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Rapid-sequence MRI for long-term surveillance for paraganglioma and phaeochromocytoma in patients with succinate dehydrogenase (SDHx) mutations

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Abstract

Introduction Patients with *SDHx* mutations need long-term radiological surveillance for the development of paragangliomas and phaeochromocytomas, but no longitudinal data exist. We assessed the performance of rapid-sequence non-contrast magnetic resonance imaging (MRI) in the long-term monitoring of patients with *SDHx* mutations.

Methods Retrospective study between 2005-2015 at a University Hospital and regional endocrine genetics referral center. Clinical and imaging data of forty-seven patients with *SDHx* mutations [*SDHB* (36), *SDHC* (6), *SDHD* (5)] who had surveillance for detection of paragangliomas by rapid-sequence non-contrast MRI (base of skull to pubic symphysis) were collected.

Results Twelve index cases (9 *SDHB*, 1 *SDHC*, 2 *SDHD*) and 35 mutation-positive relatives were monitored for a mean of 6.4 years (range 3.1 to 10.0 years). Mean age at the end of the study: *SDHB* 46.9+/-17.6 years; *SDHC* 42.3+/-24.4 years; *SDHD* 54.9 +/- 10.6 years. Excluding imaging at initial diagnosis of index cases forty-three patients underwent 116 rapid-sequence MRI scans: 83 scans were negative and 31 scans were positive for a sPGL/HNPGL in 13 patients. Most patients had multiple scans [n=number of patients (number of rapid-sequence MRI scans during screening)]; n=9 (2), n=20 (3), n=6 (4), n=1 (6). Nine patients (3 index) were diagnosed with new paragangliomas during surveillance and non-operated tumour size was monitored in 9 patients. There were two false positive scans (1.6%). Scans were repeated every 27 +/- 9 months.

Conclusions Biannual rapid-sequence non-contrast MRI is effective to monitor patients with SDHx mutations for detection of new tumours and monitoring of known tumours.

Introduction

Germ-line mutations of the subunits of the mitochondrial complex II enzyme succinate dehydrogenase (SDHA, SDHB, SDHC, SDHD and SDHAF2), *SDHx*, are associated with familial paraganglioma (PGL) of the sympathetic chain (sPGL), the parasympathetic chain of the head and neck (HNPGL), and adrenal phaeochromocytoma [1-4]. In general most phaeochromocytomas secrete catecholamines, whereas sPGLs may be functional or non-secretory, and HNPGLs are usually biochemically silent [5].

SDHx are tumour suppressor genes, characterized by loss of heterozygosity in tumour cells due to somatic mutations or loss of expression of the wild type allele [6, 7]. The underlying mechanism of tumorigenesis in *SDH*x mutations is still unclear, but non-hypoxic HIF-1alpha and HIF-2alpha activation is a key feature in pathogenesis ('pseudohypoxia' hypothesis) [8]. In *SDH*x-related tumorigenesis there is loss of SDH enzymatic activity and intracellular accumulation of succinate leading to inhibition of prolyl-hydroxylases that usually degrade HIF-1alpha [9-11]. HIF-1alpha is then able to translocate to the nucleus and activate gene expression promoting angiogenesis, cell survival, and glycolysis [10]. The role of oxygen-sensing pathways in *SDH*x tumorigenesis is also supported by observations linking living at high-altitude and an increase in disease prevalence and phenotypic severity [12, 13].

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Patients with *SDHx* mutations are at life-long risk of multifocal, recurrent and malignant PGLs. Mutations in the different subunits cause specific patterns of disease: individuals with paternally-inherited *SDHD* mutations are more likely to develop HNPGL, multifocal disease, and less frequently sPGLs [2, 5, 14, 15]; *SDHB* mutation carriers may develop sPGLs that have a higher malignant potential compared with sporadic or other syndromic PGLs [1, 14]; *SDHC* mutations are rare, with affected

individuals developing HNPGL and phaeochromocytoma that have a low risk of malignancy. Penetrance may occur over the life course, but is incomplete and variable: some *SDHx* members of the same family experience either no tumour development, or a benign or asymptomatic course, whilst others develop devastating and aggressive disease. This underscores the need for appropriate biochemical and imaging screening strategies that may be used in an affected individual over their whole life to detect tumour development, since the primary treatment is resection by an expert surgeon and where better outcomes are found when tumours are detected early [16].

Genetic testing for *SDHx* mutations has been available for approximately the last decade. Although it is widely accepted that carriers of *SDHx* mutations should be monitored for the penetrance of disease, there are no studies reporting the outcome of longitudinal monitoring as highlighted in recently published clinical practice guidelines [17]. Therefore, we report our longitudinal 10-year experience of surveillance imaging in a large cohort of *SDHx* patients attending our dedicated endocrine genetics clinic at a University Hospital using rapid sequence non-contrast magnetic resonance imaging (MRI) as a non-ionizing imaging modality appropriate for life-long follow-up to address three key clinical questions: 1), does this MRI technique detect new tumours in patients with *SDHx*?; 2), can this MRI technique be used to monitor size and extent of known disease in patients in whom definitive surgical excision has not taken place because of tumour site or patient preference?; 3), what is an appropriate time interval between imaging studies?

Methods

Patients

The study was approved as a case notes review by our institutional review board (ID number 3861). All patients with pathogenic *SDHx* mutations (n=47) attending the dedicated Endocrine Genetics clinic at the Royal Hallamshire Hospital, Sheffield Teaching Hospitals (STH) NHS Foundation Trust, a regional referral center, from October 2005 to May 2015, were included. A retrospective review of the medical notes, imaging and biochemistry was conducted. All patients have been reviewed at each clinic visit by one experienced clinician (JNP). All index cases had either excision of their presenting tumour or other treatment prior to embarking on surveillance, and the data presented here on imaging are all from the surveillance programme. All mutation-positive relatives had the first surveillance imaging following genetic diagnosis and all their imaging tests are included in the data presented here.

At our institution genetic testing is performed on patients with phaeochromocytoma aged <50 years or a family history suggesting possible genetically driven disease (such as early cardiac death), or in any patient presenting with sPGL or HNPGL. Genetic testing was performed at the accredited regional genetics laboratory as part of the National Genetics Service of the National Health Service, UK. Carriers of *SDHD* mutations were offered the screening programme if the mutation was of paternal origin as it is well-documented that only those inheriting an *SDHD* mutation from their father exhibit clinical manifestations of the syndrome [3].

All data were discussed at the weekly endocrine multidisciplinary team meeting in the presence of an endocrine radiologist, endocrinologists, endocrine surgeons and chemical pathologists, with the outcome of the studies documented as negative (normal screening), positive (paraganglioma present) or requiring further investigations. As there is no gold standard imaging modality that can

be used for long-term surveillance, i.e. without significant radiation exposure and multiple tests, the outcome of this discussion for each scan was collected and analysed. The outcome of a scan was considered false positive if the lesion was not confirmed to be a paraganglioma at subsequent imaging.

Surveillance protocol

At baseline a detailed clinical assessment was made of all newly referred patients, including a detailed clinical history, clinical examination, together with radiological and biochemical investigations. Thereafter, patients were seen approximately yearly for clinical evaluation and biochemical testing (two 24-hour collections of urinary fractionated metanephrines measured by high-performance liquid chromatography from 2005-2010 or free plasma metanephrines measured by liquid chromatography-tandem mass spectrometry, since 2010) with radiological evaluation every 2 years. For those with disease detected or lesions that require further characterization further imaging evaluation and clinical assessments were made on an individualized basis.

Imaging

MR images were acquired from skull base to the pubic symphysis, including all sympathetic and parasympathetic ganglia, on a 1.5T Siemens Avanto scanner (Siemens AG Munich) and subsequently reviewed by a single expert endocrine radiologist (MB). The imaging protocol is based on three rapid, unenhanced, non-high definition sequences (Transverse T1 spin echo in/out phase, Transverse and Coronal T2 Haste). The combination of both T1 and T2-weighted images in two planes gives a survey from skull base to pelvis. Dedicated neck and phased array body coils were used. Parameters for neck imaging; T2 5mm thickness with 1mm slice gap TR 3650ms TE 99ms matrix size 320x70, T1 5mm thickness with 1mm slice gap TR 611ms TE 12ms matrix size 320x70. Parameters for chest,

abdomen and pelvis imaging; breath hold sequences T2 Haste 7mm with 1mm slice gap TR 1100ms TE 92ms matrix size 256x80, T1 gradient echo 8mm thickness with 1mm slice gap TR 249ms TE 2.29ms (out of phase) 4.76ms (in phase). Each sequence takes usually 2-3 minutes and the average sized patient requires this to be done in three blocks. There is no requirement for intravenous contrast in the surveillance scans and the total duration of imaging is 25 to 30 minutes. Paragangliomas and phaeochromocytomas have high signal on T2-weighted images. The same protocol was used for all patients regardless of causative mutation.

Statistics

Statistical analysis was performed using one-way ANOVA (GraphPad prism 6.0). Results are reported as mean values +/- one standard deviation. A p-value of less than 0.05 was considered significant.

Results

Patients

Forty-seven patients with *SDH*x mutations were included: 36 patients with an *SDHB* mutation, 6 with an *SDHC* mutation, and 5 with an *SDHD* mutation. Twelve out of 47 patients were index cases (9 *SDHB*, 1 *SDHC*, 2 *SDHD*); the remaining 35 patients were gene-positive relatives. Two patients died during the study, one from complications of metastatic sPGL and one from an unrelated cause. At the end of the screening period, defined as the time of death (n=2) or May 2015 (n=45), there was no difference in the mean age between patients with different *SDH* subunit mutations (*SDHB* 46.9+/-17.6 years, *SDHC* 42.3+/-24.4 years, *SDHD* 54.9+/-10.6 years, p=0.5), (this lack of difference may be due to lack of power) (Table 1). There were seven different *SDHB* mutations, 1 *SDHC* and 2 different *SDHD* mutations. Mean duration of monitoring for all patients was 6.4 years (range 3.1-10.0 years).

Overall, at any time eighteen patients (12 index cases and 6 screened relatives) developed a tumour either sPGL or HNPGL [*SDHB* 31% (11/36), *SDHC* 33% (2/6), *SDHD* 100% (5/5)] (Table 1 and Table 2). Patients with *SDHB* mutations predominantly developed sPGLs. Patients with *SDHD* mutations exclusively developed HNPGLs (5/ 5 patients) and had multifocal disease (5/ 5) (Table 2). The youngest age at first presentation was 12y (*SDHD*) and 15y (*SDHB*). At the time of diagnosis of the first tumour the median age was [all patients (index)]; SDHB 28yo (28yo), SDHD 31yo (31yo).

Rapid-sequence MRI surveillance

Forty-three out of 47 patients underwent surveillance imaging with rapid-sequence MRI including all 12 index patients; four patients did not have MRI scans due to severe claustrophobia or non-attendance, were imaged by CT and are excluded from the analysis. Excluding any imaging performed during the diagnosis of the index tumours, imaging was performed on the surveillance protocol in the remaining 43 patients who underwent 116 rapid-sequence MRI scans: 83 scans were negative for sPGL/HNPGL and 31 were positive in 13 patients (Figure 1). At the end of the study there were no cases of missed PGLs, i.e. in patients who developed tumours after the first surveillance MRI (n=4; 2 noradrenaline-secreting sPGLs, 1 HNPGL confirmed with US, 1 thoracic non-secreting sPGL confirmed with dedicated MRI imaging), none of the tumours were found to be present on re-review of earlier imaging. A number of radiological tests were performed in patients who had a positive MRI either for further characterization of positive findings or for disease monitoring [CT (8 scans from 6 patients), USS neck (15 scans from 6 patients), MIBG (4) and ^{18}FDG PET CT (8)] (Table 3). USS neck was used to monitor size of HNPGLs. Rapid-sequence MRI screening was repeated every 27 +/- 9 months (median 25 months) and the majority of patients had more than one scan during surveillance [n=number of patients (number of rapid-sequence MRI scans during

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surveillance)]; n=9 (2), n=20 (3), n=6 (4), n=1 (6). The maximum diameter of new tumours diagnosed during surveillance with rapid-sequence MRI ranged between 0.6 to 3.5cm, with no differences in imaging characteristics between *SDHB*, *SDHC* and *SDHD* subunit mutations.

Index cases (Table 2, Figure 2)

Six out of 12 index patients had complete surgical resection of sPGLs (all noradrenaline-secreting) confirmed with histology prior to this study, normal biochemistry and a negative initial rapid-sequence MRI scan at surveillance baseline. Two patients (*SDHB*) were diagnosed with new sPGLs at the 2nd surveillance MRI (noradrenaline-secreting) and were referred for surgical treatment.

Four index cases with non-metastatic PGLs were not tumour-free on embarking on surveillance [subtotal resection due to multiple HNPGLs/ extensive disease (n=3, patients 12, 14, 16) or non-resected disease (n=1, patient 9)]. The rapid sequence MRI was used to follow the size of tumours and detect new disease in this group of patients; one patient developed progressive disease and was referred for surgery (patient 12), 2 patients with HNPGLs (glomus jugulare) showed slow increase of the tumours and referred for radiosurgery (patients 14, 16), and 1 patient has stable disease (patient 9, sPGL).

There has been histological confirmation of sPGL/HNPGL in all patients who had surgical treatment and in one patient with metastatic disease who had a biopsy (n=10). Although histological confirmation was not made in two other patients, one has a functioning sPGL with characteristic imaging features and diagnostic biochemistry (patient 9) and one patient has a large glomus jugulare tumour with typical radiological features that has been treated with radiosurgery (patient 15). In each case surgical treatment was either refused by the patient, or not appropriate, respectively.

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Genetically screened relatives (Table 2, Figure 2)

During surveillance six genetically screened relatives were diagnosed with either a solitary (n=4) or multiple (n=2) paraganglioma(s) on rapid-sequence MRI. The majority of patients (5/6) were diagnosed with PGLs during their first MRI scan (patients 7, 13, 15, 17, 18). All tumours were non-functioning and there was confirmation from histology (patient 7) or additional dedicated imaging. Except from one patient who underwent surgical excision (patient 7), the tumours were not resected in the remaining four because of the anatomical position and subsequent MRI scans were used to monitor size and plan management (see below). In one patient (patient 8) a small (0.6x1.2cm) thoracic non-functioning sPGL was demonstrated at the 2nd surveillance MRI, 28 months after an initial negative scan. The size of this tumour was also monitored by rapid-sequence MRIs due to the patient not wanting surgical intervention.

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Histological confirmation of a PGL has been made in all genetically screened relatives that had resection in whom the rapid sequence MRI was deemed consistent with a PGL (n=5). There are two patients with small thoracic non-secreting PGLs who have not had surgery (patients 8 and 13, see bellow). The diagnosis of sPGL in these patients is based on typical MRI features; FDG-PET was positive in one patient and negative on the second.

Treatment

Surgical treatment was offered to all patients with non-metastatic sPGL (n=10 patients that developed 12 sPGLs). Overall, 9 sPGLs were excised in 7 patients (all *SDHB*), one patient with metastatic disease (*SDHB*) was treated with chemotherapy and radiotherapy (patient 10), and in three patients the disease is monitored with imaging and biochemistry (2 *SDHB*, 1 *SDHC*; patients 8, 9, and 13) (Table 2), with strong patient preference the reason for monitoring instead of surgical treatment; in two patients with non-secreting thoracic sPGLs (patients 8 and 13) this decision was

influenced by the high surgical risk due to the presence of co-morbidities and the anatomical challenges of surgery. There were nine carotid body (CB) tumours in five patients; four patients were managed conservatively with imaging to assess tumour size because of previous surgery for a contralateral CB tumour (n=3, *SDHD*) and patient preference (n=1, *SDHD*). Five patients with glomus jugulare tumours (4 index cases) were treated with gamma knife stereotactic radiosurgery (1 *SDHB*, 1 *SDHC*, 3 *SDHD*; patients 3, 12, 14, 15, 16). One patient with a noradrenaline-secreting glomus tumour causing local pressure symptoms had gamma knife stereotactic surgery as surgical intervention was considered high risk (patient 3). Following treatment there was a gradual decrease in the level of catecholamines, with symptoms improved and imaging which showed reduction in tumour size within 2 years of intervention. A second patient (patient 12, *SDHC* mutation) with a large, HNPGL with intracranial extension was treated with a combination of tumour embolization, surgical resection, and radiosurgery to a small bone remnant. Three patients (patients 14, 15, 16) with multifocal HNPGLs had imaging surveillance followed by gamma knife therapy when an increase in tumour size was detected. Overall, gamma knife therapy led to growth arrest in 4/5 cases and tumour volume reduction in 1/5 and no complications from this intervention in up to five years of follow up.

Pituitary adenomas

The pituitary gland was included in the screening rapid sequence MRI. There were no macroadenomas detected but 2/43 patients were found to have a small pituitary abnormality, and underwent dedicated pituitary imaging revealing microadenomas: both patients carried the *SDHB* mutation c.379dupA [12% (2/17) of carriers in the cohort] aged 67 and 68. In both cases pituitary function was normal and there was no requirement for specific treatment.

Twenty-two patients were found to have incidental findings on MRI during the screening period. Three patients required a referral for a specialist opinion (respiratory physicians for a lung nodule, gynecologist for an ovarian cyst and breast surgeons), 5 patients had further imaging for characterization of a benign incidental finding, and 14 patients required no further investigations. Two rapid-sequence MRI scans were characterized as false positive based on subsequent imaging; both cases were investigated by dedicated imaging (neck US or MR) that confirmed a lymphangioma and scar tissue, respectively.

Discussion

An increasing number of patients presenting with paragangliomas are being diagnosed with *SDHx* mutations since genetic testing became standard clinical practice, and need surveillance [18], to identify tumours at an early stage when they are amenable to surgical treatment and cure [16]. Since malignant tumours have been described in children and adolescents, it is common clinical practice to offer genetic testing to relatives of affected individuals from around the second decade of life, with mutation carriers then being offered clinical, radiological and biochemical screening [15, 19]. For such life-long screening it is, therefore, important to minimize cumulative radiation exposure. Recent clinical guidelines emphasize the need for surveillance [17, 20]. Our data support the use of rapid sequence MRI for this purpose.

The clinical spectrum of paragangliomas is diverse. Without a clinical screening programme, mutation carriers are at risk of presenting late with complications of syndromes relating to catecholamine excess, local pressure effects of tumours and malignant and metastatic disease [21]. Most tumours are, however, non-functioning and therefore biochemical and clinical monitoring

alone is not enough. Measurement of free plasma metanephrines has been reported to be the most sensitive test for functional paragangliomas and phaeochromocytomas [22] combined with the measurement of the dopamine metabolite 3-methoxytyramine since some paragangliomas produce only dopamine (Table 2) [23]. For these reasons our surveillance protocol mandates yearly biochemical and clinical assessment.

There is a debate as to the gold standard for the detection of paragangliomas. A recent large French series of *SDHx* mutation carriers showed that a combination of imaging modalities (body CT, Head and neck MRA and octreotide scintigraphy) was 99% sensitive for paraganglioma detection [24]; a sub-analysis of the MRA scans from this study showed that a simplified shorter angio-MRI protocol had similar diagnostic performance to the full imaging protocol and could be used instead for the detection of HNPGLs [25]. Although CT has an excellent sensitivity, it involves the use of ionizing radiation and is not ideal for life-long surveillance. MRI does not involve ionizing radiation and is acceptable for use in younger patients and females of reproductive age, making it an ideal surveillance imaging modality for individuals with *SDHx* mutations. Shorter scanning protocols to reduce scanning time of whole body MRI have been developed and cross-sectional data show these to be effective in this clinical setting [26]. Functional imaging can further characterize any tumour, and assess for multifocal or metastatic disease [27]. In this context ^{18}F-FDG PET has been used for several years in patients with *SDHB* mutations and metastatic disease [28], but recently ^{68}Ga-DOTATATE PET/CT has been shown to be superior [29]. Other compounds such as ^{18}F-fluorodopamine (^{18}F-FDA) and ^{18}F-fluoro-dihydroxyphenylalanine (^{18}F-FDOPA) have great promise but are not currently widely available [30]. Although ^{123}MIBG imaging is less sensitive than these modalities it offers a therapeutic option (^{131}MIBG) in MIBG-avid patients with metastatic disease [28, 31].

Our rapid MRI sequences minimizes time (skull base to symphysis pubis scanned in less than half an hour), cost (intravenous gadolinium contrast is not used) and provides accurate results; we have not identified a missed case of a paraganglioma using this rapid sequence MRI for ten years. Furthermore, our data show that this technique can be used reliably to detect new tumours as well as monitor tumour growth in patients managed conservatively. Because the majority of tumours detected in our cohort were on first screening of mutation carriers, we suggest that all index case-relatives with a positive genetic test are offered imaging at the earliest opportunity, as this is the most likely time that tumours will be detected. For patients who had negative initial screening use of rapid sequence MRI approximately every two years appears to be effective and clinically safe. Patients with known tumours under surveillance should have individualized follow-up. An association of pituitary tumours and *SDHx* has been proposed [32], and our MRI protocol allows detection of pituitary tumours of size significant enough to pose a clinical management discussion. Other than the likelihood of the anatomic location of tumours, we found no differences in the MRI features of tumours due to *SDHB*, *SDHC*, or *SDHD* mutations.

Gamma knife radiosurgery appears to be an effective treatment option for some patients with HNPGLs where surgery would carry too much morbidity, including those with a previous history of neck surgery (where the predicted postoperative neurological complications are significant) and older patients with significant perioperative risk [33]. Whilst we report good outcomes from gamma knife radiosurgery it is important to note that we are the National Centre for Stereotactic radiosurgery and have treated more than 15,000 patients with this technique; it is likely that this high level of expertise had a positive impact on our patient outcomes, and good outcomes and low complications are reported from other high volume centers [34].

The strengths of this study are that it is a single-center study at a center with extensive relevant imaging and clinical expertise, where a practical rapid sequence MRI imaging protocol has been developed and used for screening for over 10 years, with all cases routinely discussed in multidisciplinary meetings in the presence of endocrine surgeons and input from all specialists informed management decisions. Although small tumours (<5mm) may suffer from partial volume effects limiting interpretation the likelihood of tumours of this size causing a clinical syndrome associated with catecholamine excess or being of malignant potential, is low. Limitations of our study include the need for multidisciplinary expertise. Although this is a large cohort, the numbers of patients with positive scans remains small precluding statistical comparisons. Furthermore, a single gold standard test that can be used for long-term screening in these patients doesn't exist and there is no imaging modality (or combination of modalities) that is without significant radiation exposure and could be used as a comparison, therefore the outcome of the review of biochemistry, clinical data and MRI imaging by the multidisciplinary team was considered the gold standard to determine the success of treatment and disease free-status. Finally, although two of the patients we describe (patients 8 and 13) have typical radiological features of sPGLs, their biochemistry was normal and they have declined surgery, and thus we do not have histological confirmation for them.

To our knowledge this is the first report of longitudinal screening in patients with *SDHx* mutations using non-contrast rapid sequence MRI. Our data support the use of this technique in the surveillance of these patients to detect new tumours and monitor size of existing tumours, and provide evidence that biannual imaging with annual biochemical testing is an effective approach.

Declaration of interest: The authors have nothing to declare

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Figure legend

Figure 1:

(1a) A 20mm lesion medial to the left adrenal gland shown in a coronal T2 haste sequence (Patient 6, noradrenaline-secreting abdominal paraganglioma).

(1b) A 27mm soft tissue lesion posterior to the pulmonary artery within the mediastinum shown in an axial gradient-echo T1 weighted sequence (Patient 9, noradrenaline-secreting thoracic paraganglioma)

(1c) Transverse gradient echo T1 sequence showing bilateral homogenous carotid body tumours at the bifurcation of the common carotid between the internal and external carotids and (1d) Axial T2 haste sequence showing extensive destructive high signal tumour centered at the right foramen jugulare (Patient 15, glomus jugulare and bilateral carotid body tumours)

(1e) Well-defined homogeneous soft tissue mass centered at the foramen jugulare shown in an axial gradient-echo T1 weighted sequence (Patient 3, noradrenaline-producing glomus jugulare).

Figure 2: Flow diagram of patient surveillance

519 **Tables**

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521 **Table 1.** Characteristics of patients with *SDHx* subunit mutations

	SDHB	***SDHC***	***SDHD***
Number of patients (females)	36 (18)	6 (5)	9 (4)
Index cases	9	1	3
Relatives	27	5	6
Age at the end of screening, mean +/- SD[1]	46.9 +/- 17.6	42.3 +/- 24.4	54.9 +/- 10.6
Age range	18-76	20-75	26-64
Index cases: age range at presentation	15-50	37	12-40
Mean age at first tumour[1] (range)	36.1 (15-70)	48.5 (37-60)	33.3 (22-56)
Mean duration of screening in years (range)	6.5 (3.0-10)	4.8 (3.2-10)	5.6 (2.8-10)
Number of patients who developed tumours in total	11	2	6
% of patients who developed tumours	31%	33%	67%
Patients who were diagnosed with tumours on surveillance programme	4	2	3
Total number of tumours	13	3	13
HNPGL (functioning)	1 (1)	2 (1)	12 (0)
sPGL (functioning)	12 (10)	1 (0)	0
Phaeochromocytoma	0	0	1

[1]No statistical difference between the *SDHB, SDHC* and *SDHD* groups

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Table 3. The results of additional imaging tests used to investigate positive screening results during monitoring

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	Number of scans	Results
Neck USS	15	12 positive (HNPGLs) 3 negative (lymph nodes, thyroglossal cyst)
MIBG	4	2 positive Patient 6: NA-secreting sPGL Patient 10: non-secreting metastatic PGL 2 negative Patient 9: NA-secreting thoracic sPGL and a HNGPL Patient 18: non-secreting multiple HNGPL
^{18}FDG PET CT	8	3 positive Patient 11: NA-secreting sPGL Patient 13: extensive HNPGL Patient 21: incidental bone lesion 5 negative Patient 2: incidental hilar mass Patient 7: non-secreting thoracic sPGL Patient 8: sPGL Patient 22: lymphangioma Patient 23: incidental lymphadenopathy

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Table 2. List of patients with *SDHx* subunit mutations who developed tumours with characteristics of disease and treatment

Patient	Mutation	Age	Tumours	Secretion	Recurrent/ Multiple or Metastatic	Size of tumour (cm)[#]	Treatment
1 [+]	*SDHB* c.72+1G>T	15	Pelvic sPGL	NA	No	(4.0)	Excision
2 [++]	*SDHB* c.600G>T	17	Pelvic sPGL	NA- both	Multiple (n=2)	(NK)	Excision (both)
		24	Abdominal sPGL			2.4	
3 [+]	*SDHB* exon 1 deletion	50	HNPGL (GJ)	NA	Locally aggressive	3.9	g-knife radiosurgery
4 [+]	*SDHB* c.137G>A	31	Abdominal sPGL	NA and DA	No	(7.0)	Excision
5 [+]	*SDHB* c.379dupA	25	Abdominal sPGL	NA and DA	No	(5.0)	Excision
6 [++]	*SDHB* c.379dupA	22	Abdominal sPGL	NA-both	Multiple (n=2)	(NK)	Excision (both)
		24	Abdominal sPGL			2.0	
7	*SDHB* c.379dupA	20	Thoracic sPGL	No	No	2.4	Excision
8	*SDHB* c.379dupA	68	Thoracic sPGL*	No	No	1.2	Monitoring for 7 years, no change
9 [+]	*SDHB* c.380T>G	44	Thoracic sPGL	NA	No	3.7	Monitoring (patient preference)
10 [+]	*SDHB* c.17_42dup26	35	Thoracic sPGL	No	Metastatic (liver spine)	8.3	I^{131} MIBG, Radiotherapy, sunitanib
11 [+]	*SDHB* c.17_42dup26	70	Abdominal sPGL	NA	No	(5.0)	Excision
12 [++]	*SDHC* c.397C>T	37	HNPGL (GJ)	DA	Locally aggressive,	>5.3	Sub-total excision, excision or recurrence, g-knife
		69	Recurrence of HNPGL	DA	progressive disease		radiosurgery
13	*SDHC* c.397C>T	60	HNPGL	No-both	Multiple (n=2)	2.2	HNPGL: Excision
			Thoracic sPGL*			2.1	sPGL: Monitoring-mild increase in 5 years
14 [+]	*SDHD* c.342T>A	40	HNPGLs (bilateral CB)	No-both	Multiple (n=3)	R: (NK), L: 1.8	Right CB: excision, Left CB: monitoring
			HNPGL (GJ)			1.5	GJ: Monitoring, g-knife radiosurgery
15	*SDHD* c.242C>T	12	HNPGL (GJ)	No	Recurrent, multiple (n=3)	3.5	GJ: Excision, g-knife radiosurgery
			HNPGLs (bilateral CB)			R: 2.2, L: 2.7	CB: Monitoring
16 [+]	*SDHD* c.242C>T	22	HNPGL (GJ)	No	Recurrent, multiple (n=2),	(NK)	GJ: Excision, g-knife radiosurgery
			HNPGL (CB)		locally aggressive (GJ)	(NK)	CB: Excision
17	*SDHD* c.242C>T	31	HNPGLs (bilateral CB)	No	Recurrent, multiple (n=2)	R: 1.8, L: 1.0	Right: excision
							Left: monitoring
18	*SDHD* c.242C>T	56	HNPGLs (bilateral CB)	No	Multiple (n=2)	R: 3.0, L: 0.6	Right: excision
							Left: monitoring

+ Index case; ++ Index case with second tumour; * sPGL on imaging, no histology; # maximum diameter by rapid sequence MRI (maximum diameter by diagnostic imaging); NK: not known; R: right; L: left; GJ: glomus jugulare; CB: carotid body tumour, NA: noradrenaline; DA: dopamine

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