RESEARCH

Genotype-phenotype associations in PPGLs in 59 patients with variants in *SDHX* genes

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Abstract

Phaeochromocytomas and paragangliomas (PPGLs) are tumours of the adrenal medulla and extra-adrenal sympathetic nervous system which often secrete catecholamines. Variants of the *SDHX* (*SDHA*, *-AF2*, *-B*, *-C*, *-D*) genes are a frequent cause of familial PPGLs. In this study from a single tertiary centre, we aimed to characterise the genotype-phenotype associations in patients diagnosed with germline variants in *SDHX* genes. We also assessed whether systematic screening of family members resulted in earlier detection of tumours. The study cohort comprised all individuals ($n = 59$) diagnosed with a rare variant in *SDHX* during a 13-year period. Patient- and pathology records were checked for clinical characteristics and histopathological findings. We found distinct differences in the clinical and histopathological characteristics between genetic variants in *SDHB*. We identified two *SDHB* variants with distinct phenotypical patterns. Family screening for *SDHB* variants resulted in earlier detection of tumours in two families. Patients with *SDHA*, *SDHC* and *SDHD* variants also had malignant phenotypes, underlining the necessity for a broad genetic screening of the proband. Our study corroborates previous findings of poor prognostic markers and found that the genetic variants and clinical phenotype are linked and, therefore, useful in the decision of clinical follow-up. Regular tumour screening of carriers of pathogenic variants may lead to an earlier diagnosis and expected better prognosis. The development of a combined algorithm with clinical, genetic, morphological, and biochemical factors may be the future for improved clinical risk stratification, forming a basis for larger multi-centre follow up studies.

Key Words

- genotype
- phenotype
- PPGL

Endocrine Connections
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Introduction

Phaeochromocytomas and paragangliomas (PPGLs: PCCs (MIM: 171300), PGL1 (MIM: 168000), PGL2 (MIM: 601650), PGL3 (MIM: 605373), PGL4 (MIM: 115310), PGL5 (MIM: 614165), PGL6 (MIM: 618464)) are tumours of the adrenal medulla and extra-adrenal sympathetic nervous system, respectively, that often secrete catecholamines. Paragangliomas (PGLs) arising from the parasympathetic

nervous system are often non-secreting tumours located in the head and neck region (termed head and neck paragangliomas (HNPPGLs)) (1). Because of the strong heritability, genetic testing is recommended in all patients with PPGLs and, when a variant is detected, also screening of their family members (1, 2, 3, 4, 5). Markers of poor prognosis in PPGLs have been identified in

different studies, including tumour size >5 cm, vascular invasion, *SDHB* variants and norepinephrine secretion (6). There is, however, little information about the clinical phenotype associated with the individual genetic variants (6, 7).

PPGLs are very heterogeneous tumours and more than 20 predisposing genes have been identified (6). In two-thirds of the PPGL patients, a pathogenic germline or somatic variant can be found and PPGLs have been divided into distinct biological subgroups (kinase signaling, pseudohypoxia and Wnt signaling) on the basis of these variants (6). Most PPGLs are non-metastatic tumours but approximately 15% are malignant (6). Malignancy status is given to metastatic disease at distant sites unrelated to the paraganglia tissues (6). The overall 5-year survival for malignant PPGLs is approximately 60% (8). Germline variants in succinate dehydrogenase (*SDH*)-A, -AF2, -B, -C, and -D (MIM: 600857; MIM: 613019; MIM: 185470; MIM: 602413; MIM: 602690) have been found to account for approximately half of the hereditary PPGLs (9) and for up to 15% of all patients with PPGLs (10).

The *SDHX* genes belonging to the *SDH* gene family encode a protein complex, which is involved in the Krebs cycle and the respiratory chain and are classified as tumour suppressors (9, 10, 11, 12). A germline variant of *SDHB* is the best-known genetic predictor of malignancy as approximately 50% of tumours progress to metastatic disease (6, 13). Two-thirds of malignant PPGLs, however, do not harbour *SDHB* variants (6) and other predictive markers of poor prognosis have been investigated. The PASS (phaeochromocytoma (PCC) of the adrenal gland scaled score) was developed to identify those PCCs (not PGLs) that have metastatic potential based on a score ≥ 4 (14). The PASS method has a number of limitations in the clinical setting as the inter- and intra-observer variability is high (6, 15, 16). A recent study found that the PASS was best used clinically to rule out, rather than rule in, malignancy potential for PPGLs due to low positive predictive value and relatively higher negative predictive value (17).

Thus, existing prognostic markers cannot fully predict PPGL behaviour (6, 18), which is a clinical challenge. To our knowledge, there is no previous systematic study of the genotype-phenotype associations in patients with solely germline variants in *SDHX* genes. In this study, we aim to characterise the possible associations between genotype on the one hand and the clinical and histopathological presentation on the other in 59 individuals diagnosed with germline variants in *SDHX* genes in the setting of a single tertiary referral centre.

Methods

Study cohort

The study cohort comprised all individuals diagnosed with a variant of one of the *SDHX* genes between 1 January 2006 and 1 March 2019 who were followed in the Department of Medical Endocrinology, Copenhagen University Hospital, Rigshospitalet. All individuals with a proven variant in the *SDHX* genes (class 3, 4, or 5) were included. The persons were identified by their social security number in the Danish National Patient Registry. Genetic screening, variant classification, and clinical workup were carried out at the Centre for Genomic Medicine, Copenhagen University Hospital, Rigshospitalet. The study was approved by the Data Protection Agency (I-Suite number 6712) and the National Committee on Health Research Ethics, Denmark (H-1803546). The approval included a dispensation for collecting an informed consent and the study was performed according to the flow chart in Fig. 1.

Genetic test results

The screening was part of the family counselling of PPGL patients and their relatives (pre-symptomatic screening). The genetic screening methods have previously been described in detail (11). In brief, genomic DNA was isolated from whole blood using a QIAmp DNA mini kit (Qiagen) according to the instructions provided by the manufacturer. From 2006 to 2014, *SDHX* genes were amplified by PCR followed by Sanger sequencing using an ABI3730 DNA analyser (Applied Biosystems). Genomic DNA was examined for large genomic rearrangements by Multiplex ligation-dependent probe amplification (MLPA) using SALSA® MLPA® Probemix P226-D1 *SDH*. Since 2014 the analysis has been done by next-generation sequencing (NGS) on a MiSeq (Illumina, San Diego, USA). The sequencing data were analysed using SequencePlot (JSI Medical Systems, Germany) where variants were called if the allele frequency was above 25%. The following genes were included in the genetic screening: *SDHA*, *SDHAF2*, *SDHB*, *SDHC*, and *SDHD*. Variants were classified according to American College of Medical Genetics (ACMG) guidelines (19). Only class 3, 4, and 5 variants were included in the diagnostic report.

Clinical records

Patient records were obtained from electronic patient records (Epic) or local archives. Clinical characteristics,

Figure 1

Flow chart of the study. CRH, corticotropin-releasing hormone; HNPGL, head and neck paraganglioma; ki-67, antigen associated with cellular proliferation; NSE, neuron-specific enolase; PASS, pheochromocytoma of the adrenal gland scaled score; PCC, pheochromocytoma; PGL, paraganglioma; PPGL, pheochromocytoma and paraganglioma; *SDHX*, succinate dehydrogenase genetic variants; SS receptor, somatostatin receptor; VIP, vasoactive intestinal peptide.

biochemistry, and imaging results at the time of PPGL diagnosis were extracted and validated by a second observer (senior clinician). One patient had previously been described (20).

Pathology reports

The pathology reports were retrieved from the national database (PatoBank) or from local archives for all patients who had undergone surgery for PPGLs. Subsequently, the reports were studied for specific histopathological findings. Malignancy of PPGLs was defined as proof of metastases based on the European Society of Endocrinology guidelines (3). Together with the genotype, Ki-67% labelling index, secretion of hormones, tumour size and location, and clinical symptoms, we included components of the PASS score to cover all possible markers of poor prognosis reported in the literature (6).

Statistical analysis

The difference between the age of disease onset in men and women was tested with the Mann–Whitney’s *U*-test and the difference between the number of men and women in the cohort by the Chi-squared test.

All other results are descriptive. Due to low numbers in the subgroups, we neither performed statistical analysis nor power calculations for these results.

All *SDHX* variant positive patients were grouped according to their respective variants. Thus, a group of two *SDHA* variant positive patients, a group of *SDHB* variant positive patients (eight families (Family B1–B8) and four unrelated patients (Bx)), four patients with *SDHC* variants (two unrelated patients and two patients from the same family), and two unrelated patients with *SDHD* variants were identified. For *SDHA*, *SDHB*, *SDHC*, and *SDHD* variants, the letters ‘A’, ‘B’, ‘C’, or ‘D’ were included in the patient identification (ID). To ensure patient anonymity, each patient within an *SDHX* positive family was given an arbitrary number and either the letter ‘F’ (female) or ‘M’ (male).

Families B1–B8 were compared by the age of onset, tumour characteristics, clinical symptoms, histopathological findings, and signs of malignant disease for the patients with PPGLs. The unrelated *SDHB* (Bx) group was compared to other *SDHB* families or family members with variants in the same exon(s) when possible. The patients who developed tumours and had class 4 and 5 genetic variants were compared by the age of onset and malignancy.

For all individuals who had not yet developed PPGLs, the total number of years without disease were calculated from the date of genetic diagnosis until 1 March 2019.

Results

The characteristics of the cohort are shown in Table 1.

In total, 59 individuals were diagnosed with an *SDHX* variant during a 13-year period. We were able to retrieve clinical records for 58 of these. One medical file was lost and for this patient we were only able to retrieve the pathology report and genetic test. A total of 24 *SDHX* variant positive patients underwent surgery (Fig. 1).

The median age at PPGL diagnosis was 42.5 years. There was no sex difference between the median age at tumour diagnosis of women (40.5 years ($n=14$)) compared to men (45.5 years ($n=10$), ($P=0.62$)) or for the number of individuals with an *SDHX* variant (30 female, 29 male, $P=0.34$) (Table 1).

Most patients were diagnosed with variants in the *SDHB* gene (51/59). Only two, four, and two individuals were positive for variants in *SDHA*, *SDHC*, and *SDHD*, respectively, while no patients were positive for *SDHAF2*. A total of 18 PPGLs and 5 PCCs were diagnosed. Proof of metastases was found in 12 out of 59 patients. Seventeen out of 51 *SDHB* variant patients were diagnosed with PPGLs. One of the *SDHA* variant positive patients ($n=2$) was diagnosed with a PGL. All *SDHC* ($n=4$) and one *SDHD* patient developed PPGLs. Three patients were diagnosed with renal cell carcinoma, two with an *SDHB* variant and one with a variant of the *SDHD* gene (Table 1).

The histopathological characteristics of the tumours are shown in Supplementary material (see section on [supplementary materials](#) given at the end of this article).

In total, the cohort had 12 probands and 41 family members with a class 4 or 5 variant and 6 individuals with a class 3 variant. Among the 41 family members, two individuals were diagnosed with tumours at the first screening and two with tumours during routine screenings after 2 and 7 years. Three family members with a class 4 or 5 variant that had been treated for tumours earlier in life and were diagnosed with the genetic variant many years later during family screening. Thirty-four family members with a class 4 or 5 variant have been followed for an average time of 48.8 months (range: 11 days to 136 months) without tumour detection.

Comparison of patients with class 4 and 5 variants ($n=19$; 1 *SDHA*, 15 *SDHB* and 3 *SDHC*) showed that 10 of 19 developed metastatic disease and that the age at tumour diagnosis was related to the genotype with family B1 presenting with tumours at a lower age than family B3 (Fig. 2). The patients with class 4 and 5 variants in *SDHB* presented mostly with PGLs; only one patient had both a PCC and a PGL. Three *SDHB* patients had non-metastatic PGLs and both cases with renal cell carcinomas were confined to the kidney. Within the *SDHB* variant group, two genetic variants showed a specific age distribution of first tumour diagnosis. In family B1, PGLs were detected between the age of 12 and 28 years and 75% were with metastatic disease. In family B3, metastatic or non-metastatic PGLs or renal cell carcinomas confined to the kidney were diagnosed between the age of 28 and 57 years. Family B4 had the same variant as family B3 and the

Table 1 Characteristics of *SDHX* variant positive patients.

Characteristics	Total (<i>n</i>)	With PPGL	No PPGL	Age (years) at first PPGL diagnosis range (median)
Females, <i>n</i>	30	14	16	17–65 (40.5)
Males, <i>n</i>	29	10	19	12–73 (46.5)
Total, <i>n</i>	59	24	35	12–73 (42.5)
Genetic variants	<i>SDHA</i>	<i>SDHB</i>	<i>SDHC</i>	<i>SDHD</i>
Total (<i>n</i>)	2	51	4	2
Patients with PPGL diagnosis, <i>n</i>	1	17	4	1
PGL, <i>n</i>	1	13	4	0
PCC, <i>n</i>	0	4	0	1
RCC, <i>n</i>	0	2	0	1
Patients, <i>n</i>	1	7	2	2
Clinical symptoms reported at PPGL diagnosis, <i>n</i>	0	8	3	0

A patient may display both a pheochromocytoma and a paraganglioma or a renal cell carcinoma (RCC), therefore the total *n* is larger than total number of patients with PPGLs.

PCC, pheochromocytoma; PGL, paraganglioma; PPGL, pheochromocytoma and paraganglioma; *SDHX*, succinate dehydrogenase genetic variants.

Figure 2

Age at first tumour diagnosis (PPGL or RCC) in patients with class 4 and 5 variants of *SDHA* ($n = 1$), *SDHB* ($n = 15$), and *SDHC* ($n = 3$) genes (open circles depict non-metastatic tumours, stars show metastatic disease). None of the *SDHD* variants were class 4 or 5. AM1, *SDHA* family, male 1; B1F1 to B8F1, *SDHB* families 1, 3, 4–8; F, female; M, male; CF3 to CM2, *SDHC* families; RCC, renal cell carcinoma; PPGL, pheochromocytoma and paraganglioma; SDH, succinate dehydrogenase.

patients were diagnosed with either renal cell carcinoma confined to the kidney or metastatic PGL at the age of 43 and 50 years.

All patients with *SDHC* variants were diagnosed after the age of 50 years. Two patients with metastatic disease were unrelated and older than the patient with the non-metastatic tumour (Fig. 2). In total, six variants were classified as class 3 (three *SDHB*, one *SDHC*, and two *SDHD*).

SDHB positive patients and carriers

Patient with variants in *SDHB* came from eight families and four were unrelated (Supplementary material). A complete histopathological characterisation is found in Supplementary material and the different genetic variants and corresponding clinical outcome are shown in Table 2.

In family B1 (class 5, deletion of exon 4 and 5 in *SDHB*), four members developed PPGLs. The tumours had different markers of poor prognosis, including metastatic disease, nuclear pleomorphism, capsular invasion, mitotic figures $>3/10$ HPF, extension into adipose tissue and Ki-67% of 15% in one case (Supplementary material). The tumours developed early in life (12–28 years). F1 and M1 were genetically diagnosed after the onset of disease, but in two family members (F4 and F5) tumours were detected during screening after 2 and 7 years of follow-up. Neither has yet had a recurrence. The family members

who were not screened prior to PPGL diagnosis had larger tumours, which secreted catecholamines, gave rise to clinical symptoms, recurrent disease, and metastases (Supplementary material and Table 3).

The homozygous proband (child of consanguineous parents) in family B2 (class 5 variant in exon 8, c.769C>G, p.Leu257Val) died at the age of 25 months from a severe respiratory infection with multi-organ failure. All heterozygous members (2F, 4M) were tumour-free.

In family B3 (class 4 variant in exon 1, c.3G>A, p.Met1?), five members were diagnosed with PPGLs (3 F, 2 M) between 28 and 57 years of age. One female (F6) and one male (M1) were diagnosed with a tumour at the first screening. The family members with PPGLs displayed markers of poor prognosis including metastatic disease and markers included in the PASS (Supplementary material) and the variant might be associated with a high risk of tumour development in adults (five out of nine family members) (Table 2). Family B4 carried the same variant as family B3 and two family members were diagnosed with tumours (M1, M2) (Supplementary material) and the PGL was disseminated at diagnosis.

One female in family B7 (class 5 variant in exon 7, c.653G>A, p.Trp218*) was diagnosed at the age of 39 years, the other carrier (M1) has until now (84 years) not been diagnosed with PPGLs. The primary tumour found in F.1 had a Ki-67% of more than 1% (50%) and poor prognostic histopathological markers, including necrosis, mitotic figures $>3/10$ HPF, vascular invasion, and nuclear pleomorphism. Later, F.1 had a new event with metastases (Supplementary material). In family B5, the tumour metastases also had a higher Ki-67% >1 (Supplementary material).

The proband (F1) of family B8 was diagnosed with a class 5 variant (c.683_684del, p.Glu228Glyfs*27) in exon 7. This tumour was smaller and had a lower Ki-67% index than the primary tumour in family B7 and no recurrence of disease (Supplementary material).

Two of four unrelated patients (Bx, 4 F) were diagnosed with PPGLs. F2 and F5 both had class 3 variants (Table 2) and developed PCCs with clinical symptoms (Table 3).

In total, seven patients with class 4 and 5 *SDHB* variants developed metastatic disease, the highest number being found in family 1 (Table 2). None of the patients with the class 3 *SDHB* variants developed metastatic disease.

Tumour types and clinical symptoms of hormone secretion in patients with an *SDHB* variant are depicted in Table 3. The predominant tumour type was PGL with symptoms of catecholamine excess and local tumour pressure.

Table 2 *SDHA-D* genetic variants and clinical outcome.

Gene	Family (genetic variant)	Variant class	Number of individuals with a variant	Number of individuals with tumour diagnosis	Tumour type				Malignant (M)	Age of individuals without tumour diagnosis
					PCC	PGL	HNPGL	RCC		
<i>SDHA</i>	A (c.762_770+17del, p. (Ala255_Gly257del) in exon 6/intron 6)	5	2	1		X		NA	Non-metastatic (N)	24
<i>SDHB</i>	B1 (c.287-?_540+?del/deletion of exon 4 and 5)	5	10	4	X	X			M (n = 3) N (n = 1) ^e	12–70 ^c
<i>SDHB</i>	B2 (c.769 C>G, p.Leu257Val in exon 8)	4 ^a	7	0				X	NA	11–54
<i>SDHB</i>	B3 (c.3G>A,p.Met1? in exon 1)	4	9	5	X	X	X		M (n = 1) N (n = 4)	32–86
<i>SDHB</i>	B4 (c.3G>A,p.Met1? in exon 1)	4	8	2	X	X	X		M (n = 1) N (n = 1)	11–76
<i>SDHB</i>	B5 (c.203G>A, Cys68Tyr in exon 3)	4	3	1	X	X			M	35–65
<i>SDHB</i>	B6 (c.343C>T, p.Arg115* in exon 4)	5	4	1			X		N	21–51
<i>SDHB</i>	B7 (c.653G>A, p.Trp218* in exon 7)	5	2	1	X	X			M	84
<i>SDHB</i>	B8 (c.683_684del, p.Glu228Glyfs*27 in exon 7)	5	5	1	X	X			N	15–75
<i>SDHB</i>	BxFl (c.416T>C, p.Leu138Pro in exon 4)	5	1	0				X	NA	28
<i>SDHB</i>	BxFl (c.24C>T, p.Ser8Ser in exon 1)	3	1	0				X	NA	56
<i>SDHB</i>	BxFl (c.424-19_424-14dup in intron 4)	3	1	1	X				N	-
<i>SDHB</i>	BxFl (c.487T>C, Ser163Pro in exon 5)	3 ^b	1	1	X				N	-
<i>SDHC</i>	CM1, CM2 (c148C>T, pArg50Cys in exon 3)	4	2	2	X				NA (n = 1) M (n = 1) ^f	-
<i>SDHC</i>	CF3(c.191_207del in exon 4)	5	1	1		X			M	-
<i>SDHC</i>	CM4 (c.496C>G, p.Leu166Val in exon 6)	3	1	1	X				N ^g	-
<i>SDHD</i>	DF1 (c.282C>T, p.Ser94Ser in exon 3)	3	1	1				X	M	-
<i>SDHD</i>	DF2 (c.359T>C, p. Leu120Ser in exon 4)	3	1	1	X				M	-

^aNo heterozygous carriers have developed tumours so far. Future follow-up examinations of heterozygous carriers may reveal if this variant can be classified as benign or pathogenic; ^bPolymorphism, allele frequency of 1.53% in European (non-Finnish) population; ^cOne individual deceased, age not included; ^dAM1: metastatic disease or synchronous tumours. ^eB1F4, B1F5: metastatic disease or synchronous tumours. B1M1: metastatic disease and new events; ^fCM2: metastatic disease or synchronous tumours; ^gCM4: Recurrent disease. B, benign; HNPGL, head and neck paraganglioma; N, non-metastatic; NA, not available; PCC, pheochromocytoma; PGL, paraganglioma; RCC, renal cell carcinoma; SDH, succinate dehydrogenase.

Table 3 Tumour type, symptoms, and hormone secretion in tumours with *SDHB* or *SDHC* variants.

Family (variant)	Individual	Diagnosis	Symptoms at diagnosis	Pressure symptoms	Hypertension	Headache	Perspiration	Nausea	Palpitation	Dizziness	Flushing	Weight loss	Paleness	Anxiety	NA	Hormone secreted at diagnosis
B1 (c.287_2_540+? del/deletion of exon 4 and 5)	F1	PGL													X	NA ^a
B3 (c.3G>A.p. Met17 in exon 1)	M1 F1	PGL PGL		X X	X X	X X	X X	X X								Noradrenaline (P) NA
B4 (c.3G>A.p. Met17 in exon 1)	F6 M3	PGL PGL		X X	X X		X X		X X	X X	X X					Chromogranin A (P) Noradrenaline (U) Adrenaline (U) Metanephrines (P) Chromogranin (P) Noradrenaline (P, U)
B5 (c.203G>A, Cys68Tyr in exon 3)	M2 F2	PGL PCC		X X	X X		X X									Noradrenaline (U)
Bx (c.424-19_424-19dup in intron 4)	F2	PGL PCC				X	X	X	X				X		X	Normetanephrine (P) Adrenaline (P)
Bx (c.487T>C, p.Ser16Pro in exon 5)	F5	PCC		X												NA
C (c.148C>T, p.Arg50Cys in exon 3)	M1	PGL													X	Chromogranin A (P)
C (c.191_207del in exon 4)	M2 F3	PGL PGL		X X			X X									Noradrenaline (U) Chromogranin A (P)
C (c.496C>G, p.Leu166Val in exon 6)	M4	Paraganglioma		X												NA ^b

None of the *SDHA* or *SDHD* variant positive individuals displayed symptoms at PPGL diagnosis. No hormones were detected biochemically for the *SDHA* variant positive patient and these data were not available for the *SDHD* variant positive patients.

^aPatient B1F-1 was not tested for catecholamines but experienced palpitations in relation to the biopsy procedure suggested catecholamine secretion by the tumour; ^bPatient C M.4 was not tested for catecholamines prior to surgery but had a sudden blood pressure increase during surgery indicative of catecholamine secretion by the tumour.

F, female; M, male; NA, not available; P, plasma; PCC, pheochromocytoma; PGL, paraganglioma; SDH, succinate dehydrogenase; U, urine.

***SDHA*, *SDHC* and *SDHD* positive patients and carriers**

A 19-year-old male with an *SDHA* variant (deletion in exon 6/intron 6) had two metastatic PPGLs which could also be interpreted as synchronous tumour events. There was one carrier in the family (female) who was healthy at the age of 24 years (Supplementary material).

All patients with an *SDHC* variant (1 F, 3 M) developed PGLs, hereof two with metastatic disease. One of these was detected in a patient carrying a class 3 variant (Table 2). All available *SDHC* histopathology records described tumour necrosis (Supplementary material). The predominant symptom at diagnosis was pressure symptoms, and the tumours secreted both chromogranin A and catecholamines (Table 3).

Two women were diagnosed with two class 3 variants in the *SDHD* gene, exon 3 and 4, respectively (Supplementary material). DF1 was diagnosed with a malignant renal carcinoma and DF2 with a metastatic PCC.

Discussion

To our knowledge, this is the first systematic study of a single tertiary centre cohort of 59 individuals with germline *SDHX* variants describing the associations between the genotype on the one hand and both the clinical and histopathological phenotype on the other. *SDHB* variants were the most frequent, which is in accordance with previous reports (9, 10, 12, 18, 21). Two patients were diagnosed with a variant in *SDHA* and four patients were diagnosed with *SDHC* variants, which corroborates previous findings that these are much less common (9, 10, 12, 22). Only two individuals were diagnosed with *SDHD* variants, although these have been described as more common in other populations (9, 10, 12).

Our patient cohort of *SDHX* variant positive individuals consisted of almost equal numbers of females (30/59) and males (29/59) which was expected from the autosomal dominant inheritance with incomplete penetrance of *SDHA*, -B, -C, -D or -AF2 variants located at chromosomes 5, 1, 1, 11, and 11, respectively. There was also no significant sex difference in whether or not the patient developed tumours. The *SDHA* positive family ($n=2$) has, to our knowledge, a novel class 4 variant not previously reported. This variant was associated with PGLs already at the age of 19 years, thus underlining the necessity for continuous screening. *SDHB* variants are

common and have a high penetrance compared to other *SDHX* variants resulting in development of abdominal PGLs, HNPGLs, and SDH deficient renal carcinomas (10, 12, 22). The class 4 and 5 variants in our cohort predisposed to metastatic or non-metastatic PGLs and renal cell carcinomas without local invasion or metastases which correlated with previous findings (9, 10, 12). In family B2, no heterozygous individual with a class 5 missense variant (c.769C>G, p.Leu257Val) in exon 8 of the *SDHB* gene has as yet developed PPGLs. Literature, however, suggests an increased risk (20), indicating a low penetrance for this specific variant. The homozygous proband's clinical course supports the international recommendation that sequencing of *SDHB*, -D, and possibly -C should be performed in patients suspected for or diagnosed with multisystem mitochondrial disorder and complex II deficiency (20).

The patients and healthy family members were included in a follow-up programme according to available international guidelines, from the year 2016 those of European Society of Endocrinology (3) with genetic testing and regular tumour screening (plasma or urinary catecholamines and chromogranin A every year, imaging every 1–2 years). This revealed tumour development before the onset of symptoms at younger ages than the proband (family B1). Tumours were consequently smaller at diagnosis and more often non-metastatic (family B1). Furthermore, one female and one male were diagnosed with PGL at the very first screening (family B3).

Distinct differences were found in the clinical picture and histopathology between various genetic variants which in the future may prove useful for the development of more individualised clinical guidelines for follow-up. Notably, families B1 and B3 showed distinct differences in age of first tumour diagnosis. Families B3 and B4 carry the same start loss variant (class 4 variant in exon 1 of *SDHB*, c.3G>A, p.Met?) that is likely pathogenic (11) and both families had clinical signs of poor prognosis. Families B5 and B7 support the hypothesis that a Ki-67 index >1% is a possible marker for malignancy in PPGLs (6, 23).

Variants in the *SDHC* gene usually predispose to non-metastatic and multiple HNPGLs (9, 10, 12). All four of our *SDHC* variant individuals developed tumours (HNPGLs and PPGLs), two with proof of metastasis and two showed catecholamine-related symptoms, which, according to the literature, are two very rare findings (9). One of the individuals with proof of metastasis had a class 3 variant further underlining that the tumour classification alone cannot predict clinical the phenotype (24). Larger studies of the genotype-phenotype associations are needed to

elucidate how the different genetic variants in PPGLs may be stratified into prognostic risk categories. Furthermore, a study suggested that the variant type (truncating vs missense) in *SDHD* had an important clinical impact (25).

Variants in the *SDHD* gene usually predispose to multiple HNPGLs and less often PPGLs (10). However, in our cohort, the *SDHD* positive individuals developed malignant renal cell carcinoma and metastatic pheochromocytoma. According to the literature, only one previous case of renal cell carcinoma has been reported in a patient with an *SDHD* variant (26).

Our study was based on the Danish National Patient Registry, which ensures that patient records are easily found. In fact, only one clinical record was missing and we obtained both genetic test results and pathology reports from all relevant individuals. The registry has previously been validated as a valuable tool for research in rare diseases (27). Our study included all patients diagnosed since 1 January 2006 in the Department of Endocrinology, Copenhagen University Hospital, Rigshospitalet, resulting in 59 patients. A national cohort would increase the power to detect differences between specific genotypes and corresponding phenotypes and possible regional differences. It has previously been shown that the distribution of *RET* variants in Denmark differs from other populations, possibly due to a founder effect (28), and that the same could be the case for *SDHX* variants, as other countries have shown differences in clinical presentation of *SDHX* mutations (29).

The clinical records obtained did not always include a description of subjective symptoms. This is a well-known drawback of retrospective studies and a potential source of recall bias or bias due to clinical practice, that is, whether the individual was asked about subjective symptoms or not. Some of the individuals have only recently been diagnosed with a familial genetic variant in *SDHX* and may not yet have developed tumours because of the short follow-up time or young age. The pathology reports obtained for this study were carried out by different pathologists and, therefore, the interpretation of the findings may be subject to observer variation.

We decided to define malignancy for PPGLs as proof of metastases based on the guidelines (3, 30). Together with the genotype, Ki-67% labelling index, secretion of hormones, tumour size, location, and clinical symptoms, we included components of the PASS score to cover all possible markers of poor prognosis reported in the literature (6). The PASS was not originally developed for PGLs and, therefore, there is some uncertainty in

applying the components of the PASS to these tumours. The clinical features and histopathological characteristics are, however, similar for PCCs and PGLs (31).

In summary, our study corroborates previous findings of poor prognostic markers (6, 12). Furthermore, we found that the genetic variants are linked to the clinical and histopathological phenotype and prognosis and therefore useful for the decision of clinical follow-up. Family screening for *SDHB* variants allowed an earlier detection of smaller tumours with better prognosis in one family (B1) and detection of tumours at the first screening in another (B3). This supports current guidelines recommending family screening. Within our cohort, two *SDHB* variants (a deletion of exon 4 and 5 and c.3G>A, p.Met1? in exon1) were identified in individuals with specific phenotypes which may be relevant in future counselling. The *SDHA*, *SDHC* and *SDHD* patients in our cohort included malignant phenotypes, underlining the necessity to screen for all *SDHX* variants and, if positive, we recommend family screening to improve early tumour detection and thereby better prognosis. The development of an objective combined algorithm with clinical, genetic, morphological, and biochemical factors may be the future for clinical individualized risk stratification.

Supplementary materials

This is linked to the online version of the paper at <https://doi.org/10.1530/EC-20-0279>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

Ulla Feldt-Rasmussen was the supervisor and performed project planning, data analysis and interpretation. Ailsa Maria Main performed data collection, data analysis, statistics, interpretation, and article writing. Åse Krogh Rasmussen was the co-supervisor and performed project planning, data analysis and interpretation. Caroline Maria Rossing performed collection of existing genetic results from database, genetic reruns by NGS, interpretation of genetic results, and provided expert counselling on clinical genetics and formulated the section on genetics under 'methods'. Line Gutte Borgwardt performed collection of existing genetic results from database, genetic reruns by NGS and interpretation of genetic results. Birgitte Grønkær Toft provided expert counselling on pathology reports.

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