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SDHC phaeochromocytoma and paraganglioma: a UK-wide case series

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SDHC phaeochromocytoma and paraganglioma: a UK-wide case series

Short title: *SDHC* variants: a UK-wide case series

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No conflicts of interest to declare.

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Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Ethics statement

This study was approved by the Guy's and St Thomas' Trust Clinical Audit Team (Service Evaluation 9649). The study conforms to the Declaration of Helsinki.

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Summary

Objective: Phaeochromocytomas (PCC) and paragangliomas (PPGL) are rare, but strongly heritable tumours. Variants in succinate dehydrogenase (SDH) subunits are identified in approximately 25% of cases. However, clinical and genetic information of patients with *SDHC* variants are under-reported.

Design: This retrospective case series collated data from 18 UK Genetics and Endocrinology departments.

Patients: Both asymptomatic and disease-affected patients with confirmed *SDHC* germline variants are included.

Measurements: Clinical data including tumour type and location, surveillance outcomes and interventions, *SDHC* genetic variant assessment, interpretation, and tumour risk calculation.

Results: We report 91 *SDHC* cases, 46 probands, and 45 non-probands. Fifty-one cases were disease-affected. Median age at genetic diagnosis was 43 years (range:11-79). Twenty-four *SDHC* germline variants were identified including six novel variants. Head and neck

paraganglioma (HNPGL, n=30, 65.2%), extra-adrenal paraganglioma (EAPGL, n=13, 28.2%) and PCC (n=3, 6.5%) were present. One case had multiple PPGLs. Malignant disease was reported in 19.6% (9/46). Eight cases had non-PPGL *SDHC*-associated tumours, six gastrointestinal stromal tumours (GIST) and two renal cell cancers (RCC). Cumulative tumour risk (95% CI) at age 60 years was 0.94 (CI:0.79-0.99) in probands, and 0.16 (CI:0-0.31) in non-probands respectively.

Conclusions: This study describes the largest cohort of 91 *SDHC* patients worldwide. We confirm disease-affected *SDHC* variant cases develop isolated HNPGL disease in nearly 2/3 of patients, EAPGL and PCC in 1/3, with an increased risk of GIST and RCC. 1/5 developed malignant disease, requiring comprehensive lifelong tumour screening and surveillance.

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SDHC phaeochromocytoma and paraganglioma: a UK-wide case series

Introduction

Phaeochromocytoma and paragangliomas (PPGL) are rare, but strongly heritable neuroendocrine tumours that are associated with a number of hereditary syndromes. European studies suggest the incidence is approximately 0.6 cases per 100,000 person years.[1] The WHO guidelines use anatomical location to classify PPGL tumours into adrenal-gland phaeochromocytoma (PCC), accounting for 80% of PPGL disease and extra-adrenal paraganglioma (EAPGL) accounting for 20%.[2,3] EAPGL are subdivided into sympathetic paraganglioma (PGL), associated with catecholamine secretion, and parasympathetic derived head and neck PGL (HNPGL) that rarely exhibit catecholamine secretion.

Over 40% of PPGL patients who undergo genetic testing are found to have a germline heterozygous pathogenic variant in a PPGL susceptibility gene.[4] Germline loss-of-function variants affecting genes encoding subunits of succinate dehydrogenase (SDH), which also functions as Complex II in the electron transport chain, are identified in 25% of PPGL cases, resulting in different autosomal dominant syndromes.[5] Loss of SDHB expression is detectable when any component of the SDH complex is inactivated, but no specific, validated SDHC-specific immunohistochemistry (IHC) exists.[6]

In the UK, patients with PPGLs are recommended to be referred for genetic counselling. Genomic testing is available if they fulfil National Genomic Test Directory criteria.[7,8] Eligible patients are currently offered an 11 PPGL panel genomic test (*SDHA, SDHB, SDHC, SDHD, SDHAF2, FH, MAX, MEN1, RET, TMEM127* and *VHL*). Variants of unknown significance (VUS) are reported in approximately 10% of PPGL diagnostic tests. If a pathogenic variant is

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identified, familial cascade genetic screening testing can be offered. Surveillance of asymptomatic relatives includes Magnetic Resonance Imaging (MRI) and biochemical testing for catecholamine secretion, to aid early detection of tumours.[7] The optimal surveillance programme is debated and numerous protocols exist.[9,10]

SDHx-associated disease characteristics vary according to the pathogenic SDH-subunit. *SDHB* variants are most prevalent, accounting for 10% of PPGL cases, *SDHD* variants 5%-9%, *SDHC* 1-2% and *SDHA* <1%.[9] *SDHB* variants are most common in patients with PCC or EAPGL, while *SDHD* variants frequently present with multifocal HNPGL.[11] The risk of aggressive, malignant disease is highest in patients *SDHB* pathogenic variants.[12] *SDHC* PPGL-associated pathogenic variants are less frequent than *SDHD* or *SDHB*, predominantly presenting with HNPGL disease at a younger age.[11,13,14] Less is known about *SDHC*-associated disease, with respect to clinical behaviour, disease penetrance, non-PGL associated pathology and optimal surveillance.

There are three objectives of this nationwide series: First to describe the demographic, clinical and treatment data for both proband and non-proband individuals. Secondly to report genetic variants implicated in *SDHC*-associated PPGL disease and use structural analyses to evaluate pathogenicity in novel variants. Thirdly, to evaluate current surveillance practice and make recommendations for future care, based on the data presented.

Materials and methods

This retrospective study presents genetic and clinical information of germline *SDHC* variants (OMIM 602413, reference sequence NM_003001.3), diagnosed before March 2020, from 18 UK centres. An invitation to participate was sent to 23 UK centres in April 2019 (see

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Appendix). Follow-up ended March 31st 2020, or if lost to follow up, date of last hospital contact, or on death. The survey was registered as a service evaluation project 9649 at Guy's and St Thomas' NHS Trust, London. A subgroup from 43 cases with *SDHC* intragenic variants had been published previously.[11]

Clinical Surveillance

Participating centres reported clinical information: age and date of diagnosis, and if affected, PPGL characteristics including size, anatomical location, malignancy (defined as the presence of disease in a lymph node or more distant site where chromaffin cells are not present, i.e. metastatic disease), required interventions and presence of other tumours. In the UK, prior to March 2019, there was no consensus on the optimum surveillance programme for individuals with *SDHx* germline variants, including *SDHC* for either affected individuals, or asymptomatic carriers. Therefore, we asked centres to report on duration, interval between review and modes of surveillance and screening outcomes. Standard biochemical tests differed by centre (24-hour urine or plasma metadrenalines (MA), normetadrenaline (NMA) and/or 3-methoxytyramine testing (3MT)).

Genetic Testing

Genetic variants were identified using standard molecular techniques (Sanger sequencing, multiplex ligase probe amplification or massively parallel sequencing via next generation sequencing or exome sequencing). Germline variants were reported according to American College of Medical Genetics and Genomics guidelines (ACMG).[15] Centres reported germline variants, proband status, whether they were from a known *SDHC* kindred and additional PPGL variants, if found. Reported variants were compared against variants in NIHR ClinVar registries.[16]

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Structural Analysis and assessment of variant pathogenicity

Structural integrity and succinate dehydrogenase (SDH) complex formation and structural prediction analyses were undertaken for all missense variants using IPS-3D, DUET and mCSM-PPI2.[17–19] These are reported alongside functional (mutPred) and pathogenicity scores, PolyPhen2 and SIFT.[20–22] Structural comparisons were performed using the porcine SDHC ortholog 1ZOY structure, with 91.7 % sequence homology, as no human SDHC structure is currently publicly available (Supplementary Figure 1).[23] Disease allele frequency was reviewed in gnomAD v3.1.1 database (https://gnomad.broadinstitute.org/).

Data Analysis

Data analysis and figure generation was undertaken using statistical computing language R version 4.0.3 (2020-10-10). Cumulative risk was estimated for pathogenic variants using survminer (ver. 0.4.8) and cmprsk (ver. 2.2-10) packages in individuals with likely pathogenic or pathogenic variants.

Results

Demographic features

A total of 91 *SDHC* variant cases from 18 UK centres were included. See Table 1. There were 41 males (45%) and 50 females (55%), median age at genetic diagnosis 43 years (range: 11-79). Forty-six (51%) cases were probands, 45 (49%) were relatives identified through cascade testing from 22 kindreds. Fifty-one cases (56%) were disease-affected, 40 (44%) were unaffected. The 51 disease-affected cases median age at diagnosis was 43 years (range: 12-79). Unaffected cases (n=40) were younger with a median age at genetic diagnosis of 38 years

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(range: 11-79). Three non-probands had *SDHC*-associated disease (HNPGL (n=2) and PA (n=1)) diagnosed prior to predictive testing.

Clinical features

There were 46 PPGL tumours diagnosed in 45 *SDHC* variant cases (Table 1), 65.2% were HNPGL, 6.5% thoracic PGL, 21.7% abdominal PGL and 6.5% PCC. HNPGLs were most frequent (n=30), located as follows: six carotid body (CBT), nine jugular, six vagal, one nasopharyngeal and eight HNPGL (site unspecified). Eleven cases (21.6%) developed *SDHC*-associated tumours: GIST (n=6, all female), Pituitary Adenoma (PA, n=3) and RCC (n=2). Of these, five cases presented with associated tumours, but no PPGL (GIST n=3, RCC n=1, PA n=1). Three GIST tumours showed SDHB immunonegativity and another was confirmed as wild-type. Eleven patients had multiple tumours: HNPGL and PA (n=2), HNPGL and thoracic PGL, thoracic PGL and adrenal adenoma, multiple RCCs, and GIST with either EAPGL (n=3), pulmonary chondroma (n=2) or oesophageal leiomyoma. Additional tumours include meningioma and bilateral breast cancer (see Supplementary Table 1 and Supplementary Table 2).

Malignant disease was identified in 19.6% (9/46) PPGL cases (Table 1). Where tumour sizes were recorded, all malignant HNPGL were >50mm, including two vagal tumours. Two cases had malignant EAPGLs, the youngest with a 45mm tumour aged 12, another aged 30 with a 78mm tumour, who died from metastatic disease aged 36. There were eight malignant *SDHC*-related tumours (six GISTs, two RCCs).

In the disease-affected group, fifty interventions were recorded from 1977 to 2019 across 37 cases (Table 1). Main treatment modalities included surgical resection (n=33), radiotherapy

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intervention (n=8) and active surveillance (n=13). Radiotherapy interventions included external beam radiotherapy, stereotactic radiotherapy and gamma knife radiosurgery. Nine HNPGL (30% of HNPGLs), two thoracic PGL, and two GIST cases remain on active surveillance.

Tumour incidence

Tumour incidence for probands with PPGL or GIST (n=37) and non-proband *SDHC* pathogenic variant cases (n=45) is shown in Figure 1. *SDHC* tumour incidence at age 40 and 60 years was 40.5% and 94.6% in probands, and 4.4% and 6.7% in non-probands respectively. Cumulative risk (95% CI) at age 20, 40 and 60 years was 0.05 (CI:0-0.12), 0.4 (CI:0.22-0.54) and 0.94 (CI:0.79-0.99) in probands, and 0, 0.08 (CI:0-0.18) and 0.16 (CI:0-0.31) in non-probands respectively (see Supplementary Figure 2). Age of diagnosis comparison between probands and non-probands (unpaired Student's t-test with two-tailed p value) p value = 0.178, 95% C.I. = 0 to 27.01. Difference between means (non-probands - probands) ± SEM = 10.91 ± 7.957.

Surveillance

Screening information dates from 2006, with centres reporting imaging and biochemistry according to local protocols, (biochemical monitoring and imaging every 12-36 months). See Table 2. Imaging modalities included whole body (WB-)MRI from skull base-pelvis, Computed Tomography and abdominal Ultrasound scan. Information regarding sequence/nature of imaging protocols was not gathered. Biochemical modalities included either 24-hour urine or plasma metanephrines measurement. Chromogranin A data was not collected unless additionally reported by the centre.

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Across the whole cohort, 68 (75%) had at least two rounds of surveillance screening using cross-sectional imaging, predominantly WB-MRI. Forty-two (46%) individuals had at least two rounds of biochemical testing. Seven of 51 disease-affected cases (13.7%, two HNPGL, five EAPGL) had raised biochemistry at diagnosis (elevated MA, or NMA or 3-MT). The overall median length of surveillance was 42 months (3.5 years, range: 4-107 months).

Surveillance data for 40 out of 45 non-proband asymptomatic individuals was available for a median period of 45.5 months (3.8 years). Five individuals were awaiting screening. Two individuals had tumours detected at baseline screen (5%), whereas 38 individuals had no tumours identified (95%). Twenty individuals had at least two rounds of cross-sectional imaging, 23 had at least two rounds of biochemical testing, without additional tumours identified (Table 2).

The eldest non-proband, diagnosed aged 79, had raised NMA and an EAPGL and GIST identified on MRI. After EAPGL surgery, the individual had annual MRI surveillance, with the GIST remaining stable. Another non-proband aged 60 had a CBT and thoracic PGL diagnosed on MRI and fully characterised by ^{18}FDG-PET scan. The CBT was excised and the thoracic PGL has been monitored by MRI. Their PGLs showed no malignant features, with sizes smaller than average in this cohort: thoracic PGL 21mm; EAPGL 38mm (EAPGL and PCC tumour size average 45.5 $\pm$ 25.1mm); HNPGL 22mm (HNPGL tumour size average 32.9 $\pm$ 16.9mm) (Supplementary Table 1 and Supplementary Table 2).

Surveillance data for 46 proband individuals (following diagnostic investigations) was available for a median period of 26 months (2.17 years, range: 4-107 months). Thirty-five probands had at least one subsequent surveillance screen: 34 individuals had at least one screen

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with cross-sectional imaging, 34 individuals had at least one screen including biochemical testing. Twenty-four individuals underwent at least two rounds of cross-sectional imaging. No new PPGLs were identified.

Genetic Variants

Twenty-four genetic *SDHC* variants were identified, including four single exon deletions, 19 intragenic variants and one intronic variant distributed along the entire coding sequence of the gene (see Table 3 and Figure 2). Eighteen *SDHC* variants have previously been described. Six *SDHC* variants are novel: three exon deletions (exon 4 deletion, c.371_372del. p.(Leu124fs), and exon 5 deletion), two missense variants (*SDHC*:c.200T>A, p.(Met67Lys) and *SDHC*:c.257G>A, p.(Gly86Asp)), and an intronic variant *SDHC*:c.(241+9)-(241+10)del.

The 5'-UTR variant *SDHC*:c.-38G>A initially reported as a VUS, was reclassified as benign (Table 3). Three cases had additional *SDHx* variants, none classified as pathogenic. One individual with PCC aged 39 had two pathogenic variants, *SDHC*:c.148C>T, p.(Arg50Cys) and *RET*:c.2410G>A, p.(Val804Met). They underwent adrenalectomy and thyroidectomy, where no C-cell hyperplasia or medullary thyroid cancer was found. PCC occurs rarely in RET V804M cases.[24] LOH analysis of PCC tumour showed normal *SDHC* results, indicating RET V804M may have driven PCC development.

All 11 missense coding variants were assessed according to ACMG guidelines, five (45.4%) met criteria for likely pathogenic variants, whilst five (45.4%) are VUSs and one is considered likely benign (Table 4). Overall, 16 out of 24 *SDHC* variants identified are considered pathogenic or likely pathogenic, six are VUSs and two are benign. Genotype-phenotype correlations are included in the appendix.

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Structure-phenotype correlation

All missense variants are predicted to have a destabilising effect on SDHC complex formation (Table 4). The two novel variants *SDHC*:c.200T>A, p.(Met67Lys) and *SDHC*:c.257G>A, p.(Gly86Asp) are predicted to destabilise protein structure and SDH complex formation. Both variants are predicted to be 'damaging' and 'pathogenic' by PolyPhen2 and SIFT scores. HNPGL tissue from c.257G>A, p.(Gly86Asp) showed SDHB immunonegativity. The missense mutation most disruptive to complex formation (c.148C>T, p.(Arg50Cys)) disrupts the N-terminus flexible loop, which is critical for the for both the assembly and stability of Complex II.[23] The most destabilising missense mutation (c.377A>G, p.(Tyr126Cys)) results in a switch of bulky, hydrophobic tyrosine for a polar cysteine and interrupts strong stacking interactions within a hydrophobic pocket, which are important for the stability of the helical membrane anchor.[23] The two missense mutations predicted to not affect protein structure stability (c.214C>T, p.(Arg72Cys) and c.380A>G, p.(His127Arg)) both correspond to heme complex binding residues, with the latter being directly coordinated to the iron centre. The fact that the variant residues retain a degree of polarity, and that other coordinating residues remain intact, may explain the relative redundancy in protein stability in these variants. One variant *SDHC*:c.490A>T, p.(Met164Leu) was predicted to have a stabilising effect on protein structure, predicted to be 'tolerated'/'benign' by structural analysis and classified as 'likely benign' by ACMG guidelines. In this case, the PCC tumour showed SDHB immunopositivity.

Discussion

This multi-centre, nationwide UK case-series describes the largest *SDHC* cohort to date. The median age of genetic diagnosis for disease-affected cases was 43 years (range: 12-79), similar

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to that reported by Schiavi *et al.* (median 46 years, range: 13-73), younger than cases with sporadic HNPGL (median 53 years, range: 15-83).[13]

As reported previously, we recorded a wide spectrum of tumours in *SDHC*-associated disease.[11] We confirm the majority of *SDHC* cases develop isolated HNPGL (65.2%), with EAPGL in 28.2%, and PCC in 6.5%. HNPGL are more common in *SDHD* (85%) than *SDHC* but less common in *SDHB* (35%). [9] Previous studies reported 36% *SDHC* cases had multiple PPGLs, however only one UK *SDHC* case had multiple PPGLs.[9] While *SDHC* cases have been described as developing benign HNPGLs, six cases developed malignant HNPGLs (20%), >50mm at diagnosis.[13,25] Two metastatic EAPGL cases presented young (12 and 30 years), with tumours >45mm. These features are all predictors of aggressive disease. In a recent meta-analysis, patients with *SDHC* tumours were reported to have a metastatic risk of 23% (CI: 0.10-0.45) over an averaged follow-up period of 4.8 years.[26] Our marginally lower overall metastatic PPGL rate of 19.6%, may reflect our slightly shorter follow-up period, averaged at 3.1 years. The risk of malignant disease at 19.6% remains lower than the pooled risk in *SDHB* (31%), but higher than in *SDHD* (8%).[26]

Our case series identified rarer tumours associated with *SDHC*: GIST, PA and RCC in 21.5% of cases.[27] GIST was the second most common tumour after PPGL. Interestingly, five of the six cases with GIST had related tumours, e.g. EAPGL (confirming Carney-Stratakis syndrome), or pulmonary chondroma and oesophageal leiomyoma, evidence of incomplete Carney's Triad. Unfortunately, there was no *SDHB* IHC testing in RCC or PA cases, so SDH-deficiency remains unconfirmed. Whilst PA have been reported in *SDHC* cases, they occur commonly in the general population at a frequency of 1 in 1,064 cases.[28] The association of

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pituitary tumours as a feature of *SDHC*-related disease may therefore represent an incidental finding.

We describe six novel germline *SDHC* variants: Three pathogenic exon deletions, two missense VUS and one intronic variant. *In silico* structural analyses of all missense variants correlated with pathogenicity classification for previously identified variants and demonstrated disruption of key structural and functional elements of the SDH complex. Structural analyses predict novel variants *SDHC*:c.200T>A, p.(Met67Lys) and *SDHC*:c.257G>A, p.(Gly86Asp) to be pathogenic, correlating with SDHB immunonegativity in the *SDHC*:c.257G>A, p.(Gly86Asp) case.

Reclassification of variants is a dynamic process and can have important implications for clinical practice. One patient with an EAPGL has the non-coding variant *SDHC*:c.-38>A, designated a VUS in 2008, but reclassified as a benign variant in 2018. *SDHC:*c.148C>T, p.(Arg50Cys), and c.377A>G, p.(Tyr126Cys) were initially reported as VUSs, but recently reclassified (in 2018 and 2021) as 'likely pathogenic', affecting nine probands. Reclassification events are increasing, with one commercial laboratory reporting 7.7% of unique VUS being reclassified over 10 years.[29] Upgrades from a VUS to a pathogenic variant will have clinical consequences. Downgrades may be helpful in releasing patients from unnecessary surveillance. Overall, 16 out of 24 *SDHC* variants identified are currently considered pathogenic or likely pathogenic, with six remaining as VUSs and two now considered benign.

Screening outcomes were reported for all *SDHC* variant cases. Relatively few cases with disease had a positive biochemical result (13.7%). This is unsurprising, reflecting the propensity for *SDHC* patients to develop HNPGL, which are often non-secretory, underlining

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the importance of imaging in PPGL detection. During follow-up, no metachronous tumours were identified in probands, but tumour growth and progression of metastatic disease was observed.

All tumours found in asymptomatic non-proband cases were identified at initial work-up: three PGLs and a GIST in two cases, detected by MRI. Reassuringly their PGL tumour sizes were smaller than the averages across the cohort, indicating surveillance had detected disease earlier, with possible beneficial outcomes. However, with only one GIST case in non-probands, the penetrance of this tumour remains unclear. The range of treatments employed reflects the changing practice for treating HNPGL disease. HNPGLs are slow-growing tumours with a doubling time of 5.8 years.[30] Many patients with HNPGLs are now being considered for nonsurgical therapy instead of operative intervention, which risks increased morbidity to achieve surgical cure.[31] The broadened range of radiation techniques available and their success, for example ablative stereo-tactic radiosurgery, has led to increased use of these interventions. Discussion via a National MDT for managing HNPGLs in *SDHC*, in view of the 20% HNPGL malignancy risk, could provide a mechanism to enhance practice and outcomes.

This study reports *SDHC* tumour incidence at age 40 and 60 years was 40.5% and 94.6% in probands, and 4.4% and 6.7% in non-probands respectively. This is in keeping with the published findings of Benn *et al.*, who report predicted lifetime penetrance of 8.3% (95% CI 3.5% to 18.5%), among patients selected for known *SDHC* pathogenic or likely pathogenic variants.[32] This study highlights low cumulative tumour risk in non-probands, 16% (95% CI:0-0.31) at 60 years compared to a previous estimate of 25% (95% CI 0-0.57).[11] This may reflect the younger average age of non-probands at 38 years, compared to the average age of affected cases at 43, with tumours yet to develop in non-probands. Certainly, for proband cases, tumours

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presented throughout life and metastatic disease was seen at all ages, emphasizing lifelong tumour susceptibility.

The aim of any surveillance programme in *SDHC* patients at risk of PPGL, GIST and RCC is to detect early disease and reduce the chance of metastatic spread.[11] However, with low tumour incidence in non-probands and lower metachronous tumour risk in probands than previously documented, surveillance frequency might safely be lengthened from recommended biennial cross-sectional imaging, thus reducing costs and the burden to patients of frequent scans.[7] Recent screening studies also confirm low tumour rates in asymptomatic *SDHC* carriers with 4% (1/28) having PPGL diagnosed at baseline,[33] matching our experience of 5% (2/40) cases identified.

In 2016, the European Society for Endocrinology published a clinical practice guideline for patients operated on for PPGL tumours, including any genetic variants. It recommended assaying plasma or urinary MN and 3MT every year, and performing imaging tests every 1-2 years in patients with biochemically inactive PPGL, to screen for local or metastatic recurrence or new tumours.[34] While this has been broadly adopted for patients with other *SDHx* variants (i.e. *SDHB*)[9], evidence from our surveillance data suggests this was not routinely achieved for *SDHC* cases across the UK prior to 2020. Following the results of this study, we recommend:

1. Lifelong tumour specific follow-up (to monitor recurrent or metastatic disease, as determined by specialist multidisciplinary teams) for affected patients.
2. For metachronous PPGL and RCC detection, annual clinical review with plasma metanephrines, and WB-MRI (skull base, neck, thorax, abdomen and pelvis) 3-5 yearly. To identify GISTs, gastric symptoms should be enquired of at clinical review and a full blood count (FBC) measurement taken to assess for anaemia. For patients with

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suggestive symptoms or anaemia, Oesophageal-Gastro-Duodenoscopy (OGD) should be considered.

3. Non-probands commence annual clinical review with biochemistry and FBC from 10 years, and WB-MRI, as above, from 15 years.
4. Patients with a *SDHC* VUS and PPGL continue tumour specific follow-up. For their potential metachronous tumour risk, annual clinical review with biochemistry and FBC for 10 years, and WB-MRI, as above. Assessment of *SDHC* VUS status to determine if lifelong WB-MRI surveillance is indicated after 10 years (provided there are no high-risk features, e.g. multiple PPGLs or familial disease).
5. SDHB immunohistochemistry, molecular analysis (LOH studies or somatic testing), histopathological review and consideration of tumour methylome and metabolomics profiling in PPGL and additional tumours, to aid variant classification and determine whether tumours are truly *SDHC*-related.[27]

Despite this study being the largest *SDHC* patient cohort reported, we recognise its limitations, including retrospective data collection, incomplete clinical information and the challenge of case ascertainment. Our short follow-up period may not have captured all tumour occurrences or metastatic disease and the small numbers in the cohort may have insufficient power for some differences to be realised. At present, there is no UK register for patients with *SDHx* disease, their surveillance outcomes or impact of surveillance on morbidity and mortality. Such a register would also benefit from access to centralised genomics data and cancer registration, which would provide a richer overview of *SDHC*-associated disease.

In summary, this UK multi-centre study reports the largest dataset of 91 affected and unaffected *SDHC* germline variant carriers to date, with six novel *SDHC* variants out of 24 identified. The

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clinical manifestations of disease in this cohort have increased our understanding of *SDHC* disease and demonstrate the importance of lifelong surveillance of these patients, who have a 1/5 risk of metastatic disease. It is imperative that prospective data is collected to help tailor surveillance programmes for the benefit of asymptomatic *SDHC* patients and to provide accurate information for genetic counselling of patients about their lifetime risk of disease.

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Tables (included as editable excel files within the submission)

Table 1: Clinical characteristics of 91 *SDHC* variant cases, tumour diagnoses, treatments and outcomes.

Table 1:

[a] Other includes skull base, glomus, HNPGL site not clearly specified.

[b] SDHB IHC or LOH studies were not performed on PA or RCC tumours.

[c] Some cases had multiple tumours - see Supplementary Table 1.

Table 2: Screening modalities and results, for proband and non-proband individuals.

Table 2:

[a] Definitions of disease status: Positive indicates new diagnosis or new focus of metastatic disease. Stable indicates radiologically stable disease. Negative indicates no evidence of disease in screen, whether biochemically or radiologically.

[b] See main text for description of imaging and biochemical modalities. For non-probands, investigations undertaken within 6 months are considered within a single screen.

[c] One patient had undergone screening in 2012, but results were not available.

[d] Other forms of investigation included: Computed Tomography with biochemical testing (CT/B), Bronchoscopy (Br), I123/I131 metaiodobenzylguanidine (MIBG) imaging with MRI (MIBG/MRI) or Ultrasound scan with biochemical testing (US/B) and with MRI (US/B/MRI).

Table 3: List of all intragenic or regulatory site variants in *SDHC* [reference sequence NM_003001.3].

Table 3:

Additional co-variants in *SDHB*, *SDHD* have also been annotated. Variants listed as 'novel', have, to the best of our knowledge, not been published elsewhere.

[a] One patient with *SDHC* c.148C>T variant has a concurrent pathogenic low penetrance *RET* protooncogene variant, c.2410G>A, p.(Val804Met).

[b] This is an intronic variant. Its possible effect on splicing has not been investigated further.

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Table 4: Structural and pathogenicity prediction scores for all *SDHC* missense variants described in this cohort.

Table 4:

[a] The coexisting *SDHD* variant c.34G>A is registered on ClinVar as likely benign.

[b] The coexisting *SDHB* variant c.8C>G is registered on ClinVar as a benign variant.

[c] The coexisting *SDHD* variant c.319C>T is registered on ClinVar as a VUS.

[d] Allele frequencies are as reported in gnomAD v3.1 (accessed via https://gnomad.broadinstitute.org/ Nov 2020). Variants reported from position chr 1.161328466.

Figure Legends (Figures included as separate files within the submission)

Figure 1: Tumour incidence of *SDHC*-associated disease across proband and non-proband groups.

Figure 2: Lollipop plot depicting the distribution of *SDHC* variants. Diagram illustrating 71 germline *SDHC* variants along the amino acid sequence, colour-coded by the mutation class: start loss (11), truncating (24) and missense (36) genetic variants. The Y axis represents the number of occurrences for each *SDHC* variant in our cohort. The variant *SDHC:*c.397C>T, p.(Arg133Ter) is the most prevalent variant displayed, occurring 14 times.

Supporting information and Appendix (Supporting tables and figures included as editable excel files within the submission)

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Supporting information:

[1] Supplementary Table 1: Patients with multiple *SDHC*-related tumours and their outcomes.

Supplementary Table 1: Abbreviations: NR = No Record. GIST= Gastrointestinal tumour. CBT= carotid body tumour. All patients with GIST are female, median age 33 (range 22-79) years. [a] HNPGL average size across cohort 32.9 ± 16.9mm, EAPGL/PCC average size across cohort 45.5 ± 25.1mm.

[2] Supplementary Table 2: Tumours in Non-proband *SDHC* cases.

Supplementary Table 2: NR = No record. HNPGL average size across cohort 32.9 ± 16.9mm, EAPGL/PCC average size across cohort 45.5 ± 25.1mm.

[3] Supplementary Table 3: Clinical characteristics and disease phenotypes listed by *SDHC* variant.

Supplementary Table 3: Cases with * have malignant disease. [a] Cases are separated by commas. [b] One patient with *SDHC* c.148C>T, p.(Arg50Cys) variant has a concurrent pathogenic low penetrance *RET* protooncogene variant, c.2410G>A, p.(Val804Met). Abbreviations: HN HNPGL unknown site, LCBT left carotid body tumour, RCBT right carotid body tumour, L left glomus, RG right glomus, LJ left jugulare, RJ right jugulare, LV left vagus, RV right vagus, V vagus (site unspecified), NPG nasopharyngeal, LSB left skull base, GIST gastrointestinal stromal tumour, RCC renal cell cancer, PA pituitary adenoma, [c] Pituitary adenomas were prolactinomas.

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[4] Supplementary Figure 1: BLAST alignment of Human Succinate dehydrogenase cytochrome b560 subunit, SDHC (Q99643) and Pig SDHC (D0VWV4).[35] Structural prediction analyses were based on the high-resolution 1ZOY structure of succinate dehydrogenase from *Sus scrofa* (https://www.rcsb.org/structure/1zoy, PMID: 15989954).

[5] Supplementary Figure 2: Cumulative tumour risk and 95% CI for probands and non-probands with pathogenic variants in *SDHC* with median ages for both groups highlighted. NB: Due to limited number of older non-probands, the final value of the corresponding line is likely to be over-inflated (95% CI: 0-89.6%).

Appendix

[1] Supplementary information regarding centres approached. An invite was initially sent to 23 genetics centres in the UK and these departments linked us to relevant endocrine services involved in the care of their *SDHC* families**.** Genetic centres in Aberdeen, Bristol, Dundee, Exeter, St George's Hospital (SW London) and University Hospital Southampton NHS Trust, Wessex did not have any known *SDHC* variant cases. The All Wales Genetic service was able to identify *SDHC* cases, but unable to provide any clinical information due to difficulties in locating information from patient records. There were 28 cases from London centres (Guy's and St Thomas' NHS Trust, King's College Hospital NHS Foundation Trust, Bart's Health NHS Foundation Trust, North West London Genetic Service, including Imperial College Healthcare Foundation NHS Trust and University College London Hospital NHS Foundation Trust). Outside of London, there were 63 cases in total from the following centres: Oxford University Hospital NHS Foundation Trust, Cambridge University Hospital Foundation NHS Trust, University Hospitals Birmingham NHS Trust, Nottingham University Hospitals NHS

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Trust, Sheffield Teaching Hospitals NHS Foundation Trust, Leeds Teaching Hospitals NHS Trust, University Hospitals of Leicester, Manchester University NHS Trust, Newcastle Upon Tyne Hospitals NHS Foundation Trust, Edinburgh Royal Infirmary, Glasgow Royal Infirmary and Belfast City Hospital, Northern Ireland. The largest contributing centre in London was GSTT and outside of London was Newcastle.

[2] Supplementary information regarding genotype-phenotype evaluation. Bayley *et al.* reported that truncating *SDHB* and *SDHD* variants were significantly over-represented in affected cases, with *SDHB/D* truncations associated with higher risk of developing PPGLs, compared with missense variants.[36] In our *SDHC* cohort, truncating variants were not associated with PPGL over other disease sites (two-sided Fischer's exact test, $p = 0.999$, CI: 0.246-4.028), malignant disease (p=0.999, CI: 0.232-5.080), or development of non-PGL malignancy (GIST and RCC, p= 0.683, CI: 0.069-3.637) (Supplementary Table 3). Given the small sample size per variant, it is not meaningful to associate predicted pathogenicity with tumour incidence in this cohort, although this has been demonstrated in *SDHB* and *SDHD* variants.[11]

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Table 1: Clinical characteristics of 91 *SDHC* variant cases, tumour diagnoses, treatments and outcomes

Characteristics	*SDHC* cases
Male:Female	41:50
Median age at genetic diagnosis (years, range)	43 (11-79)
Median age at tumour diagnosis (years, range)	43 (12-79)
Total number of cases affected by PPGL or *SDHC*-related tumours	51
Number of tumours and site (n, %)	
Total PPGL tumours	46
Total HNPGL tumours and site	30 (65.2%)
Carotid Body Tumour	6
Jugular	9
Vagal	6
Nasopharyngeal	1
Other [a]	8
Metastatic HNPGL disease (n, %)	6 (20%)
HNPGL Tumour size (mm)	32.9 ± 16.9
Number of EAPGL tumours and site (n, %)	13 (28.2%)
Thoracic PGL	3 (6.5%)
Abdominal and retroperitoneal PGL	10 (21.7%)
Metastatic EAPGL	2 (4.3%)
Phaeochromocytomas (PCC)	3 (6.5%)
Metastatic PCC	1 (33%)
EAPGL/PCC Tumour size (mm)	45.5 ± 25.1
Patients with multiple PPGL	1
Total metastatic PPGL disease (n, %)	9 (19.6%)
Type of metastases	
Lymph node	3
Bone	4
Liver	2
Lung	1
SDHC-related tumours [b]	11
GIST	6
Pituitary Adenoma (PA)	3
Renal Cell Cancer (RCC)	2
Total malignant *SDHC*-related tumours	8 (72.7%)
Total number of tumours in *SDHC* cases [c]	57
Intervention/active surveillance	
Pre-operative embolisation	3
Surgery	33
Radiotherapy	8
MIBG	3
Radionuclide therapy ($Yttrium^{90}$)	1
Targeted therapy -Imatinib +/- Sunitinib (GIST treatment)	2
HNPGL - on active surveillance	9
Thoracic PGL - on active surveillance	2
GIST - on active surveillance	2
PPGL deaths	1

Table 1: [a] Other includes skull base, glomus, HNPGL site not clearly specified.

[b] SDHB IHC or LOH studies were not performed on PA or RCC tumours.

[c] Some cases had multiple tumours - see Supplementary Table 1.

Table 2: Screening modalities and results, for proband and non-proband individuals

Group		First screen		Second screen		Third screen		Fourth Screen		Fifth Screen	Total surveillance
Non-proband	**Total (n = 45)**	**45**	→	**34**	→	**26**	→	**20**	→	**16**	
	Positive [a]	2		0		0		0		0	
	Stable	0		2		2		2		2	
	Negative	38		23		17		13		9	
	Not available/pending	5		9		7		5		5	
	MRI and biochemistry [b]	35		9		11		11		7	
	MRI alone	3		3		2		1		0	
	Biochemistry alone	1		14		5		1		4	
	Not available/pending	4		5		5		7		5	
	Other [d]	2		3		3		0		0	
	Other screening modalities (per case) [d]	US/B, US/B/MRI		US/B, US/B, US/B		CT, US/B, US/B					
	Median interval (months, range)			16.5 (11-27)		12 (8-26)		16 (9-32)		15 (12-63)	
	Median surveillance duration (months, range)										45.5 (12-103)
Proband	**Total (n = 46)**	**46**	→	**35**	→	**27**	→	**22**	→	**15**	
	Positive [a]	14		5		1		1		1	
	Stable	1		3		5		3		2	
	Negative	19		15		12		10		4	
	Not available/pending [b]	12		12		9		8		8	
	MRI and biochemistry	23		15		13		8		3	
	MRI alone	7		4		3		5		3	
	Biochemistry alone	1		6		3		1		1	
	Not available/pending	11		8		8		8		8	
	Other [d]	4		2		0		0		0	
	Other screening modalities [d]	CT/MRI, Br, MIBG/MRI, CT/B		CT/B, Br							
	Median interval (months, range)			12 (4-78)		12 (4-29)		12 (1-31)		11 (2-14)	
	Median surveillance duration (months, range)										26 (4-107)

Table 2: [a] Definitions of disease status: Positive indicates new diagnosis or new focus of metastatic disease. Stable indicates radiologically stable disease. Negative indicates no evidence of disease in screen, whether biochemically or radiologically.

[b] See main text for description of imaging and biochemical modalities. For non-probands, investigations undertaken within 6 months are considered within a single screen.

[c] One patient had undergone screening in 2012, but results were not available.

[d] Other forms of investigation included: Computed Tomography with biochemical testing (CT/B), Bronchoscopy (Br), I^{123}/I^{131} metaiodobenzylguanidine (MIBG) imaging with MRI (MIBG/MRI) or Ultrasound scan with biochemical testing (US/B) and with MRI (US/B/MRI).

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Table 3: List of all intragenic or regulatory site variants in *SDHC* [reference sequence NM_003001.3].

Gene	*SDHC* Exon	Variant *SDHC/SDHx*	Result *SDHC/SDHx*	Protein change *SDHC/SDHx*	Number of probands	Number of cases	ClinVar ID or reference	Reported effect *SDHC/SDHx*	Allele frequency [c]
SDHC	Promoter	c.-38G>A	Does not affect function	p.=	1	1	368837	Benign	*Not described*
	1 (-30)	c.1A>G	Start codon lost	p.(Met1?)	3	4	407060	Pathogenic	1.32E-05
		c.1A>T	Start codon lost	p.(Met1?)	2	7	653751	Pathogenic	*Not described*
	2 (21)	Exon 2 deletion	Copy Number Variant	p.?	1	1	Burnichon *et al. 2009.*	Pathogenic	*Not described*
		c.43C>T	Stop gain	p.(Arg15Ter)	2	7	41776	Pathogenic	1.97E-05
	3 (78)	c.78-1G>A	Acceptor splice site variant	p.=	1	1	185473	Likely Pathogenic	6.57E-06
		c.148C>T [a]	Missense variant	p.(Arg50Cys)	6	12	135194	Likely Pathogenic	6.57E-06
		c.148C>T and *SDHD* c.34G>A	Missense variant/Missense variant	p.(Arg50Cys)/p.(Gly12Ser)	1	1	135194; 6895	Likely Pathogenic and Likely benign	6.57E-06
		c.164A>G	Missense variant	p.(His55Arg)	1	1	239449	VUS	6.57E-06
	4 (180)	Exon 4 deletion c.(179+92_180-1)_(241+107_242-1)del	Copy Number Variant	p.?	1	1	**Novel**	**Novel Pathogenic**	*Not described*
		c.200T>A	Missense variant	p.(Met67Lys)	1	1	**Novel**	**Novel VUS**	*Not described*
		c.214C>T	Missense variant	p.(Arg72Cys)	0	2	653952	Likely Pathogenic	1.31E-05
		c.215G>A	Missense variant	p.(Arg72His)	2	8	407044	VUS	*Not described*
		c.224G>A and *SDHB* c.8C>G	Missense variant/Missense variant	p.(Gly75Asp)/p.(Ala3Gly)	1	1	189841; 12791	Conflicting (Likely Pathogenic/VUS) and Benign	*Not described*
		c.(241+9)_(241+10)del [b]	Unknown	p.=	1	1	**Novel**	**Novel VUS**	*Not described*
	5 (242)	Exon 5 deletion	Copy Number Variant	p.?	1	1	**Novel**	**Novel Pathogenic**	*Not described*
		c.257G>A	Missense variant	p.(Gly86Asp)	1	1	**Novel**	**Novel VUS**	*Not described*
		c.263C>T	Missense variant	p.(Ser88Leu)	1	1	407056	VUS	1.31E-05
		c.345dupA	Frame-shift elongation	p.(Ala116SerfsTer2)	1	1	Andrews *et al. 2018*	Pathogenic	*Not described*
		c.371_372del	Frame-shift elongation	p.(Leu124fs)	1	2	**Novel**	**Novel Pathogenic**	*Not described*
		c.377A>G	Missense variant	p.(Tyr126Cys)	1	1	428933	Llikely pathogenic	2.63E-05
		c.377A>G and *SDHD* c.319C>T	Missense variant/Missense variant	p.(Tyr126Cys)/p.(Leu107Phe)	1	1	428933; 578681	Likely pathogenic and VUS	2.63E-05
		c.380A>G	Missense variant	p.(His127Arg)	3	5	187084	Pathogenic	6.57E-06
		c.397C>T	Stop gain	p.(Arg133Ter)	7	14	183753	Pathogenic	6.58E-06
	6 (406)	Exon 6 deletion	Copy Number Variant	p.?	4	14	Andrews *et al. 2018*	Pathogenic	*Not described*
		c.490A>T	Missense variant	p.(Met164Leu)	1	1	184146	Conflicting (Likely Benign/VUS)	1.86E-04
TOTAL					46	91			

Table 3: Additional co-variants in SDHB, SDHD have also been annotated. Variants listed as 'novel', have, to the best of our knowledge, not been published elsewhere.

[a] One patient with c.148C>T variant has a concurrent pathogenic low penetrance *RET* protooncogene variant, c.2410G>A, p.(Val804Met).

[b] This is an intronic variant. Its possible effect on splicing has not been investigated further.

[c] Allele frequencies are as reported in gnomAD v3.1.1 (accessed via https://gnomad.broadinstitute.org/ on 01/07/2021).

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Table 4: Structural and pathogenicity prediction scores for all *SDHC* missense variants described in this cohort

SDHC Exon	*SDHC*Variant	Allele frequency [d]	INPS-3D Kcal/mol	mCSM-PPI2 Kcal/mol	DUET Kcal/mol	mutPred	PolyPhen HVAR rank	SIFT	Predicted pathogenicity	Effect as per ACMG guidelines	Evidence
3 (78)	c.148C>T [a]	6.57E-06	-0.297	-1.926	-2.101	0.78	0.88582	Damaging	Pathogenic	Likely pathogenic	PS3_Moderate, PS4_Moderate, PM2, PP3
	c.164A>G	6.57E-06	-0.532	-0.141	-1.456	0.933	0.84481	Damaging	Pathogenic	VUS - not enough evidence	PS4_Moderate, PM2, PP3
4 (180)	c.200T>A	*Not described*	-1.277	-0.256	-0.546	0.909	0.67921	Damaging	Pathogenic	VUS - not enough evidence	PM2, PP3
	c.214C>T	1.31E-05	0.146	-1.001	-0.611	0.89	0.86255	Damaging	Pathogenic	Likely Pathogenic	PS3_Moderate, PS4_Moderate, PM1_Supporting, PM2, PP3
	c.215G>A	*Not described*	-0.043	-0.558	-1.517	0.877	0.86255	Damaging	Pathogenic	Likely Pathogenic	PS4_Moderate, PM2, PM5, PP3
	c.224G>A [b]	*Not described*	-0.486	-0.058	-1.354	0.943	0.92359	Damaging	Pathogenic	VUS - not enough evidence	PS4_Moderate, PM2, PP3
5 (242)	c.257G>A	*Not described*	-0.164	-2.107	-2.245	0.79	0.995	Damaging	Pathogenic	VUS - not enough evidence	PM2, PP3
	c.263C>T	1.31E-05	-0.083	-0.23	0.452	0.552	0.087	Tolerated	Pathogenic	VUS - not enough evidence	BP4
	c.377A>G [c]	*2.63E-05*	-1.939	-0.563	-2.138	0.823	0.92359	Damaging	Pathogenic	Likely pathogenic	PS4_Moderate, PM1_Supporting, PM2, PP3
	c.380A>G	6.57E-06	0.873	-0.285	-1.309	0.945	0.97372	Damaging	Pathogenic	Likely pathogenic	PS4_Moderate, PM2, PP4, PP3
6 (406)	c.490A>T	1.86E-04	-0.179	-0.15	0.207	0.476	0.01387	Tolerated	Benign	Likely benign	PS4, BP4

Table 4: Variants reported from position chr 1.161328466.

[a] The coexisting SDHD variant c.34G>A is registered on ClinVar as likely benign.

[b] The coexisting SDHB variant c.8C>G is registered on ClinVar as a benign variant.

[c] The coexisting SDHD variant c.319C>T is registered on ClinVar as a VUS.

[d] Allele frequencies are as reported in gnomAD v3.1.1 (accessed via https://gnomad.broadinstitute.org/ on 01/07/2021).

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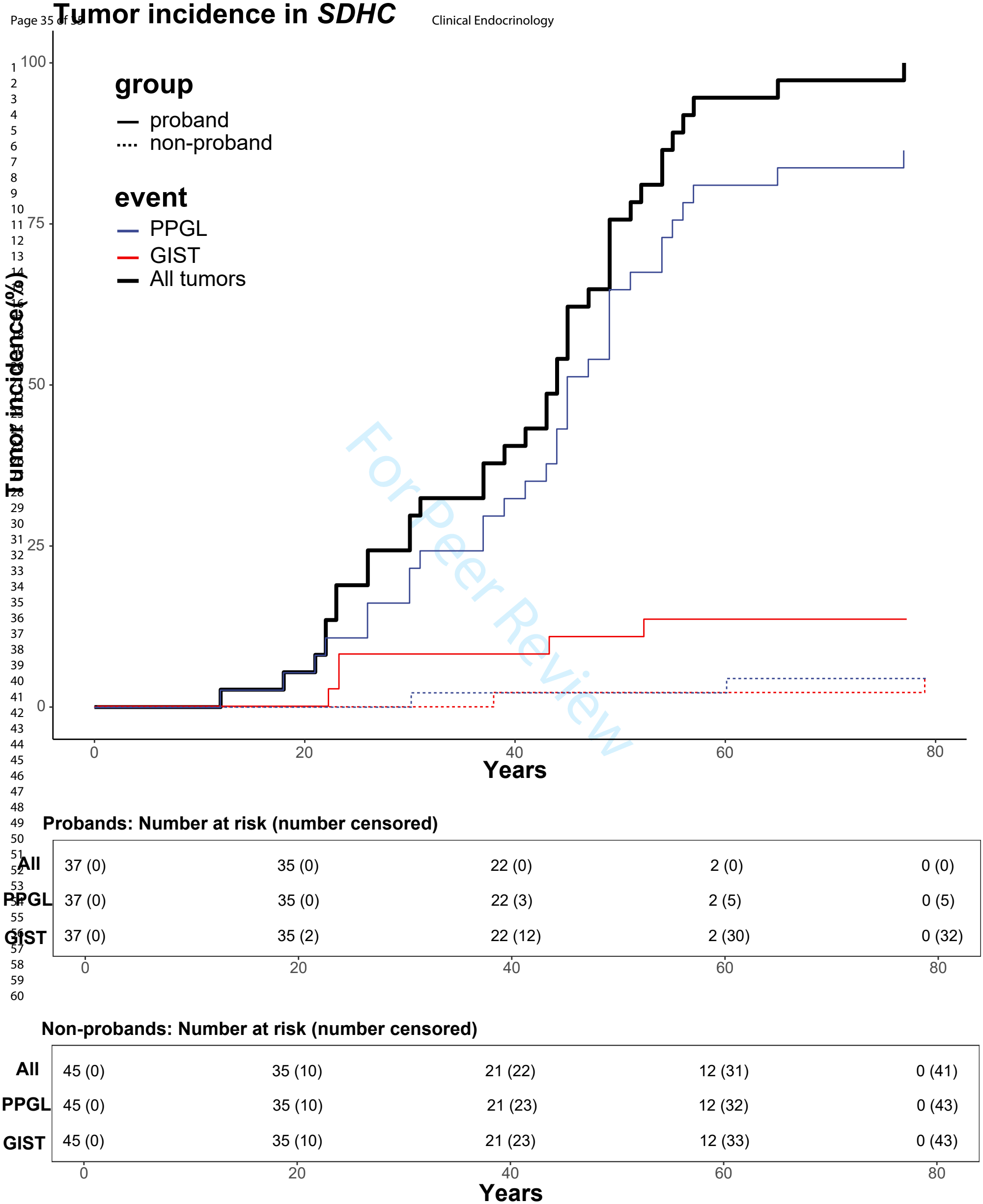

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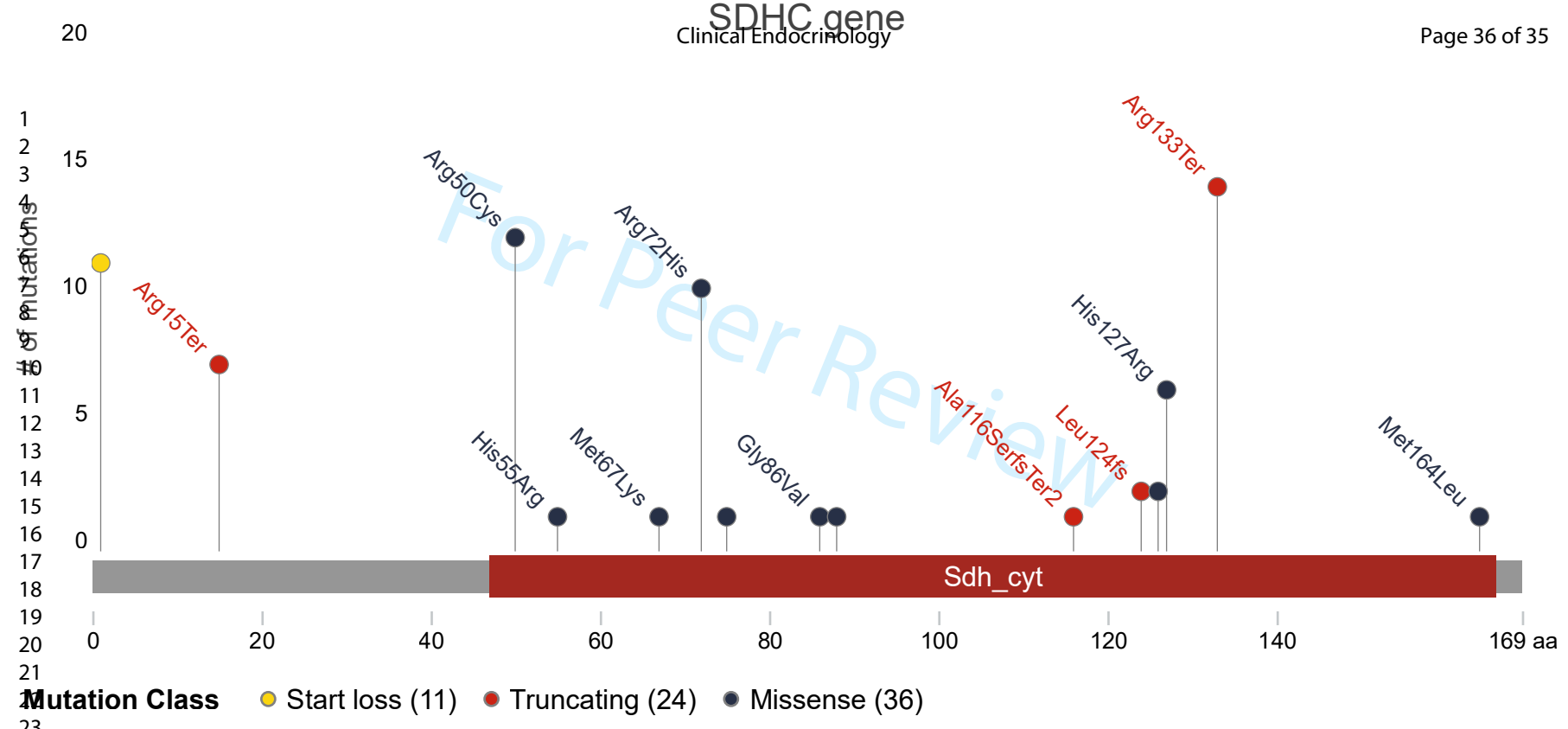

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