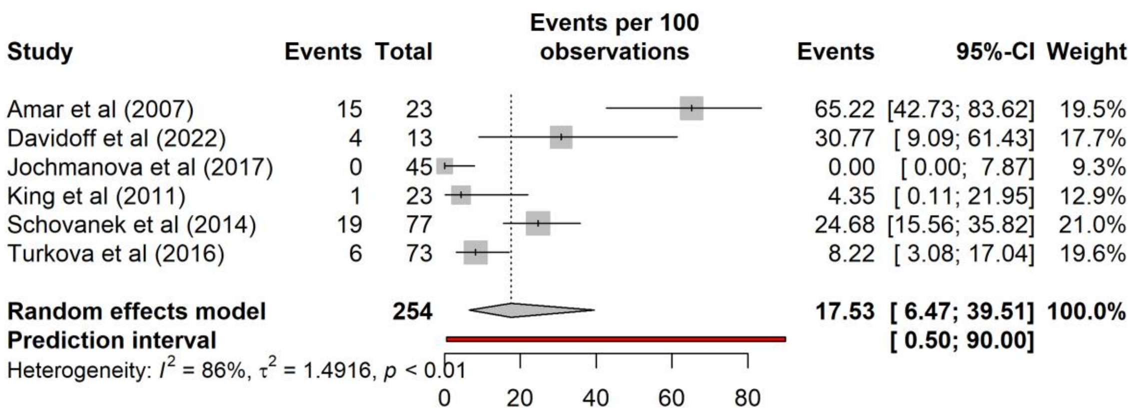

Figure 7. Five-year mortality of *SDHB* pathogenic variant carriers with metastatic disease.

professionals may wish to consider more frequent surveillance for patients with these risk factors. Finally, our meta-analysis confirms a poor prognosis for *SDHB* PV carriers once metastatic disease has occurred, with a 5-year mortality of 18%.

Conclusion

This review has clinical relevance for genetic counseling and surveillance of all *SDHB* PV carriers. Nonproband/nonindex carriers have a 4% chance of having developed PPGL by age 20 years, rising to 35% by age 80 years. For carriers who develop PPGL, there is a 9% chance of metastatic progression. Since *SDHB* PV carriers who have developed one tumor (probands and nonproband/nonindex cases combined) have a 24% chance of developing a second tumor, follow-up surveillance is important even when the first tumor is surgically removed. In the setting of a lifelong risk of developing PPGLs and subsequent risks of metastatic progression, developing a second tumor and mortality from disease, our review supports lifelong surveillance as an important recommendation for all centers, as advocated by clinical practice guidelines (3).

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Author Contributions

D.F.D designed the study, acquired the data, performed data analyses, and drafted/approved all versions of the manuscript. R.C.B. and R.D.A.L. designed the study, aided with interpretation of data, and edited/approved the manuscript. D.B. and V.H.M.T. aided with interpretation of data and edited/approved the manuscript.

Disclosures

The authors have nothing to declare.

Data Availability

Some or all data sets generated during and/or analyzed during the current study are available from the corresponding author on request.

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Supplementary Material Outcomes of SDHB Pathogenic Variant Carriers

Supplementary Methods: Modified newcastle ottowa scale

Study (author, year):

____

Selection

1) Did the sample represent the population of interest? (circle one)

a) yes truly representative of the average type of patient/ population in the community ☐

b) yes somewhat representative of the average type of patient/ population in the community ☐

c) selected group of users

d) no description of the derivation of the cohort

Methodology

1) Assessment of genetic information (circle one)?

a) genetic variants obtained from original data ☐

b) genetic information from secondary sources or not adequately described

Outcome

1) Assessment of outcome (circle any that apply)?

a) independent blind assessment stated in the paper, or confirmation of the outcome by reference to secure records (x-rays, medical records, etc.) ☐

b) record linkage (e.g. identified through ICD codes on database records) ☐

c) self report (i.e. no reference to original medical records or x-rays to confirm the outcome)

d) no description

2) Was follow-up long enough for outcomes to occur (circle one)?

a) yes (at least 5 years of follow-up)

b) no

3) Adequacy of follow up of cohorts (circle one)?

a) complete follow up - all subjects accounted for

b) subjects lost to follow up unlikely to introduce bias - small number lost - > 10% (select an adequate %) follow up, or description provided of those lost)

c) follow up rate < 10% and no description of those lost

d) no statement

Appendix 1: Clinical penetrance assessment of our data for non-proband *SDHB* PV carriers

Aim

Our aim was to perform a clinical penetrance assessment of non-proband *SDHB* PV carriers from the population described in Davidoff et al (5).

Methods

Age of PPGL diagnosis was defined as the age where a clinical diagnosis was made based on history, biochemistry and radiology evaluation. Penetrance of PPGL was determined by the Kaplan-Meier method after excluding probands. Data was analysed in the statistical software SPSS.

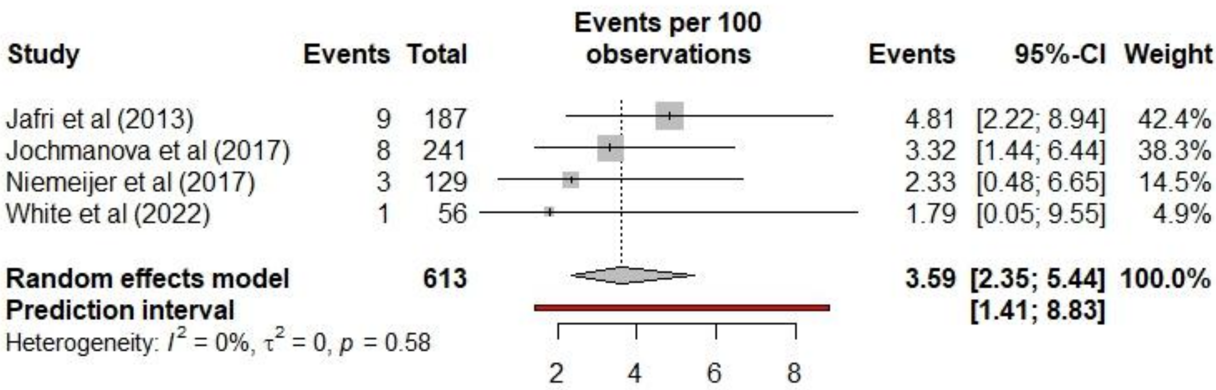
Results

There were 148 non-proband carriers. The penetrance of PPGL by age 20 was 5% (n=7), by age 40 was 11% (n=17), by age 60 was 17% (n=25) and by age 80 was 20% (n=29).

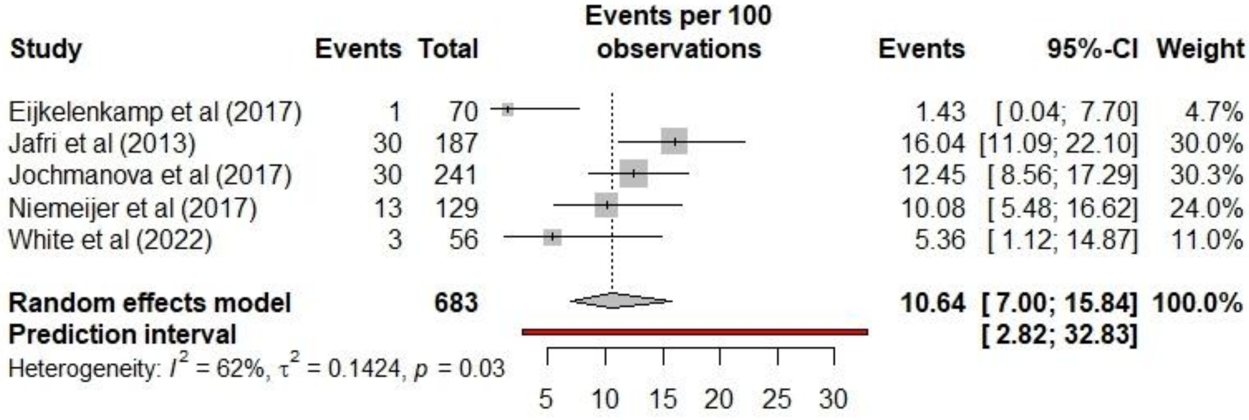
Appendix 2: Clinical penetrance assessment for non-proband/ non-index *SDHB* PV carriers following exclusion of our data

Exclusion of penetrance data from Davidoff et al gave similar results as shown in the figures of Appendix 2 below. The pooled penetrance of PPGL by age 20 remained 4% and the 95% CI and prediction intervals were similar (95% CI, 2-5%; prediction interval 1-9%, n=613, 4 studies). The I^2 remained 0%. By age 40 the pooled penetrance remained 11% and the 95% CI and prediction intervals were similar (95% CI, 7%-15%; prediction interval, 3%-33%; n=683, 5 studies). Moderate variability remained between studies with I^2 of 62%. By age 60 pooled penetrance was similar at 26% and the 95% CI and prediction intervals were also similar (95% CI, 19%-33%; prediction interval, 9%-53%; n=1054, 6 studies). High variability remained between studies with I^2 of 82%. By age 80 pooled penetrance increased from 35% to 41% but the 95% CI and prediction intervals remained wide (95% CI, 33-49%; prediction interval, 0%-98%; n=741, 3 studies). There was still high variability between studies with I^2 of 80%.

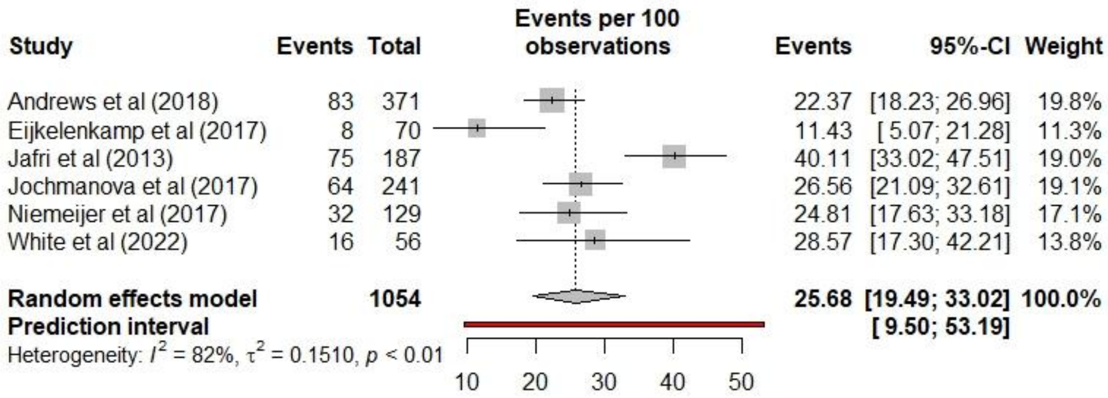
Age 20



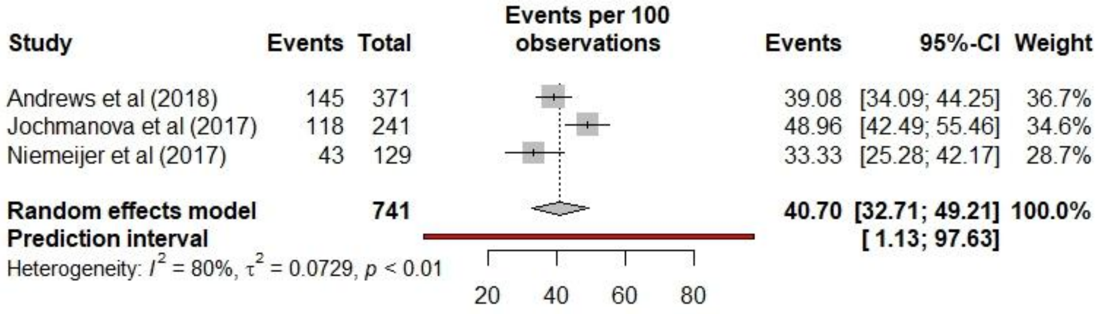
Age 40


Study
Events
Total
Events per 100 observations
Events
95%-CI
Weight
Eijkelenkamp et al (2017) 1 70 1.43 [0.04; 7.70] 4.7%
Jafri et al (2013) 30 187 16.04 [11.09; 22.10] 30.0%
Jochmanova et al (2017) 30 241 12.45 [8.56; 17.29] 30.3%
Niemeijer et al (2017) 13 129 10.08 [5.48; 16.62] 24.0%
White et al (2022) 3 56 5.36 [1.12; 14.87] 11.0%
Random effects model 683 10.64 [7.00; 15.84] 100.0%
Prediction interval [2.82; 32.83]
Heterogeneity: I^2 = 62%, τ^2 = 0.1424, p = 0.03
5 10 15 20 25 30

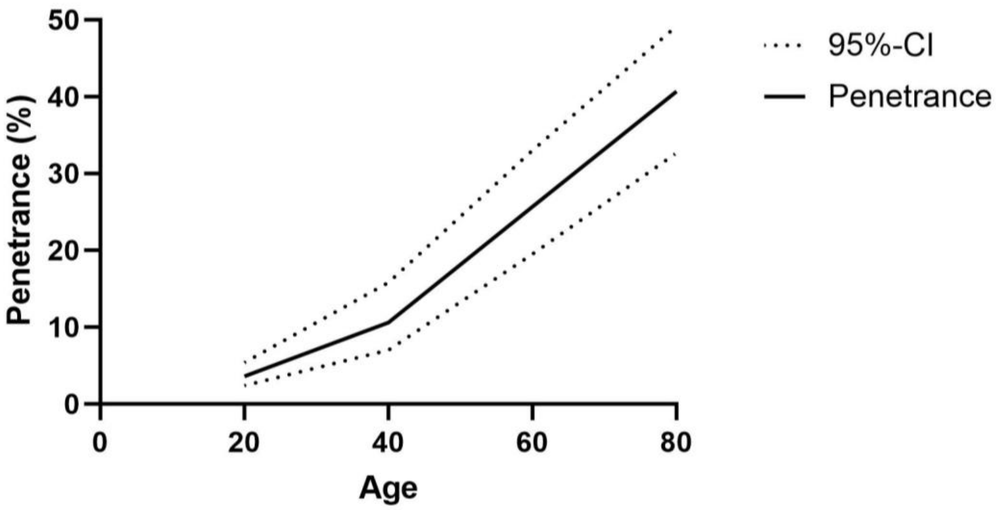
Age 60


Study
Events
Total
Events per 100 observations
Events
95%-CI
Weight
Andrews et al (2018) 83 371 22.37 [18.23; 26.96] 19.8%
Eijkelenkamp et al (2017) 8 70 11.43 [5.07; 21.28] 11.3%
Jafri et al (2013) 75 187 40.11 [33.02; 47.51] 19.0%
Jochmanova et al (2017) 64 241 26.56 [21.09; 32.61] 19.1%
Niemeijer et al (2017) 32 129 24.81 [17.63; 33.18] 17.1%
White et al (2022) 16 56 28.57 [17.30; 42.21] 13.8%
Random effects model 1054 25.68 [19.49; 33.02] 100.0%
Prediction interval [9.50; 53.19]
Heterogeneity: I^2 = 82%, τ^2 = 0.1510, p < 0.01
10 20 30 40 50

Age 80



Overall pooled penetrance of PPGL for *SDHB* PV carriers, excluding index cases



Clinical Chemistry 67:4
696–705 (2021)

the Clinical Chemist

Genetic Metabolic Series

Measuring Tumor Succinate and Fumarate to Resolve Pathogenicity of an *SDHA* Variant

Dahlia F. Davidoff,[a,b,c] Catherine Luxford,[a,b] Edward Kim,[a,b] Talia Novos,[d] Andrea R. Horvath,[d] Anthony J. Gill,[b,e] Trisha Dwight,[a,b] Roderick J. Clifton-Bligh,[a,b,c,*] and John R. Burgess[f,g]

Case

A 25-year-old man presented with palpitations, sweating, chest tightness, dyspnea, and headache. His history included hypertension treated with 50 mg of atenolol each morning. There was no family history of endocrine neoplasia. Computed tomography (CT) showed a 40 mm hyperdense suprarenal lesion. A provisional diagnosis of pheochromocytoma was made. 18 F-fluorodeoxyglucose positron emission tomography/computed tomography (^{18}F-FDG-PET/CT) and ^{68}gallium octreotate PET/CT both demonstrated intense tracer avidity in the lesion (Fig. 1, A and B). There was no evidence of loco-regional or distant metastatic disease.

Plasma norepinephrine was increased at 6.8 nmol/L (reference interval 1.0–5.0 nmol/L), and plasma epinephrine and dopamine were normal. Plasma normetanephrine was increased 1951 pmol/L (reference limit <900 pmol/L), and plasma metanephrine was normal 130 pmol/L (reference limit <500 pmol/L).

After 4 weeks' preparation with phenoxybenzamine, the patient underwent right nephrectomy and adrenalectomy with resection of an adjacent tumor mass. Histopathology showed an extra-adrenal paraganglioma with evidence of capsular invasion. Immunohistochemistry for succinate dehydrogenase subunits A and B (SDHA and SDHB, respectively) was abnormal (i.e., negative staining). Genetic testing demonstrated a germline *SDHA* heterozygous variant [NM_004168: c.1A>G, p.(Met1Val)], classified as "variant of uncertain significance" Class III according to the American College of Medical Genetics Criteria (1).

SDH converts succinate to fumarate in the tricarboxylic acid cycle. Therefore, to resolve whether this variant was pathogenic, both succinate and fumarate were measured in a formalin-fixed paraffin embedded (FFPE) sample from the resected PGL. Methodology of sample preparation and liquid chromatography–tandem mass spectrometry (LC–MS/MS) has been described, and this sample was included in previous group data statistics (2). An experienced anatomical pathologist confirmed the specimen contained >60% tumor. Mean tumor succinate: fumarate ratio (SFR) was 431.5 ± 93.2 (6 samples each representing sequential 50 µm cross-sections of tumor), above a previously published cutoff of 63.12 (2). Interassay and intraassay coefficients of variation were 26.82 and 5.92%, respectively.

The patient remains recurrence free 4 years postsurgery.

Discussion

Pheochromocytoma and paraganglioma (PPGL) are rare but heritable neuroendocrine tumors occurring in 2–8 per million persons per year, with at least one-

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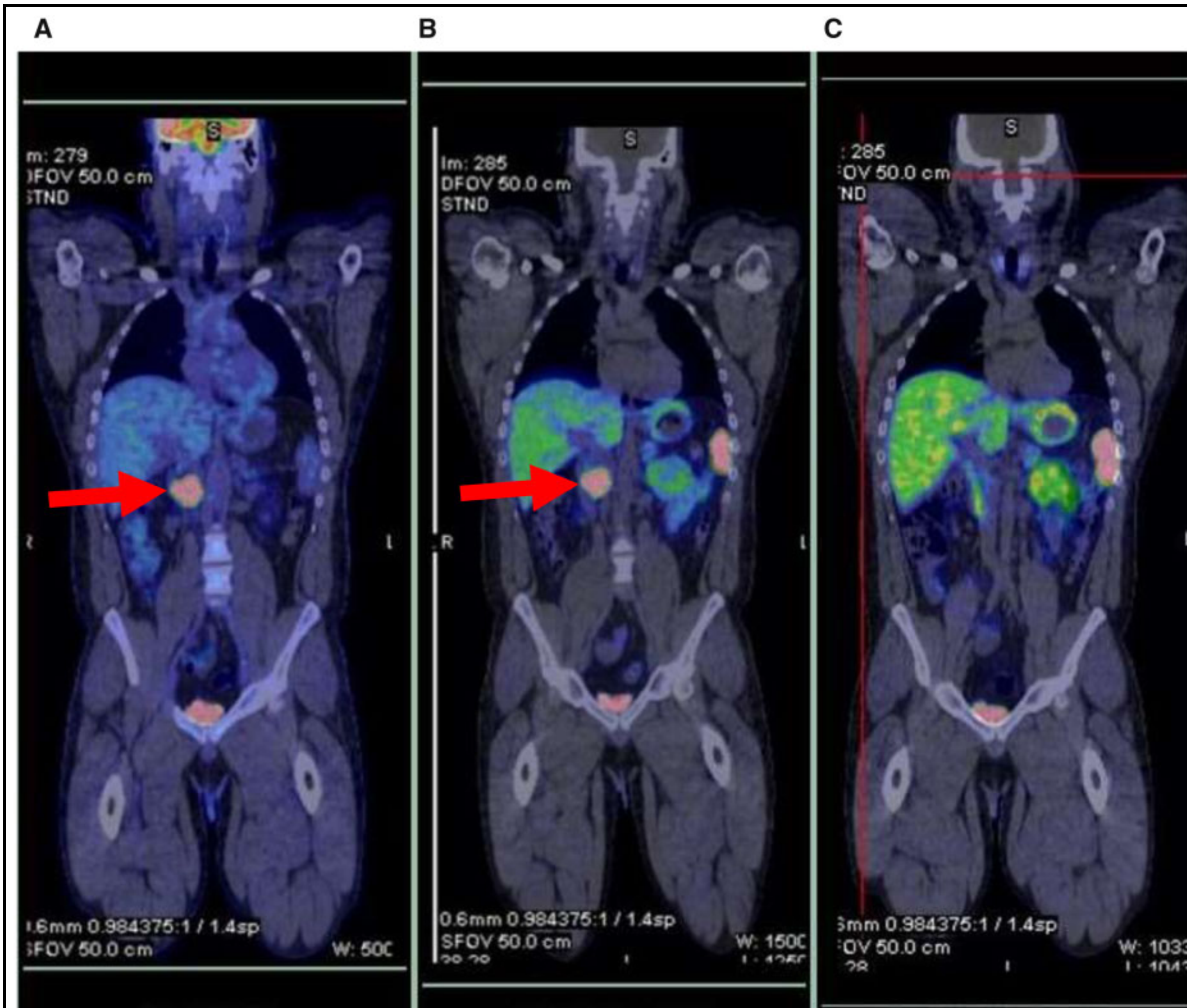

Fig. 1. A and B), Demonstrate a right sided extra-adrenal paraganglioma (red arrow) on the ^{18}F-FDG-PET and ^{68}gallium octreotate PET/CT, respectively. C), ^{68}gallium octreotate PET/CT performed 4 years postoperatively.

third of cases associated with a germline mutation in one the susceptibility genes, most commonly those encoding the 4 SDH subunits A–D (3). The catalytic component of SDH comprises FAD-binding SDHA complexed with SDHB, whereas SDHC and SDHD anchor the complex to the mitochondrial membrane (Fig. 2) (3). In PPGLs associated with *SDHx* variants, SDH deficiency causes succinate accumulation and stabilization of hypoxia inducible factors known as "pseudohypoxia" (2).

PPGL often present with clinical features of catecholamine excess. Measurement of catechol-*O*-methyltransferase metabolites of epinephrine, norepinephrine, or dopamine (metanephrine, normetanephrine, and 3-methoxytyramine, respectively) by LC–MS-MS has high diagnostic accuracy for PPGL (4).

Variants of uncertain significance are more common in *SDHA* than for other *SDHx* genes (5) increasing the need to properly classify pathogenic *SDHA* variants according to standardized methods. To this end, an increased SFR indicates functional loss of SDH activity (2, 3), whereas loss of SDHA and SDHB immunohistochemistry indicates loss of protein expression. Small-molecule metabolite ratios have been shown to be adequately maintained during the FFPE process (2). Richter et al. reported a cutoff SFR of 97.7 for fresh frozen SDH-associated PPGL (3) and our group reported a cutoff of 63.12 for FFPE SDH-associated PPGL (2). In this case, increased tumoral SFR provided orthogonal confirmation that the *SDHA* variant identified in germline DNA was indeed pathogenic. Increased SFR has also been associated

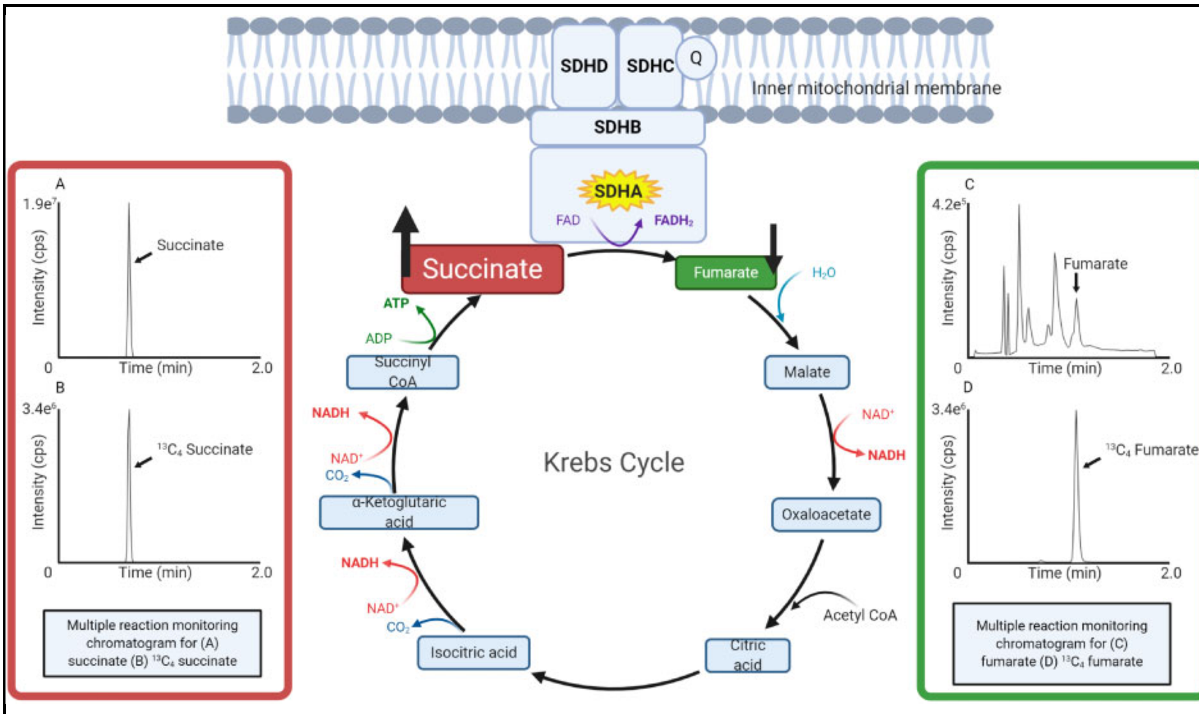

Fig. 2. Succinate dehydrogenase converts succinate to fumarate in the tricarboxylic acid cycle. Red box insert showing the patient's LC-MS/MS chromatograms for, succinate (top panel) and succinate internal standard (bottom panel). Green box insert showing the patient's LC-MS/MS chromatograms for, fumarate (top panel) and fumarate internal standard (bottom panel). To measure tumor succinate and fumarate, the FFPE sample was prepared by adding extraction solution (20% LC-MS grade H_2O, 80% LC-MS grade methanol containing 0.1 μg/mL 13C4 fumarate and 0.5 μg/mL 13C4 succinate), vortexing and incubating mixtures for 45 min at 70 °C followed by 5 min on ice (2). Metabolites were extracted from melted paraffin by collecting and combining supernatants from two consecutive centrifugations at 14 000*g* for 10 min at 4 °C (2). Samples were dried by evaporating methanol for 2 h using a Speedvac and then stored at −80 °C (2). Analysis of extracts was performed on a Prominence high performance liquid chromatography system (Shimadzu) coupled to an API QTRAP 6500+ mass spectrometer (SCIEX) operated in negative electrospray ionization (2). Analytes were detected by monitoring multiple reaction monitoring *m/z* transitions of 116.9 > 73.1 and 116.9 > 99.1 as quantifier and qualifier transitions, respectively, for succinate, 121.0 > 76.1 for the 13C4 succinate internal standard, 114.9 > 71.0 for fumarate and 119 > 74 for the 13C4 fumarate internal standard (2).

with an increased risk of metastases in *SDH*-mutated PPGLs (3).

Human Genes: *SDHA*, succinate dehydrogenase subunit A; *SDHx*, succinate dehydrogenase gene variant.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.*

T. Novos, statistical analysis; R. Horvath, financial support, provision of study material or patients; A.J. Gill, provision of study material or patients.

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CASE REPORT

JBMR PLUS Open Access ASBMR

Failure of Oral Risedronate Therapy to Prevent Spontaneous Vertebral Fracture in a Patient Ceasing Denosumab: A Cautionary Case

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ABSTRACT

Denosumab is a highly effective treatment for postmenopausal osteoporosis, significantly improving BMD and reducing risk of fracture. However, denosumab's effect is transient with the risk of a rebound increase in bone turnover following withdrawal of this potent RANKL inhibitor. This poses challenges, particularly in individuals seeking to discontinue denosumab, such as those experiencing a direct complication of prolonged antiresorptive therapy or those in whom an antiresorptive drug holiday would be ordinarily considered. Bisphosphonate strategies to mitigate postdenosumab bone loss are being actively studied. We describe the case of a 73-year-old woman who developed a spontaneous vertebral fracture following denosumab discontinuation, despite prolonged treatment with bisphosphonate therapy both before her course of denosumab (20 years of use) and following denosumab discontinuation (1 year of use). This is a cautionary case seeking to highlight uncertainties around the safe withdrawal of denosumab therapy despite intervening treatment with bisphosphonates. © 2020 The Authors. *JBMR Plus* published by Wiley Periodicals, Inc. on behalf of American Society for Bone and Mineral Research © 2020 The Authors. *JBMR Plus* published by Wiley Periodicals LLC on behalf of American Society for Bone and Mineral Research.

KEY WORDS: ANTIRESORPTIVES; BISPHOSPHONATES; DENOSUMAB; OSTEOPOROSIS; RISEDRONATE; SPONTANEOUS VERTEBRAL FRACTURE

Introduction

Denosumab is a human IgG2 monoclonal antibody to RANKL.[1] By binding to RANKL, denosumab prevents the RANKL/RANK receptor interaction on osteoclasts, resulting in reduced osteoclast formation and function.[1] Denosumab has revolutionized the management of postmenopausal osteoporosis because of its dramatic impact on BMD and its reduction of fracture risk.[2] Current guidelines recommend the consideration of an intermission in antiresorptive therapy after prolonged treatment following careful evaluation of patient characteristics, BMD, and prior history of fracture.[3] The risk of atypical femur fracture (AFF) in patients on antiresorptive therapy is time-dependent, with the risk increasing steadily after 5 years of treatment.[4] Patients on prolonged denosumab therapy,[5] particularly those on lengthy prior treatment with bisphosphonate,[6] are also at risk of AFF. We describe a case of a postmenopausal woman who had received a quarter century of antiresorptive therapy, first with oral bisphosphonate therapy for 20 years, followed by 5 years of denosumab. After careful consideration, she was transitioned from denosumab to an oral bisphosphonate in an effort to initiate an antiresorptive treatment intermission. Despite cautious treatment with a bisphosphonate and her many years of preceding bisphosphonate treatment, she sustained a spontaneous single vertebral fracture 13 months after denosumab discontinuation.

Clinical Vignette

A 73-year-old woman with a lengthy history of osteoporosis and an extensive fracture history received 25 years of treatment with antiresorptive therapy. For the first 20 years, she received alendronate, followed by denosumab for 5 years. Because of her prolonged history of antiresorptive use, in the absence of recent minimal trauma fractures and with improved BMD values performed by DXA (lumbar spine density, 0.885 g/cm^2, *T*-score, 1.5 SD; left femoral neck density, 0.613 g/cm^2, *T*-score, 2.1 SD; left total hip, 0.683 g/cm^2, *T*-score, 2.1 SD), an antiresorptive treatment holiday was planned. She was subsequently transitioned from denosumab to a weekly oral bisphosphonate risedronate

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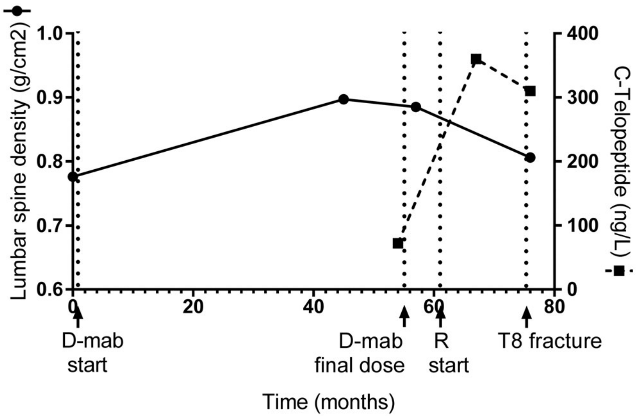

Fig 2. BMD and bone turnover marker response to denosumab and risedronate. D-mab = denosumab; R = risedronate.

to protect from a rebound loss of bone density.[7,8] Of note, she had a remote history of vertebral and pelvic fractures occurring over three decades, but not recently. A bone scan prior to transition to risedronate showed non-avid old vertebral fractures. Her lengthy prior treatment with bisphosphonate was also considered to potentially alleviate a rebound increase in bone turnover postdenosumab withdrawal. Six months after switching from denosumab to the oral bisphosphonate, bone turnover markers reassuringly remained within the reference range with P1NP 35 µg/L (15 to 115) and CTx 360 ng/L (100 to 1000) as assessed with a Roche cobas immunoassay analyzer (Roche Diagnostics, Mannheim, Germany).

Thirteen months after transitioning to the oral bisphosphonate, the patient sustained a T8 minimal trauma fracture after lifting a heavy metal pan (Fig. 1A and 1B). The patient reported compliance to risedronate. Bone turnover marker analysis performed 6 weeks after the fracture were stable compared to previous results with P1NP 38 µg/L (15 to 115) and CTx 310 ng/L (100 to 1000). A secondary osteoporosis screen was unremarkable including replete 25-hydroxyvitamin D of 134 nmol/L (50 to 140) and normal corrected calcium of 2.44 mmol/L (2.15 to 2.55), serum phosphate of 1.31 mmol/L (0.8 to 1.5), intact PTH of 20 ng/L (15 to 68), thyroid-stimulating hormone of 0.70 mIU/L (0.40 to 5.00), negative celiac serology by deamidated gliadin and tissue transglutaminase IgA and IgG, and normal serum protein electrophoresis and immunofixation. However, a DXA showed a decline in lumbar spine bone density (0.806 g/cm2, *T*-score, 2.2; 8.9% decline over 19 months) and minor reductions in the left total hip left femoral neck density (Fig. 2, Table 1). Given the decline in BMD, denosumab treatment was resumed and will continue indefinitely.

Discussion

Denosumab is a highly effective treatment for postmenopausal osteoporosis, which results in continued increases in BMD over time[9] and is well-tolerated.[10] In the Freedom Extension [Clinicaltrails.gov: NCT00089791 (FREEDOM) and NCT00523341 (Extension): An Open Label, Single Arm, Extension Study to Evaluate the Long Term Safety and Sustained Efficacy of Denosumab (AMG162) in the Treatment of Postmenopausal Osteoporosis] Trial, the mean change in lumbar spine BMD from baseline to 5 years was +13.7%, comparable to our patient's improvement and by 10 years, up to +21.7%.[9] However, denosumab

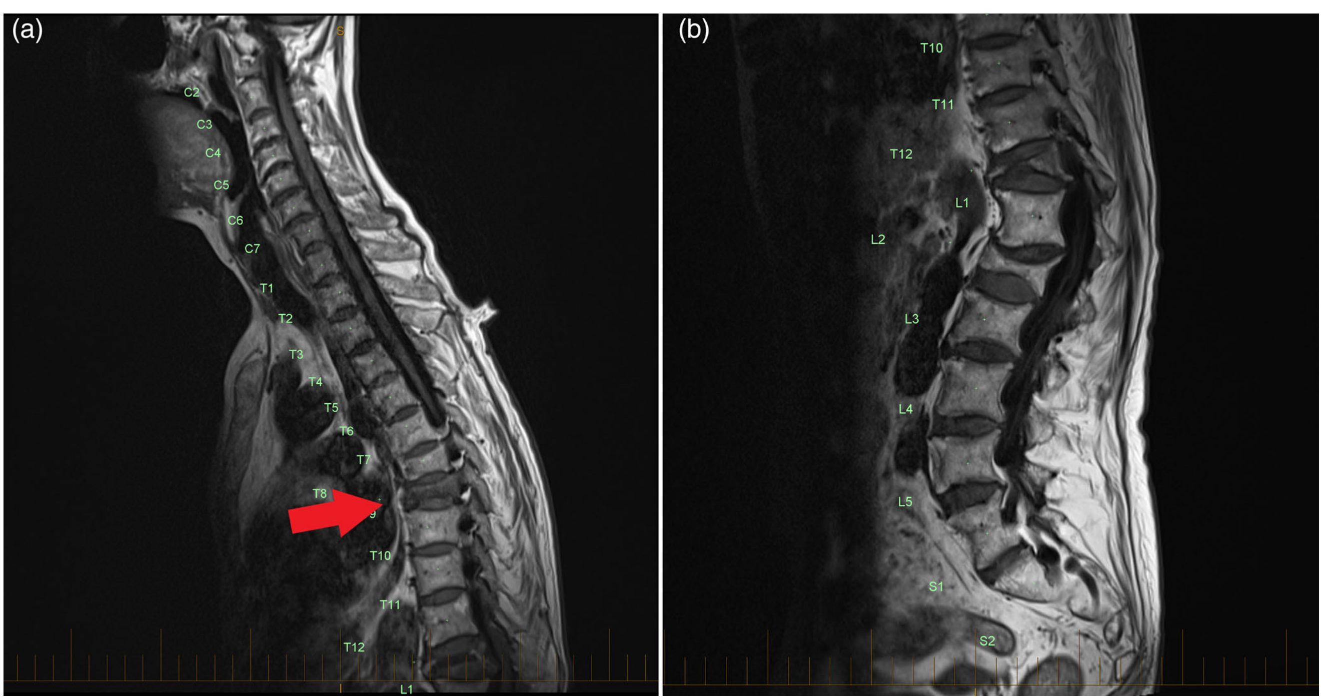

Fig 1. T_1-weighted MRI imaging of the thoracic (*A*) and lumbar spine (*B*) showing an acute compression fracture identified in T8 (arrow) and evidence of prior vertebral compression fractures in T7, T12, and L2.

Table 1. Change in Lumbar Spine BMD, CTx, and P1NP Over Time

Months from baseline	Lumbar spine density (g/cm^2)	Lumbar spine *T*-score	Lumbar spine density (% change) from baseline	CTx (ng/L)	Total P1NP (µg/L)
0	0.776	−2.5	NA	-	-
45	0.897	−1.4	15.6%	-	-
54	-	-	-	72	20
57	0.885	−1.5	14.0%	-	-
67	-	-	-	360	35
76	0.806	−2.2	3.9%	310	38

withdrawal is problematic with a risk of rapid loss in bone density gained[11–13] and a risk of vertebral fractures, especially in those with prior vertebral fracture.[13-15]

Strategies for mitigating postdenosumab bone loss are under examination, but the literature suggests that an oral bisphosphonate may be the most indefinitely approach at this time.[7,8] Post-FRAME (Fracture Study in Postmenopausal Women with Osteoporosis) Trial data found that following denosumab cessation, treatment with oral risedronate ($n = 5$) provided a 41% retention of the increase in BMD achieved with denosumab.[7] The DAPS (Denosumab Adherence Preference Satisfaction) Study further addressed the question of transition to an oral bisphosphonate with a 24-month open-label randomized cross-over study.[8] A majority of postmenopausal women with osteopenia who switched to alendronate after 24 months of denosumab maintained or gained bone density in the DAPS Study in the lumbar spine, total hip, and femoral neck (84.1%, 92.4%, and 78.3% of participants, respectively).[8] Bisphosphonates may vary in the potency of their antiresorptive effect and the CTx rise following denosumab may be mitigated by a potent bisphosphonate, whereby alendronate may be more effective than risedronate.[16] Studies of BMD retention in patients who switched from denosumab to zoledronic acid have been conflicting. A series of six postmenopausal women found a lack of bone density retention in patients transitioned to zoledronic acid,[17] whereas post-FRAME data showed that the 11 women who switched to zoledronic acid had 73% retention of the gains in BMD after 1 year.[7]

The rapid loss of bone postdenosumab is not uniform among denosumab users, and the risk is considered greatest in patients who were bisphosphonate-naïve prior to denosumab,[18,19] and those who achieved a marked increase in BMD on denosumab treatment.[8] Optimal bisphosphonate treatment strategies to mitigate postdenosumab bone loss are under investigation.[20]

In our patient, two decades of prior treatment with a bisphosphonate and a postdenosumab course of risedronate failed to prevent a rebound vertebral fracture following denosumab cessation. The vertebral fracture was speculated to relate to a rebound phenomenon postdenosumab in light of the bone density decline and increase in bone turnover markers following denosumab cessation. Interestingly, our patient's CTx following denosumab was not particularly high, and fell within the reference range for postmenopausal women. Our management was consistent with current recommendations to consider an intermission in antiresorptive therapy after prolonged treatment, in individuals who do not exhibit a high fracture risk, those without recent fracture, or with *T*-score > -2.5 SD, and to consider bisphosphonate transition postdenosumab withdrawal.[3] The aim of an antiresorptive "drug holiday" is to reduce the risk of medication adverse events such as atypical femoral fracture.[3] Nonetheless, the patient still fractured postdenosumab.

This case highlights the uncertain efficacy of bisphosphonate use to adequately suppress bone turnover following denosumab discontinuation and raises three important points: (i) Patients with previous history of vertebral fracture, no matter how remote, are at greatest risk of postdenosumab spontaneous vertebral fractures despite bisphosphonate use; (ii) standard markers of bone turnover may not adequately herald the onset of spontaneous vertebral fractures or decline in bone density following denosumab; and (iii) the dynamics of bone loss following denosumab withdrawal are poorly understood and unpredictable. Until further research emerges regarding the safe withdrawal of denosumab in patients on long-term antiresorptive treatment, a high degree of caution is necessary. An individualized assessment of the risk: benefit ratio of long-term treatment with denosumab is required, bearing in mind that this an otherwise highly effective fracture-preventing treatment.

Disclosures

There are no disclosures.

Peer Review

The peer review history for this article is available at https://publons.com/publon/10.1002/jbm4.10396.

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