Contrasting clinical manifestations of *SDHB* and *VHL* associated chromaffin tumours

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Abstract

Mutations in succinate dehydrogenase-B (*SDHB*) and the von Hippel-Lindau (*VHL*) genes result in an increased risk of developing chromaffin tumours via a common aetiological pathway. The aim of the present retrospective study was to compare the clinical phenotypes of disease in subjects developing chromaffin tumours as a result of *SDHB* mutations or *VHL* disease. Thirty-one subjects with chromaffin tumours were assessed; 16 subjects had *SDHB* gene mutations and 15 subjects had a diagnosis of *VHL*. *VHL*-related tumours were predominantly adrenal pheochromocytomas (22/26; 84.6%), while *SDHB*-related tumours were predominantly extra-adrenal paragangliomas (19/25; 76%). Median age at onset of the first chromaffin tumour was similar in the two cohorts. Tumour size was significantly larger in the *SDHB* cohort in comparison with the *VHL* cohort ($P=0.002$). Multifocal disease was present in 9/15 (60%) of the *VHL* cohort (bilateral pheochromocytomas) and only 3/16 (19%) of the *SDHB* cohort, while metastatic disease was found in 5/16 (31%) of the *SDHB* cohort but not in the *VHL* cohort to date. The frequency of symptoms, hypertension and the magnitude of catecholamine secretion appeared to be greater in the *SDHB* cohort. Renal cell carcinomas were a feature in 5/15 (33%) of the *VHL* cohort and 1/16 (6%) of the *SDHB* cohort. These data indicate that *SDHB*-related tumours are predominantly extra-adrenal in location and associated with higher catecholamine secretion and more malignant disease, in subjects who appear more symptomatic. *VHL*-related tumours tend to be adrenal pheochromocytomas, frequently bilateral and associated with a milder phenotype.

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Introduction

Recent studies have shown that ~25% of apparently sporadic pheochromocytomas and paragangliomas are due to germ line mutations in one of several familial syndrome genes, including von Hippel-Lindau (*VHL*), *RET*, succinate dehydrogenase-B (*SDHB*), *SDHC*, *SDHD* or *NF1* (Neumann *et al.* 2002, 2004,

Amar *et al.* 2005). Pheochromocytomas are tumours arising from catecholamine-producing chromaffin cells in the adrenal medulla, while paragangliomas are extra-adrenal chromaffin tumours of either sympathetic (secretory) or parasympathetic (mainly non-secretory) origin, located between the base of skull and the pelvis. *VHL* disease and familial paraganglioma syndrome

type 4 are caused by mutations in the *VHL* and *SDHB* genes respectively (Latif *et al.* 1993, Astuti *et al.* 2001). Functional investigations of the *VHL* and *SDHB* gene products have revealed overlapping functions. Firstly, inactivation of *VHL* and *SDHB* results in activation of hypoxic gene response pathways through stabilization of the HIF-1 and HIF-2 transcription factors (Maxwell *et al.* 1999, Selak *et al.* 2005). However, *VHL* mutations associated with a pheochromocytoma only phenotype (type 2C *VHL* disease) retain the ability to regulate HIF transcription factors (Clifford *et al.* 2001) implicating additional pathways in the pathogenesis of pheochromocytoma. Subsequently, it was demonstrated that *VHL* and *SDHB* mutations are both associated with a failure of normal developmental apoptosis of pheochromocytoma precursor cells (Lee *et al.* 2005). However, despite the similar functional consequences of *VHL* and *SDHB* inactivation, clinical differences have been described and analysis of *VHL*- and *SDHB*-associated tumours have shown some differences in patterns of gene expression (Pollard *et al.* 2006).

SDHB gene mutations have already been associated with extra-adrenal disease, a high risk of malignancy and renal cell carcinoma (Neumann *et al.* 2004, Vanharanta *et al.* 2004, Benn *et al.* 2006, Timmers *et al.* 2007, Ricketts *et al.* 2008, Srirangalingam *et al.* 2008). *VHL* disease has been associated with both adrenal and extra-adrenal chromaffin tumours (type 2), low rates of malignancy and renal cell carcinoma (Maher *et al.* 1990, Walther *et al.* 1999, Neumann *et al.* 2001, Kaelin 2004). Correlating the genetic dysfunction to differences in clinical phenotype of disease may provide valuable clues to differentiate the two syndromes.

In this study, we have compared the detailed clinical presentations of pheochromocytomas and paragangliomas in these two syndromes with a common aetiological pathway. We note an early onset of disease in both cohorts but significant differences with respect to location of disease, size of tumours, multifocality and metastatic potential of disease. We also note differences with respect to associated symptoms and catecholamine secretion.

Materials and Methods

Subjects for this study were identified at St Bartholomew's Hospital from records collated by SLC, with additional subjects identified by LW, SAA and ABG. *SDHB* subjects from other UK centres who were related to members of the initial St Bartholomew's Hospital cohort were included in this study. *VHL*

subjects, with a history of chromaffin tumours were included in the study, if they had a *VHL* mutation identified or they fulfilled the clinical criteria for disease as delineated by Melmon and Rosen (1964). Subjects were included in the study if they had complete imaging from the base of the skull to the pelvis and measurements of urinary or plasma catecholamines. The case-notes and hospital databases were examined for the required data, which included: age at first symptom/diagnosis, family history, disease penetrance, site and size of tumour, symptoms, secretion of catecholamines, multifocal disease, metastatic disease, treatment and disease outcome. The tumour size was taken as the widest diameter recorded on examination of the pathological sample or radiologically, if the tumour had not been removed. A comparison between cohorts was made along with a subgroup analysis of associated features corrected for tumour size and genetic mutation. The *SDHB* subjects in this study have been reported previously (Astuti *et al.* 2001, 2003, Lawrence *et al.* 2004, Srirangalingam *et al.* 2008), as have some of the *VHL* subjects with respect to pancreatic lesions (Mukhopadhyay *et al.* 2002). The study was classified as a service evaluation and therefore did not require formal ethical approval. Each subject provided informed consent within the screening process, which included appropriate genetic counselling.

Pheochromocytomas were defined as tumours arising from chromaffin tissue in the adrenal medulla, while all extra-adrenal chromaffin tumours were designated paragangliomas. Paragangliomas originate in the abdomen, pelvis, thorax or head and neck regions. Multifocal disease was defined as a subject having multiple tumour foci, presenting synchronously or metachronously to the original tumour in areas normally containing chromaffin tissue. Metastatic disease was defined as evidence of distant spread in tissue not normally containing chromaffin tissue i.e. bone, liver, lung or lymph nodes.

The statistical software package GraphPad Prism version 4 was used for this analysis. Mean data with standard deviations or median data with interquartile range (IQR) are presented where appropriate. Two-tailed tests with a significance level of 5% were used. The χ^2 or Fisher's exact test was employed to test for association between categorical variables. Continuous data were assessed for normality and appropriate parametric (*t*-test) or non-parametric (Wilcoxon signed-rank test) statistical testing applied. Odds ratios were calculated in relation to mutation type and location of tumours.

Results

Basic demographics

Thirty-one subjects were included in this study consisting of 16 *SDHB* mutation carriers and 15 subjects with *VHL* disease followed-up between 1975 and 2008. Median follow-up time overall was 11.0 years (IQR 3.5–15.0, range 1.1–31); 8.7 years (IQR 3.3–15.8, range 1.1–31) in the *SDHB* cohort and 11 years (IQR 3.5–15.0, range 3–28) in the *VHL* cohort. The index case in family 3 was excluded because the original data were unavailable. Further demographics are given in Table 1.

There were 11 index subjects in the *SDHB* cohort and 9 index subjects in the *VHL* cohort. Evidence of familial disease was only noted in 2/11 (18%) *SDHB* index cases compared with 4/9 (44%) *VHL* index-cases. Apparently sporadic presentations of disease occurred in 6/11 (55%) of *SDHB* index subjects compared with 2/9 (22%) of *VHL* index subjects. In 3/11 *SDHB* subjects and 3/9 *VHL* subjects, there was a suggestive but inconclusive history of familial disease.

The median age at first diagnosis of a chromaffin tumour was similar between the groups; 26.5 years (IQR 16–40.5) in the *SDHB* mutation carriers versus 19 years (IQR 12–29) in the *VHL* group ($P=0.15$, Mann–Whitney test). The initial manifestation of

disease in *VHL* group was a chromaffin tumour in 9/15 (60%) subjects, retinal angiomas in 4/15 (27%) subjects, cerebellar haemangioblastoma and renal cell carcinoma in 1/15 (7%) subjects each. Where a chromaffin tumour was not the first presentation of disease, this lagged the initial diagnosis by a median period of 3.5 years (IQR 2–14).

Mutations

Ten mutations in the *SDHB* gene (nine exonic and one intronic) and five mutations in the *VHL* gene were noted. Of the 15 *VHL* subjects, 12 subjects were mutation positive (*subject* 29 – genetic linkage suggested 99% chance of carrying affected mutation carried by affected father (*subject* 28) but mutation analysis not done), while three fulfilled clinical criteria for *VHL* disease. Mutations are listed in Table 2.

Tumour characteristics

Twenty-five tumours in the *SDHB* mutation carriers and twenty-six tumours in the *VHL* group were noted (Fig. 1). Of the *SDHB*-related tumours, 19/25 (76%) were extra-adrenally located compared with 6/25 (24%) adrenal phaeochromocytomas. This is in contrast to the *VHL*-related tumours, where the majority, 22/26 (84.6%) were adrenal phaeochromocytomas and only 4/26 (15.4%) tumours were extra-adrenally located. A further breakdown of tumour distribution is given in Table 2. The majority of extra-adrenal tumours were abdominal paragangliomas in *SDHB* mutation carriers, whereas extra-adrenal disease was equally distributed between the abdomen and head-and-neck regions in the *VHL* group (Fig. 1). There were no cases of bilateral adrenal phaeochromocytomas in the *SDHB* cohort in comparison with 9/15 (60%) in the *VHL* cohort. Of these, 5/15 (33%) *VHL* subjects had synchronous phaeochromocytomas and 4/15 (27%) had asynchronous tumours. As a result, multifocal disease was more common in the *VHL* group in comparison with the *SDHB* group; 9/15 (60%) compared with 3/16 (19%) respectively. The median diameter of tumours associated with *SDHB* mutation carriers was significantly higher at 6.0 cm (IQR 4.5–7.3) than tumours in the *VHL* group of 3.7 cm (IQR 2.9–5.0) ($P=0.0016$, Mann–Whitney test). The odds ratio of developing a phaeochromocytoma or an extra-adrenal paraganglioma according to genetic mutation was calculated. In comparison with an *SDHB* mutation carrier, a subject with *VHL* disease has an odds ratio of 17.42 (95% CI, 4.1–71.1; $P<0.0001$) of developing an adrenal phaeochromocytoma.

Table 1 Basic demographics of the succinate dehydrogenase-B and von Hippel-Lindau cohorts

	<i>SDHB</i> cohort	<i>VHL</i> cohort
Number of subjects	16	15
Males	9	10
Females	7	5
Index subjects	11	9
Followed-up between/year	1975–2007	1980–2008
Median follow-up time/years	8.7 (IQR 3.3–15.8)	11 (IQR 3.5–15.0)
Number of families	3 (7 subjects)	4 (10 subjects)
Isolated subjects ^a	9	5
Definite family history	2/11 (18%)	4/9 (44%)
No family history (apparently sporadic)	6/11 (55%)	2/9 (22%)
Number of tumours	25	26
Multifocal disease	3/16 (19%)	9/15 (60%)
Metastatic disease	5/16 (31%)	0/15 (0%)

Index subjects are defined with respect to present study i.e. index subject in this cohort (true index subject may not be followed up in this cohort).

^aIsolated subjects in this study, however, may have a family history outside of this cohort.

Table 2 Genetic mutations and disease phenotype in the succinate dehydrogenase-B and von Hippel-Lindau cohorts

Subject	Family	Gender	Mutations	Site	Mutation type	Amino acid	Age at diagnosis	Disease	Additional information
SDHB mutation carriers									
1 ^a		F	c.268 C>T	3	Nonsense	p.Arg90X	41	AP	No further disease
2 ^a		M	c.487 T>C	5	Missense	p.Ser163Pro	40	H, AP, P	Died 20 years after diagnosis second stroke
3 ^a		M	c.590C>G.	6	Missense	p.Pro197Arg	24	Pe, mets	Mother had CBT – died aged 32 years
4 ^a		F	c.423+20 T>A	4(I)	Splice site ^b	–	41	P	No further disease
5 ^a		M	c.423+20 T>A	4(I)	Splice site ^b	–	48	P	Carotid artery dissection, PFO, PTE, stroke
6 ^a		F	c.141 G>A	2	Nonsense	p.Trp47X	10	AP, RCC	Chemo for RCC mets
7 ^a		M	c.292 T>C	4	Missense	p.Cys98Arg	47	Pe, mu, mets	Died second PGL mets
8 ^a	1	M	c.136 C>T	2	Nonsense	p.Arg46X	18	AP	No further disease
9		M	c.136 C>T	2	Nonsense	p.Arg46X	26 ^c	AP, mets	Died metastatic PGL
10		F	c.136 C>T	2	Nonsense	p.Arg46X	39 ^c	T	Awaiting excision
11 ^a		M	c.72+1 G>T	1	Splice site	–	14	P	No further disease
12 ^a		F	c.406 del A	4	Frameshift	p.Ile136X	29	AP, mets	Died PGL mets intra-operatively
13	2	F	c.406 del A	4	Frameshift	p.Ile136X	14	P	No further disease
14 ^a		M	c.137G>A	2	Missense	p.Arg46Gln	11	AP, P, T, mu, mets	Mother had PGL mets, died aged 38 years
15	3	F	c.590C>G	6	Missense	p.Pro197Arg		AP	No further disease
16		M	c.590C>G	6	Missense	p.Pro197Arg	18	Pe	Lost to follow-up
VHL group									
17 ^a		M	–	–	–	–	58	P	RCC
18 ^a		M	c.555C>G	3	Nonsense	p.Tyr185X	30	AP	Retinal angiomas aged 16
19 ^a		M	–	–	–	–	32	H	Cerebellar HB aged 28
20 ^a		M	c.499 C>T	3	Missense	p.Arg167Trp	14	P, mu	Left phaeochromocytoma identified on adrenal catheter
21 ^a	4	M	–	–	–	–	23	P, mu	
22 ^a		F	c.499C>T	3	Missense	p.Arg167Trp	8	P, mu	Asynchronous
23		M	c.499C>T	3	Missense	p.Arg167Trp	25	P, mu	Synchronous
24		M	c.499C>T	3	Missense	p.Arg167Trp	14	P	
25	5	F	c.499C>T	3	Missense	p.Arg167Trp	12	P, mu	
26 ^a		M	c.509T>G	3	Missense	p.Val170 Gly	29	P, mu	Synchronous
27		F	c.509T>G	3	Missense	p.Val170 Gly	12	P	

Table 2 continued

Subject	Family	Gender	Mutations	Site	Mutation type	Amino acid	Age at diagnosis	Disease	Additional information
28 ^a	6	M	c.353T>G	2	Missense	p.Leu118Arg	13	P, mu, AP, H	Asynchronous
29		F	c.353T>G	2	Missense	p.Leu118Arg	19	P, mu	Asynchronous
30 ^a	7	F	c.464T>A	3	Missense	p.Val155Glu	24	P, mu	Synchronous
31		M	c.464T>A	3	Missense	p.Val155Glu	8	P	Awaiting surgery

F, female; M, male; AP, abdominal paraganglioma; P, pheochromocytoma; Pe, pelvic paraganglioma; T, thoracic paraganglioma; H, head-and-neck paraganglioma; PGL, paraganglioma; CBT, carotid body tumour; RCC, renal cell carcinoma; Mu, multifocal; Mets, metastatic; PFO, patent foramen ovale; PTE, pulmonary thrombo-embolism; (I) Intronic mutation.
^aIndex subjects.
^bSplice site based on predictive models (genotype–phenotype relationship not confirmed).
^cDisease-affected subjects identified at screening. Subjects 17, 19 and 21, fulfil Melmon & Rosen criteria for *VHL* disease (Melmon & Rosen 1964). Subject 29, genetic linkage analysis – 99% chance of being affected, however, mutation analysis not done.

Figure 1 Distribution of chromaffin tumours amongst the *SDHB* and *VHL* cohorts. Proportion for each tumour location given as percentage of total number of tumours in that cohort i.e. 25 tumours in the *SDHB* cohort and 26 tumours in the *VHL* cohort.

Symptoms

The difference between the groups in median duration of symptoms prior to initial chromaffin tumour did not reach significance at 1.3 years (IQR 0.2–2.8) for the *SDHB* mutation carriers and 0.09 years (IQR 0.0–3.5) for the *VHL* group ($P=0.088$, Mann–Whitney test). *SDHB* mutation carriers were more frequently hypertensive (11/16; 69%) than subjects in the *VHL* group (5/15; 30%) ($P=0.048$, χ^2 test). A breakdown of symptom frequency for each group is given in Fig. 2. The classic triad symptoms (headache, palpitations and diaphoresis) were the commonest features of disease; however, the *SDHB* mutation carriers appeared more symptomatic overall. While the classical triad of symptoms was present in 8/16 (50%) of *SDHB* mutation carriers, the full set of symptoms was not present in any *VHL* subject ($P=0.002$, Fisher’s exact test).

Catecholamines

Catecholamines were raised in the majority of subjects with both syndromes. Twelve of fifteen (80%) *SDHB* mutation carriers and thirteen of fourteen (93%) of the *VHL* group had raised catecholamine levels (Table 3). Of those subjects in both cohorts with elevated catecholamines, noradrenaline levels were raised in all subjects, while adrenaline levels were raised in 3/15 (20%) compared with 1/15 (7%) and dopamine levels were raised in 1/15 (8%) compared with 3/15 (20%) subjects in the *SDHB* and *VHL* groups respectively.

Figure 2 Comparison of disease phenotypes between the *SDHB* and *VHL* cohorts. (A) Percentage of subjects diagnosed at a given age. (B) Percentage of subjects with common symptoms. Black bars indicate *SDHB* subjects and grey bars indicate *VHL* subjects.

However, further analysis of the magnitude of the noradrenaline rise over the upper limit of the normal range (99th centile) in these subjects reveals that *SDHB* mutation carriers produced significantly more noradrenaline than *VHL* group ($P=0.0126$, Mann-Whitney test). Statistical analysis was not undertaken for adrenaline and dopamine secretion as the numbers of subjects with elevated levels were too small for comparison.

Functional imaging

Radiolabelled MIBG scanning was carried out in 12 *SDHB* mutation carriers and 12 of the *VHL* group. Proportionally, more of the *VHL* group demonstrated MIBG uptake in comparison with the *SDHB* mutation carriers i.e. 11/12 (92%) vs 6/12 (50%) subjects, though this difference did not reach statistical

significance ($P=0.068$, Fisher's exact test). Out of the five *VHL* subjects with synchronous adrenal phaeochromocytomas, only 2/5 (40%) had bilateral uptake on radiolabelled MIBG imaging. One *SDHB* subject with a thoracic paraganglioma did not show evidence of radiolabelled MIBG uptake but had positive uptake with FDG PET scanning.

Metastatic disease

Metastatic paragangliomas were noted in 5/16 (31%) *SDHB* mutation carriers at a median of 4 years (range 1.5–25) after the initial tumour. To date, no member of the *VHL* group has developed evidence of malignant disease.

Subgroup analysis

A subgroup analysis was carried out to correct for possible confounding factors by matching subjects for tumour size (within 1 mm) and ensuring that no subject within the subgroup had the same mutation. This comparison resulted in the inclusion of seven subjects from each group with tumours ranging in size from 3.5 to 6.5 cm. The trend for higher levels of noradrenaline secretion (magnitude rise over the upper limit of the normal range; median level 6.1 vs 1.8), frequency of the classic triad of symptoms (2/7 vs 0/7) and an increased malignancy rate (2/7 vs 0/7) was noted for the *SDHB* subjects compared with *VHL* subjects, but these differences were not statistically significant ($P=0.37$, 0.46 and 0.46 respectively).

Associated disease

As reported previously, one *SDHB* mutation carrier developed a metastatic type II papillary renal cell carcinoma 16 years after the initial presentation of an abdominal paraganglioma at the age of 10 years. The metastatic renal carcinoma in this subject has responded to the multi-target tyrosine kinase inhibitor, *sunitinib*. Along with other manifestations of *VHL* disease (which we have not discussed), 5 out of 15 (33%) *VHL* subjects developed clear-cell renal carcinomas.

Treatment

All the *SDHB* mutation carriers have had surgery for their tumours and five subjects required repeat surgery for recurrent tumours or metastatic disease. Fourteen out of the fifteen *VHL* group have had surgery for their tumour, while four subjects required further surgery for multifocal disease. Two *VHL* subjects have tumours *in situ*. Subject 31 has an adrenal phaeochromocytoma

Table 3 Catecholamines/metabolite secretion and radiolabelled MIBG imaging

Subject	Disease	Catecholamines (urinary)				Catecholamines (plasma)			Additional information	Radiolabelled MIBG
		NA	A	D		NA	A			
1	AP	+	–	–		–	–			–
2	H (bilateral) AP, P, mu					+	–			+
3	Pe, mets	+	–	–		+	–			+
4	P	+	–	–		+	–			+
5	P	+	+	–		–	–		Pre-operative urinary VMA negative	–
6	AP, RCC, mets									
7	Pe, mu, mets	+	–	+		+	+			–
8	AP	+	–	–		–	–			+
9	AP, mets	+	–	–		+	+			–
10	T	–	–	–		–	–			–
11	P	+	–	–		–	–		Chromaffin tumour not suspected pre-operatively	+
12	AP, mets									
13	P	–	–	–						–
14	AP, P, T, mu, mets	+	–	–		+	–			+
15	AP	+	–	–						
16	Pe	+	–	–						
17	P	–	–	–		–	–			+
18	AP	+	–	–					Chromaffin tumour not suspected pre-operatively	+
19	H									
20	P, mu	+	–	–						+
21	P, mu	+	+	–						+
22	P, mu	+	–	+						+
23	P, mu	+	–	–						+
24	P	+	–	–						+
25	P, mu	+	–	–						+
26	P, mu									+
27	P	+	–	–		+	–		Chromaffin tumour not suspected pre-operatively	–
28	P, mu, AP, H									
29	P, mu	+	–	–						+
30	P	+	–	–						+
31	P	+	–	+						

+ signifies elevated level or positive uptake (MIBG); – signifies non-elevated level or negative uptake (MIBG); blank box indicates test not done. NA, noradrenaline; A, adrenaline; D, dopamine; AP, abdominal paraganglioma; P, pheochromocytoma; Pe, pelvic paraganglioma; T, thoracic paraganglioma; H, head-and-neck paraganglioma; RCC, renal cell carcinoma; Mu, multifocal; Mets, metastatic.

and is awaiting surgery, while *subject 28* has a non-functioning neck paraganglioma which is ^{123}I MIBG negative but FDG-PET positive which remains under observation. Out of the five *SDHB* mutation carriers with metastatic paragangliomas, two have had ^{131}I MIBG therapy, while two have had chemotherapy. Out of the two *SDHB* subjects requiring radiolabelled MIBG therapy, one has responded to therapy while the other subject has had progressive disease. The two subjects requiring chemotherapy (MIBG non-avid disease) did not respond to therapy.

Outcome

Out of the 16 *SDHB* subjects, four subjects are dead. In three cases, this was directly disease related and in the other subject this was indirectly disease related (multiple strokes secondary to hypertension). The 12 remaining subjects continue under follow-up. To date, 9 out of the 16 *SDHB* mutation subjects have had no further disease after their initial tumour. There was no associated mortality in the *VHL* cohort with chromaffin tumours.

Discussion

In this study, we have made a comparison of the clinical manifestations of pheochromocytomas and paragangliomas in a cohort of 16 subjects with *SDHB* mutations and 15 subjects with *VHL* disease. Despite a common aetiological pathway in the pathogenesis of disease, there appear to be distinct clinical features in terms of tumour distribution, symptomatology, associated disease and malignancy rates. Limitations of this study include the small sample size and variations in follow-up regimens between cohorts. Differences in tumour size and the inclusion of family members with the same mutation may introduce further sources of bias. A subgroup analysis, correcting for tumour size and genetic mutations, noted a trend for higher noradrenaline secretion, symptom frequency and malignancy rates with *SDHB*-related tumours, but was probably under-powered and did not confirm these differences statistically. The higher malignancy rate in the *SDHB* mutation carriers made it difficult to meaningfully correct our data for tumour size as some of these subjects suffered rapid tumour development between annual surveillance scans.

This study demonstrates marked differences in the chromaffin tumour characteristics between the syndromes. *SDHB* mutation carriers have a predominance of extra-adrenal disease (76%) mainly in the form of abdominal paragangliomas (48%), whereas the *VHL*

group have a predominance of adrenal disease (85%), mainly in the form of bilateral adrenal pheochromocytomas (60%). Multifocal disease is limited to 19% of *SDHB* mutation carriers compared with 60% of the *VHL* group. The tumour size was significantly larger in the *SDHB* mutation carriers compared with the *VHL* group (6.0 vs 3.7 cm). The higher rates of malignancy seen with *SDHB* mutation carriers may correlate with larger tumour size. Given that *VHL* disease surveillance has been in place longer and that there is heightened abdominal surveillance may result in earlier identification of chromaffin tumours (Maher et al. 1990, Choyke et al. 1995) in this cohort.

The age at initial diagnosis of a pheochromocytoma or paraganglioma was not significantly different between the groups but emphasizes the presentation of these tumours at an early age. A history of familial disease was more evident in the *VHL* cohort than in the *SDHB* mutation carriers. We have demonstrated previously the incomplete penetrance of disease in *SDHB* mutation carriers (Srirangalingam et al. 2008) and indeed a history of apparently sporadic disease does not rule out the possibility of an *SDHB* mutation as noted in 55% of *SDHB* mutation carriers in this study. The wide range of manifestation of *VHL* disease and the high penetrance of features such as cerebellar haemangioblastomas and retinal angiomas, tend to make family disease more apparent in comparison with disease in *SDHB* mutation carriers.

In these small cohorts, obvious genotype–phenotype correlations with respect to chromaffin tumours were not apparent. However, genotype–phenotype correlations in *VHL* disease in comparison with *SDHB* disease are more established (Ong et al. 2007). These note that missense mutations involving amino acids on the surface of the VHL protein are associated with higher rates of pheochromocytomas (Ong et al. 2007).

This study demonstrates that the *VHL* group appears to be significantly less symptomatic than the *SDHB* subjects. Both hypertension and the classic triad of symptoms were significantly less prevalent though the duration of symptoms were comparable. It is possible that documentation of symptoms of chromaffin tumours is more comprehensive in *SDHB* mutation carriers, where pheochromocytomas and paragangliomas are the predominant disease manifestation in comparison with *VHL* disease where it is one of many manifestations. The absence of symptoms in a significant proportion of subjects with *VHL* disease-associated pheochromocytomas has been noted previously (Walther et al. 1999). Again, the established surveillance programme for *VHL* disease may mean that disease is identified before symptoms develop.

The smaller size of tumours in the *VHL* group may correlate with less metabolically activate disease and hence less symptoms. Catecholamines were raised in a similar number of subjects in the 2 cohorts; however, the degree to which the catecholamines were raised may explain these differences. In both cohorts, noradrenaline was the predominant catecholamine secreted, however, the magnitude of the secretion was higher in the *SDHB* population that may correlate with symptomatology (Walther *et al.* 1999).

Functional imaging with radiolabelled MIBG was positive in significantly more subjects with *VHL* disease than in *SDHB* mutation carriers (92% vs 50%). *SDHB*-related tumours are larger, more malignant and may be expected to be more dedifferentiated (Timmers *et al.* 2007). Such tumour may lack the relevant transporter to take up MIBG. Importantly, despite high MIBG uptake in the *VHL* cohort overall, in the cases of synchronous bilateral adrenal tumours only 40% demonstrated bilateral uptake.

The two cohorts contrast significantly with respect to malignancy rates. In *SDHB* mutation carriers, malignant paragangliomas were evident in 31% of subjects, while no subjects have developed malignant disease in the *VHL* cohort to date. These data correlate with the larger tumour size noted in the *SDHB* mutation cohort compared with the *VHL* cohort. The literature confirms the malignant phenotype of *SDHB*-related disease in numerous studies (Neumann *et al.* 2004, Benn *et al.* 2006, Timmers *et al.* 2007, Srirangalingam *et al.* 2008). Though malignant disease can be associated with *VHL* disease, it is significantly less frequent (Neumann *et al.* 1993).

VHL disease is associated with a wide range of manifestations. In relation to the present study, it is of interest that chromaffin tumours were the initial manifestation of disease in 60% of *VHL* group followed by retinal angiomas in 27% of subjects. Renal cell carcinomas appear to be a manifestation

of disease in both cohorts. However, *VHL* disease is associated with a high risk of clear-cell renal carcinoma (Maher *et al.* 1990), whereas *SDHB* mutations are associated with a lower risk and a variety of renal cell carcinoma cell types (Vanharanta *et al.* 2004, Srirangalingam *et al.* 2008). In both cohorts, the renal cell carcinomas have responded to therapy with various multi-target tyrosine kinase inhibitors which work via inhibition of the VEGF-HIF pathway which is implicated in the pathogenesis of these tumours (U Srirangalingam, personal observations).

The aggressive nature of disease associated with *SDHB* mutations is illustrated by the fact that there have been four deaths in this cohort, three of which were as a result of metastatic disease. Despite the co-morbidity associated with *VHL* disease, no subjects have died within this cohort to date.

Disease surveillance regimes with respect to chromaffin tumours in *VHL* disease are well established and include annual history and examination, measurement of plasma or urinary catecholamines or metanephrines and CT or MRI scan of the abdomen (Maher *et al.* 1990, Choyke *et al.* 1995). Annual imaging of the abdomen in *VHL* subjects is driven by the surveillance requirements for renal cell carcinoma, and will also image both adrenals and much of the abdominal sympathetic chain. Table 4 summarizes our present practice for chromaffin tumour surveillance in *SDH* mutation carriers and subjects with *VHL* disease. Some patients with *VHL* disease have paragangliomas outside the abdomen and so additional imaging of the sympathetic chain in the neck, chest and pelvis is prudent and we suggested this should be done on a 3-yearly basis. For *SDHB* mutation carriers, we introduced annual surveillance in 2001 (Chew 2001), but there remains some debate about the most cost-effective method of imaging the entire sympathetic chain.

Table 4 Disease surveillance regimes for von Hippel-Lindau and succinate dehydrogenase-B-related chromaffin tumours

Genetic mutation	Catecholamines/ metanephrines	Imaging (preferably MRI)	
		Region	Frequency
<i>SDHB</i>	Annually	Abdomen	Annually
		Neck/thorax/pelvis	2 yearly
<i>SDHD</i> ^a	Annually	Neck/thorax/abdomen/pelvis	3 yearly
<i>VHL</i>	Annually	Abdomen	Annually
		Neck/thorax/pelvis	3 yearly

Annual review includes clinical assessment, symptom enquiry, blood pressure and relevant clinical examination. All subjects should have imaging from the base of the skull to the pelvis on initial surveillance. Disease surveillance recommended from the age of 5 years for all mutations carriers. If a lesion is noted, or surveillance is suggestive of disease, imaging should be carried out early.
^aChange to *SDHB* surveillance regime for *SDHD* mutation associated with increased risk of malignant disease (Timmers *et al.* 2008).

Despite the common pathway involved in the pathogenesis of these two syndromes and the similarities in presentation with respect to age at diagnosis of the first chromaffin tumours, predominance of noradrenaline secretion and the association with renal cell carcinoma, there are several marked differences with respect to disease location, frequency of symptoms, magnitude of catecholamine secretion, other extra-paraganglial associations, malignancy and mortality rates. While these findings may be explicable in part, as a result of different management practices, it is likely that the aggressive disease phenotype that can be associated with *SDHB*-related disease plays a role. Such findings should improve disease identification and future surveillance strategies.

Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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