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# The phenotype of *SDHB* germline mutation carriers: a nationwide study

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## Abstract

**Objective:** Succinate dehydrogenase B subunit (*SDHB*) gene germline mutations predispose to pheochromocytomas, sympathetic paragangliomas, head and neck paragangliomas and non-paraganglionic tumors (e.g. renal cell carcinoma, gastrointestinal stromal tumor and pituitary neoplasia). The aim of this study was to determine phenotypical characteristics of a large Dutch cohort of *SDHB* germline mutation carriers and assess differences in clinical phenotypes related to specific *SDHB* mutations.

**Design:** Retrospective descriptive study.

**Methods:** Retrospective descriptive study in seven academic centers.

**Results:** We included 194 *SDHB* mutation carriers consisting 65 (33.5%) index patients and 129 (66.5%) relatives. Mean age was  $44.8 \pm 16.0$  years. Median duration of follow-up was 2.6 years (range: 0–36). Sixty persons (30.9%) carried the exon 3 deletion and 46 (23.7%) the c.423 + 1G > A mutation. Fifty-four mutation carriers (27.8%) had one or multiple head and neck paragangliomas, 4 (2.1%) had a pheochromocytoma and 26 (13.4%) had one or more sympathetic paragangliomas. Fifteen patients (7.7%) developed metastatic paraganglioma and 17 (8.8%) developed non-paraganglionic tumors. At study close, there were 111 (57.2%) unaffected mutation carriers. Statistical analyses showed no significant differences in the number and location of head and neck paragangliomas, sympathetic paragangliomas or pheochromocytomas, nor in the occurrence of metastatic disease or other tumors between carriers of the two founder *SDHB* mutations (exon 3 deletion vs c.423 + 1G > A).

**Conclusions:** In this nationwide study of disease-affected and unaffected *SDHB* mutation carriers, we observed a lower rate of metastatic disease and a relatively high number of head and neck paragangliomas compared with previously reported referral-based cohorts.

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## Introduction

Parangliomas (PGLs) are rare vascular, neuroendocrine tumors of paraganglia. They derive from either sympathetic chromaffin tissue of the adrenal medulla (also termed pheochromocytoma (PCC)) and extra-adrenal locations (also termed sympathetic PGL (sPGL)) or from parasympathetic tissue of the head and neck (HNPG) (1). PGLs can occur spontaneously or as part of a hereditary syndrome. Most familial cases of PCC and/or PGL and 10–20% of sporadic cases carry germline mutations. In the Netherlands, succinate dehydrogenase (*SDH*) germline mutations are responsible for most hereditary cases. The *SDHA*, *SDHB*, *SDHC* and *SDHD* genes encode for the four subunits of succinate dehydrogenase (also mitochondrial complex II), a key respiratory enzyme that links the Krebs cycle and the electron transport chain (2). The *SDHAF2* gene encodes SDH complex assembly factor 2 (SDHAF2), essential for flavination of the SDHA protein and SDH enzyme activity (3). These various germline mutations have distinct phenotypic effects. *SDHD*-related PGL/PCCs are usually characterized by multiple PGLs, predominantly located in the head and neck region with a low frequency of malignancy. In contrast, *SDHB*-related disease is often diagnosed as a single tumor (4). Furthermore, *SDHB* mutation carriers more frequently develop sPGLs, PCCs and metastatic disease than mutation carriers in the other subunits of the *SDH* gene (5, 6, 7). Although initial malignancy rates as high as 31–97% were reported for *SDHB*-related PGL (5, 6, 7, 8, 9), we recently reported risks of metastatic disease in *SDHB* mutation carriers that were considerably lower. A systematic review and meta-analysis reported by Van Hulsteijn *et al.* demonstrated that the pooled prevalence of metastatic disease was 13% in populations including both asymptomatic *SDHB* mutation carriers and mutation carriers with manifest PGL, and 23% in studies that included only mutation carriers with manifest disease (10).

*SDH* mutations have also been linked to non-paraganglionic tumors. In a recent study we strengthened the etiological association of *SDH* genes with pituitary neoplasia, renal tumorigenesis and gastric gastrointestinal stromal tumors. We also found that pancreatic neuroendocrine tumors may be part of the *SDH*-related tumor spectrum (11).

Two founder mutations in *SDHB* have been identified in Dutch PGL families, the c.423+1G>A splice site mutation and the c.201-4429\_287-933del, p.(Cys68fs) mutation, also annotated as a deletion of exon 3 (12, 13). The aim of this study was to obtain a better impression

of the phenotype of *SDHB* mutation carriers, especially of the two founder mutations. Therefore, we investigated the clinical and biochemical characteristics of disease-affected and unaffected *SDHB* germline mutation carriers in a nationwide study in seven academic centers in the Netherlands.

## Subjects and methods

In this retrospective nationwide study, all *SDHB* germline mutation carriers diagnosed before 2014 were included in the analysis. All included persons gave written informed consent and in case of persons under 18 years of age, written informed consent was obtained from their parents. Follow-up ended on July 1, 2014 or, when lost to follow-up, the date of the last contact with the endocrinologist or otolaryngologist/head and neck surgeon. We evaluated the genetic, clinical, radiological and biochemical data of *SDHB* mutation carriers identified in seven of the eight clinical genetics centers of the Netherlands: Leiden University Medical Center (Leiden), University Medical Center Groningen (Groningen), Radboud University Medical Center (Nijmegen), VU University Medical Center (Amsterdam), Erasmus Medical Center (Rotterdam), Academic Medical Center (Amsterdam) and University Medical Center Utrecht (Utrecht). Maastricht University Medical Center was not able to participate for technical reasons. However, they only had identified one germline *SDHB* mutation carrier. Data from 47 *SDHB* mutation carriers from the Leiden University Medical Center are previously described by van Hulsteijn *et al.* (14).

In the academic centers, genetic counseling and DNA testing for mutations in the *SDH* genes are offered to patients with PCC/sPGL and a positive family history for HNPG or PCC/sPGL, patients with an isolated PCC/sPGL at an early age (younger than 50 years), and all patients with an HNPG. If a mutation in the *SDHB* gene is identified, at-risk family members of the index patients are subsequently invited for genetic counseling and DNA testing for the family-specific *SDHB* mutation. Screening for germline *SDHB* mutations is performed by direct sequencing using the Sanger method on an ABI 377 Genetic Analyser (Applied Biosystems) and by multiplex ligation-dependent probe amplification (MLPA) using the P226 MLPA kit (MRC Holland, Amsterdam, the Netherlands). *SDHB* germline variants are classified as in the international guidelines by Plon *et al.* (15). In this manuscript we report pathogenic or likely

pathogenic variants, including missense mutations in highly conserved regions that are likely pathogenic, as germline mutations.

All *SDHB* germline mutation carriers were investigated according to structured protocols used for standard care in the Netherlands for patients with a PGL ([www.oncoline.nl/familiair-paraganglioom](http://www.oncoline.nl/familiair-paraganglioom)). They were offered annual clinical surveillance for PGL at the departments of otorhinolaryngology and endocrinology. For mutation carriers older than 18 years of age, screening consisted of magnetic resonance imaging (MRI) of the head and neck region once every three years, and MRI or computed tomography (CT) scans of thorax, abdomen and pelvis once every two years. Annual biochemical screening included the measurement of (nor)epinephrine, vanillylmandelic acid (VMA), dopamine, (nor)metanephrine and/or 3-methoxytyramine (3-MT) in two 24-h urinary samples (depending on the academic center which urinary measurement(s) were done), and/or plasma free (nor)metanephrine. In case of excessive catecholamine secretion (i.e. any value above the upper reference limit), radiological assessment by MRI or CT scans of thorax, abdomen and pelvis and/or  $^{123}\text{I}$  metaiodobenzylguanidine (MIBG)-scans/positron emission tomography (PET) with 2-deoxy-2-[fluorine-18]fluoro-D-glucose ( $^{18}\text{F}$ -FDG PET)-scans/ $^{18}\text{F}$ -L-dihydroxyphenylalanine ( $^{18}\text{F}$ -DOPA) PET-scans were performed to identify potential sources of excessive catecholamine production outside the head and neck region. In cases without available tumor histology, tumors were classified as paraganglionic based on their specific characteristics on CT and/or MRI. When in doubt, additional nuclear medicine imaging studies were performed in order to confirm the diagnosis.

At the time of this study, there were no national, structured protocols for surveillance in *SDHB* mutation carriers younger than 18 years of age. Therefore, the method and interval of surveillance in this age category varied between centers. In case of a diagnosis of sPGL, PCC or HNPGL, treatment or intensified periodic examination was offered, guided by the clinical course. In general, for a PCC or sPGL an operation was the preferred treatment of choice. In case of an HNPGL, treatment was guided by the clinical symptoms, tumor characteristics and patient characteristics. Wait and scan policy, radiotherapy or resection were possible treatment options.

An unaffected mutation carrier was defined as a germline mutation carrier without evidence of disease (i.e. HNPGL, sPGL and/or PCC). A disease-affected mutation carrier was defined as a germline mutation carrier with disease, i.e. HNPGL, sPGL and/or PCC.

Malignant disease was defined as the presence of metastases, that is, the presence of chromaffin tissue in locoregional lymphnodes or in non-chromaffin organs distant from the primary tumor, because there are no histological features of the primary tumor that reliably distinguish benign from malignant PGLs.

The study was approved by the Medical Ethics Committee of the Leiden University Medical Center (LUMC; number P13.161), participating centers complied with their local medical ethics committee requirements.

### Data analysis

IBM SPSS Statistics version 20.0 (SPSS) was used for data analysis. Chi-square tests were used to test whether proportions differed significantly, except when an expected cell size was less than five, in which case Fisher's exact was employed. For comparison of disease risks for index patients and relatives Kaplan–Meier curves (One Minus Cum Survival) were plotted. Results are presented as mean  $\pm$  s.d. Differences were considered statistically significant at  $P \leq 0.05$  (two-sided).

### Results

A total of 194 *SDHB* germline mutation carriers were included: 61 from the Leiden University Medical Center (Leiden), 61 from the University Medical Center Groningen (Groningen), 29 from the Radboud University Medical Center (Nijmegen), 17 from the VU University Medical Center (Amsterdam), 18 from the Erasmus Medical Center (Rotterdam), four from the Academic Medical Center (Amsterdam) and four from the University Medical Center Utrecht (Utrecht).

In total, 83 men (42.8%) and 111 women (57.2%) were included. The median duration of the follow-up was 2.6 years (range: 0–36). Eleven persons (5.7%) were lost to follow-up: six for unknown reasons, three chose not to pursue any follow-up, one emigrated and one continued the follow-up in a non-participating hospital. Seven persons (3.6%) died: three because of intercurrent disease (lung cancer, metastasized breast cancer and myocardial infarction), one due to progressive disease of a malignant HNPGL (jugular body tumor) with bone metastases, and three due to progressive disease due to a malignant sPGL.

In total, our cohort consisted of 83 (42.3%) disease-affected mutation carriers and 111 (57.2%) unaffected mutation carriers. From the 111 unaffected mutation carriers, 104 have had complete radiological screening

(CT/MRI of the head and neck region and CT/MRI of the thorax/abdomen/pelvis). Seven have had either a CT/MRI of the head and neck region (two mutation carriers) or a CT/MRI of the thorax/abdomen/pelvis (five mutation carriers). From the 83 disease-affected mutation carriers, 74 have had complete radiological screening. Nine mutation carriers have had either a CT/MRI of the head and neck region (two mutation carriers) or a CT/MRI of the thorax/abdomen/pelvis (seven mutation carriers). However, all the mutation carriers, who did not have had complete radiological screening by CT/MRI, did had another (total body) imaging study (i.e.  $^{123}\text{I}$  MIBG-scans/ $^{18}\text{F}$ -FDG PET-scans/ $^{18}\text{F}$ -DOPA PET-scans).

There were 65 index patients and 129 relatives of index patients. Of the 129 relatives, 109 persons (84.5%) were unaffected mutation carriers. Four index patients were not affected with HNPGL, PCC or sPGL because these patients had DNA testing for other reasons (one with multiple congenital anomalies, one with two renal cell carcinomas (RCCs) and a gastric gastrointestinal stromal tumor (GIST), one was thought to have an HNPGL, but during radiological follow-up the diagnosis of HNPGL was reversed to no evidence of a tumor and the fourth patient was thought to have a PCC, but this turned out to be a non-functioning adrenal adenoma).

## Genetics

Details of *SDHB* mutations are outlined in Table 1. Sixty (30.9%) were carriers of the exon 3 deletion and 46 (23.7%) were carriers of the c.423+1G>A mutation. The c.654G>A, p.(Trp218\*) mutation was present in 19 persons (9.8%) and the c.653 G>C, p.(Trp218Ser) mutation in 11 persons (5.7%).

## Clinical features

The mean age at first evaluation at the outpatient clinic was  $44.8 \pm 16.0$  years (range 11–76). In total, our cohort comprised of 65 (33.5%) index patients and 129 (66.5%) of their relatives.

Clinical characteristics at the end of follow-up of the cohort as a whole and for four most prevalent Dutch *SDHB* mutations (deletion exon 3, c.423+1G>A, c.654G>A and c.653 G>C) are outlined in Table 2.

Of the whole cohort, 54 mutation carriers (27.8%) were clinically affected with one or multiple HNPGLs. Mean age of diagnosis of HNPGL was  $45.9 \pm 14.2$  years (range: 11–77). Carotid body tumors were the most

**Table 1** *SDHB* germline mutations.

| DNA mutation                  | <i>SDHB</i> predicted protein change | Number of subjects (%) |
|-------------------------------|--------------------------------------|------------------------|
| Exon 3 deletion               | p.?                                  | 60 (31)                |
| c.423+1G>A                    | p.?                                  | 46 (24)                |
| c.654G>A                      | p.(Trp218*)                          | 19 (10)                |
| c.653G>C                      | p.(Trp218Ser)                        | 11 (6)                 |
| c.574T>C                      | p.(Cys192Arg)                        | 8 (4)                  |
| c.200+1G>A                    | p.?                                  | 6 (3)                  |
| c.137G>A                      | p.(Arg46Gln)                         | 4 (2)                  |
| c.328A>C                      | p.(Thr110Pro)                        | 4 (2)                  |
| c.418G>T                      | p.(Val140Phe)                        | 4 (2)                  |
| c.725G>A                      | p.(Arg242His)                        | 3 (1.5)                |
| c.649C>T                      | p.(Arg217Cys)                        | 3 (1.5)                |
| c.590C>G                      | p.(Pro197Arg)                        | 3 (1.5)                |
| c.686_725del                  | p.(Glu229fs)                         | 3 (1.5)                |
| c.343C>T                      | p.(Arg115*)                          | 3 (1.5)                |
| c.292T>C                      | p.(Cys98Arg)                         | 2 (1)                  |
| Deletion promoter and exon 1  | p.?                                  | 1 (0.5)                |
| Deletion promoter till exon 8 | p.0                                  | 2 (1)                  |
| Exon 2 deletion               | p.?                                  | 2 (1)                  |
| Exon 1 deletion               | p.?                                  | 2 (1)                  |
| c.713delT                     | p.(Phe238fs)                         | 1 (0.5)                |
| c.727T>A                      | p.(Cys243Ser)                        | 1 (0.5)                |
| c.761C>T                      | p.(Pro254Leu)                        | 1 (0.5)                |
| c.626C>T                      | p.(Pro209Leu)                        | 1 (0.5)                |
| c.380T>C                      | p.(Ile127Thr)                        | 1 (0.5)                |
| c.325A>C                      | p.(Asn109His)                        | 1 (0.5)                |
| c.1A>G                        | p.?                                  | 1 (0.5)                |
| c.119A>C                      | p.(Lys40Thr)                         | 1 (0.5)                |

prevalent HNPGLs (in 11.3%), followed by jugular body tumors (in 7.2%) and vagal body tumors (in 6.2%). Twenty-seven carriers (50.0%) had an operation for their HNPGL and 15 (27.8%) received radiotherapy.

Four patients (2.1%) were clinically affected with a PCC. Mean age of diagnosis of PCC was  $36.2 \pm 16.3$  years (range 19–56). Clinical characteristics are detailed in Table 3.

Twenty-six mutation carriers (13.4%) were clinically affected with one or more sPGLs. Mean age of diagnosis of sPGL was  $33.4 \pm 12.7$  years (range: 10–66). None of the 26 mutation carriers suffered from an HNPGL. More than half of the patients with an sPGL had elevated hormone levels. Five carriers had two sPGLs. The sPGLs were mainly located in the abdominal/pelvic region (28 tumors); there were only three thoracic PGLs. Eight persons carried the exon 3 deletion, five the c.423+1G>A mutation, two the c.343C>T mutation and another two the c.200+1G>A mutation. Twelve of the 26 carriers with one or more sPGLs had metastatic disease and three of them died due to progressive metastatic disease. Clinical characteristics and biochemical phenotypes are detailed in Table 4.



**Table 2** Clinical phenotypes of specific *SDHB* germline mutations.

|                                  | Total cohort (n=194) | Exon 3 deletion (n=60) | c.423+1G>A (n=46)      | c.654G>A (n=19) | c.653G>C (n=11) |
|----------------------------------|----------------------|------------------------|------------------------|-----------------|-----------------|
| Gender                           |                      |                        |                        |                 |                 |
| Man                              | 83 (42.8%)           | 29 (48.3%)             | 18 (39.1%)             | 8 (42.1%)       | 2 (18.2%)       |
| Woman                            | 111 (57.2%)          | 31 (51.7%)             | 28 (60.9%)             | 11 (57.9%)      | 9 (81.8%)       |
| Age (mean±s.d.) <sup>a</sup>     | 44.8±16.0            | 43.2±15.3              | 51.0±14.5              | 44.0±18.1       | 49.1±11.7       |
| Family history positive          | 129 (66.5%)          | 40 (66.7%)             | 35 (76.1%)             | 18 (94.7%)      | 8 (72.7%)       |
| HNPGL                            | 54 (27.8%)           | 18 (30.0%)             | 11 (23.9%)             | 1 (5.3%)        | 3 (27.3%)       |
| 1 HNPGL                          | 47                   | 15                     | 10                     | 1               | 3               |
| 2 HNPGL                          | 6                    | 2                      | 1                      | 0               | 0               |
| 3 HNPGL                          | 1                    | 1                      | 0                      | 0               | 0               |
| CBT                              | 22 (11.3%)           | 6 (10.0%)              | 3 (6.5%)               | 1               | 2 (18.2%)       |
| Left                             | 11                   | 3                      | 3                      | 0               | 1               |
| Right                            | 9                    | 4                      | 0                      | 1               | 1               |
| Bilateral                        | 2                    | 0                      | 0                      | 0               | 0               |
| VBT                              | 12 (6.2%)            | 4 (6.6%)               | 3 (6.5%)               | 0               | 1 (9.1%)        |
| Left                             | 6                    | 2                      | 0                      |                 | 1               |
| Right                            | 6                    | 2                      | 3                      |                 | 0               |
| Bilateral                        | 0                    | 0                      | 0                      |                 | 0               |
| JBT                              | 14 (7.2%)            | 7 (11.7%)              | 5 (10.9%)              | 0               | 0               |
| Left                             | 8                    | 5                      | 3                      |                 |                 |
| Right                            | 5                    | 1                      | 2                      |                 |                 |
| Bilateral                        | 1                    | 1                      | 0                      |                 |                 |
| Tymp                             | 10 (5.2%)            | 4 (6.7%)               | 1 (2.2%)               | 0               | 0               |
| Left                             | 5                    | 1                      | 1                      |                 |                 |
| Right                            | 5                    | 3                      | 0                      |                 |                 |
| Bilateral                        | 0                    | 0                      | 0                      |                 |                 |
| Other (HNPGL)                    | 1 (right tonsil)     | 0                      | 0                      | 0               | 0               |
| Age HNPGL <sup>b</sup>           | 45.9±14.2            | 47.0±14.8              | 50.6±11.2              | 27.2            | 44.8±14.3       |
| Operation HNPGL                  | 27 (50.0%)           | 8 (44.4%)              | 4 (36.4%)              | 0               | 1 (33.3%)       |
| Radiotherapy HNPGL               | 15 (27.8%)           | 8 (44.4%)              | 4 (36.4%)              | 0               | 0               |
| PCC                              | 4 (2.1%)             | 1 (1.7%)               | 0                      | 0               | 1 (9.1%)        |
| Left                             | 3                    | 1                      |                        |                 | 1               |
| Right                            | 1                    | 0                      |                        |                 |                 |
| sPGL <sup>c</sup>                | 26 (13.4%)           | 8 (13.3%)              | 5 (10.9%)              | 1 (5.3%)        | 1 (9.1%)        |
| Operation sPGL                   | 25                   | 8 (100%)               | 5 (100%)               |                 | 1 (100%)        |
| Malignant PGL/PCC                | 15 (7.7%)            | 5 (8.3%)               | 1 (2.2%)               | 1 (5.3%)        | 1 (9.1%)        |
| Other tumors <sup>d</sup>        | 17 (8.8%)            | 5 (8.3%)               | 7 (15.2%) <sup>g</sup> | 0               | 0               |
| Mamma ca.                        | 1                    | 0                      | 1                      |                 |                 |
| Renal cell ca.                   | 3 <sup>e</sup>       | 2                      | 1                      |                 |                 |
| Basal cell ca.                   | 2                    | 0                      | 1                      |                 |                 |
| Melanoma                         | 2                    | 1                      | 1                      |                 |                 |
| Lung ca.                         | 1                    | 0                      | 1                      |                 |                 |
| Prostate ca.                     | 1                    | 0                      | 0                      |                 |                 |
| Colon ca.                        | 2                    | 0                      | 2                      |                 |                 |
| Meibomian gland                  | 1                    | 0                      | 0                      |                 |                 |
| Synovial sarcoma                 | 1                    | 1                      | 0                      |                 |                 |
| Ovarian ca.                      | 1                    | 0                      | 1                      |                 |                 |
| Gastric GIST                     | 2 <sup>f</sup>       | 0                      | 1                      |                 |                 |
| Micro-PRL                        | 1                    | 0                      | 0                      |                 |                 |
| Pituitary incidentaloma          | 1                    | 1                      | 0                      |                 |                 |
| Disease status at last follow-up |                      |                        |                        |                 |                 |
| NED                              | 133 (68.6%)          | 42 (70.0%)             | 32 (69.6%)             | 16 (84.2%)      | 8 (72.7%)       |
| AWD                              | 43 (22.2%)           | 13 (21.7%)             | 9 (19.6%)              | 1 (5.3%)        | 3 (27.3%)       |
| LTF                              | 11 (5.7%)            | 3 (5.0%)               | 2 (4.3%)               | 1 (5.3%)        | 0               |
| DOD                              | 4 (2.1%)             | 2 (3.3%)               | 1 (2.2%)               | 1 (5.3%)        | 0               |
| DID                              | 3 (1.5%)             | 0                      | 2 (4.3%)               | 0               | 0               |

<sup>a</sup>Mean age at presentation at the outpatient clinic in an academic hospital; <sup>b</sup>age at diagnosis HNPGL; <sup>c</sup>total cohort: 26 patients with 1 or more sPGLs.

Of these 26 patients, five patients had 2 sPGLs; <sup>d</sup>number of patients (some patients developed multiple tumors); <sup>e</sup>there was one patient with two foci of renal cell carcinoma (RCC) on the left side and one RCC on the right side. The other 2 patients both had 1 foci of a RCC; <sup>f</sup>one patient developed three renal cell carcinomas (2 foci on the left side and one on the right side) as well as a gastrointestinal stromal tumor (GIST); <sup>g</sup>one patient with rectal cancer and ovarian cancer, one patient with three RCC as well as a GIST.

AWD, alive with disease; ca., carcinoma; CBT, carotid body tumor; DID, dead of intercurrent disease; DOD, dead of disease; GIST, gastrointestinal stromal tumor; HNPGL, head and neck paraganglioma; JBT, jugular body tumor; LTF, loss to follow-up; NED, no evidence of disease; PCC, pheochromocytoma; PRL, prolactinoma; sPGL, sympathetic paraganglioma; Tymp, tympanicum body tumor; VBT, vagal body tumor.

**Table 3** Clinical characteristics of the 4 patients with a pheochromocytoma.

| Case | Sex | <i>SDHB</i> mutation | Location | Presenting symptoms                        | Age <sup>a</sup> | Biochemical phenotype<br>(urinary measurements) | Biochemical<br>phenotype<br>(blood) | Outcome                   |
|------|-----|----------------------|----------|--|------------------|---|-------------------------------------|---------------------------|
| 1    | M   | Exon 2 deletion      | Right    | Hypertension, flushes,<br>palpitations     | 40               | NMN elevated, M normal                          | NA                                  | NED                       |
| 2    | F   | c.343C>T             | Left     | Collaps                                    | 28               | NA  | NA                                  | NED                       |
| 3    | F   | Exon 3 deletion      | Left     | None, brother with <i>SDHB</i><br>mutation | 56               | M, NMN, 3-MT slightly<br>elevated               | NA                                  | NED                       |
| 4    | F   | c.653G>C             | Left     | Hypertension, flushes                      | 19               | NAV   | NAV                                 | AWD (vagal<br>body tumor) |

<sup>a</sup>Age at diagnosis of pheochromocytoma.

F, female; M, male; MN, metanephrine; NA, not assessed; NAV, not available; NED, no evidence of disease; NMN, normetanephrine.

Out of the whole cohort of *SDHB* germline mutation carriers, 15/194 (7.7%) developed metastatic PGL. Clinical characteristics, treatment and outcome of the patients with metastatic disease are displayed in detail in Table 5. Treatment of the primary tumor existed of surgery in all patients. None of the 47 mutation carriers described previously have developed metastatic disease since our publication in 2014 (14).

Seventeen mutation carriers (8.8%) developed a total of 21 non-paraganglionic tumors. Three patients developed a total of five (histology confirmed) renal tumors. Four of those tumors were described previously and classified as SDH-deficient renal carcinomas (11, 16, 17). Two patients developed a RCC on one side (one clear cell carcinoma and one SDH-deficient carcinoma), and one patient developed two foci of a RCC on the left side and one on the right side (all three SDH-deficient renal carcinomas). This latter patient also developed an SDH-deficient gastric GIST and has been described previously (11). There was one other patient with an SDH-deficient gastric GIST. Furthermore, there were two patients with a basal cell carcinoma, two with a melanoma, one with a squamous cell lung carcinoma, one with (metastasized) breast cancer, one with prostate cancer, one with a meibomian gland (adeno) carcinoma and one with a (metastasized) synovial sarcoma. In addition, two patients had a rectal cancer and one had ovarian cancer (granulosa cell tumor).

Besides these malignancies, one person developed a microprolactinoma and one person had a non-functioning pituitary incidentaloma, both of which underwent radiological follow-up without available biopsy or surgically-resected material.

Of these 17 mutation carriers with non-paraganglionic tumors, only three patients had also paraganglionic tumors (all three patients had an HNPGL).

The clinical characteristics of the index patients vs relatives are outlined in Table 6 and the age-related disease risk for index patients (proband) vs relatives is outlined in Fig. 1.

To explore potential differences in clinical phenotypes related to the specific mutations within the *SDHB* gene, carriers of the two most common *SDHB* mutations in the Netherlands (exon 3 deletion and c.423+1G>A) were compared. Statistical analyses showed no significant differences in number and location of HNPGLs, sPGLs or PCCs, nor in the occurrence of malignant disease or other tumors.

## Discussion

In this nationwide multicenter study we assessed the phenotypes of 194 *SDHB* germline mutation carriers. Our cohort consisted of 83 (42.8%) disease-affected mutation carriers and 111 (57.2%) unaffected mutation carriers. Fifty-four carriers (27.8%) were clinically affected with one or multiple HNPGLs. Only four patients (2.1%) were clinically affected with a PCC and 26 (13.4%) with one or more sPGLs. Fifteen patients (7.7%) developed metastatic disease.

Previous studies have reported much higher rates for developing PCC and sPGLs, 18–52% and 59–84% respectively (5, 6, 8, 18). For various reasons, it is quite difficult to directly compare our results with those reported in the literature. The majority of previously published studies include a high proportion of index patients. This may result in ascertainment bias and therefore overestimation of the risk of developing HNPGL, PCC, sPGL or malignant disease. A recently published study by the French network on PGL/PCC in *SDHx* mutation carriers included 124 *SDHB* mutation carriers, 39 (31%) of whom were index patients and 85 persons (69%)

**Table 4** Characteristics of 26 patients with sympathetic paragangliomas.

| Case | Sex | <i>SDHB</i> mutation | Location sPGL                                      | Age <sup>a</sup> (year) | Malignant disease | Tumor reduction therapy   | Outcome  |
|------|-----|----------------------|--|-------------------------|-------------------|---|--|
| 1    | F   | c.343C>T             | Retroperitoneal and presacral                      | 31                      | No                | Surgery   | No evidence of disease                                     |
| 2    | F   | Exon 3 deletion      | Para-aortic  | 41                      | No                | Surgery (non-radical)   | Alive with disease   |
| 3    | M   | c.200+1G>A           | Retroperitoneal (pararenal)                        | 42                      | Yes               | Surgery, <sup>131</sup> I-MIBG therapy, radiotherapy                  | Alive at age 52, with disease                              |
| 4    | M   | Exon 3 deletion      | Retropancreatic                                    | 11                      | No                | Surgery   | No evidence of disease                                     |
| 5    | M   | Exon 3 deletion      | Thoracic (vertebra Th6) and intra-abdominal        | 10 and 32               | Yes               | Surgery, chemotherapy radiotherapy <sup>131</sup> I-MIBG therapy, RFA | Alive at age 37, without evidence of disease               |
| 6    | F   | Exon 1 deletion      | Renal hilum  | 28                      | No                | Surgery   | No evidence of disease                                     |
| 7    | M   | Exon 3 deletion      | Para-aortic abdominal                              | 42                      | No                | Surgery   | No evidence of disease                                     |
| 8    | F   | c.423+1G>A           | Retroperitoneal                                    | 36                      | No                | Surgery   | No evidence of disease                                     |
| 9    | F   | c.725G>A             | Para-adrenal                                       | 40                      | Yes               | Surgery, Lutetium octreotate therapy                                  | Alive at age 51, with disease                              |
| 10   | F   | c.423+1G>A           | Para-iliac (2 lesions)                             | 19                      | No                | Surgery   | No evidence of disease                                     |
| 11   | M   | c.423+1G>A           | Para-aortic abdominal                              | 31                      | No                | Surgery   | No evidence of disease                                     |
| 12   | F   | c.653G>C             | Retroperitoneal                                    | 66                      | Yes               | Surgery, <sup>131</sup> I-MIBG therapy                                | Alive at age 78, with disease                              |
| 13   | M   | Exon 3 deletion      | Retroperitoneal                                    | 37                      | Yes               | Surgery   | Alive at age 40, with disease                              |
| 14   | M   | Exon 3 deletion      | Bladder and retroperitoneal                        | 27                      | No                | Surgery   | No evidence of disease                                     |
| 15   | M   | c.423+1G>A           | Para-aortic abdominal                              | 38                      | No                | Surgery   | No evidence of disease                                     |
| 16   | M   | c.325A>C             | Para-aortic abdominal                              | 30                      | Yes               | Surgery, <sup>131</sup> I-MIBG therapy                                | Alive at age 46, with disease                              |
| 17   | M   | c.200+1G>A           | Bladder  | 45                      | Yes               | Surgery, radiotherapy, chemotherapy (CVD)                             | Alive at age 47, with disease                              |
| 18   | M   | c.574T>C             | Liver hilum  | 24                      | No                | Surgery   | No evidence of disease                                     |
| 19   | F   | c.727T>A             | Retroperitoneal (para-aortic)                      | 52                      | Yes               | Surgery, radiotherapy   | Died at age 63, due to intercurrent disease                |
| 20   | F   | c.343C>T             | Thoracic   | 14                      | No                | Surgery   | Loss to follow-up  |
| 21   | F   | c.686_725del         | Para-aortic abdominal and para-vertebral (Th3/Th4) | 39                      | No                | Follow-up   | Alive with disease   |
| 22   | M   | c.626C>T             | Bladder  | 42                      | Yes               | Radiotherapy, Firstmappp trial (started June 2014)                    | Alive at age 52, with disease                              |
| 23   | F   | c.423+1G>A           | Pararenal  | 31                      | No                | Surgery   | No evidence of disease                                     |
| 24   | M   | Exon 3 deletion      | Presacral  | 28                      | Yes               | Surgery, <sup>131</sup> I-MIBG therapy, radiotherapy                  | Dead of disease: died at age 32 due to progressive disease |
| 25   | F   | c.654G>A             | Bladder  | 19                      | Yes               | Surgery (primary bladder PGL) sunitinib (metastases)                  | Dead of disease: died at age 62 due to progressive disease |
| 26   | F   | Exon 3 deletion      | Para-vertebral abdominal                           | 33                      | Yes               | Surgery, <sup>131</sup> I-MIBG therapy, radiotherapy                  | Dead of disease: died at age 37 due to progressive disease |

<sup>a</sup>Age at diagnosis of sympathetic paraganglioma; \*catecholamine measurements at time of primary tumor not available; \*\*catecholamine excess developed with lymph node metastases, not at time of primary tumor; \*\*\*catecholamine excess developed at time of malignant disease, not at time of primary tumor.

3-MT, 3-methoxytyramine; CVD, cyclophosphamide, vincristine, dacarbazine; D, dopamine; E, epinephrine; F, female; Firstmappp, randomized, double-blind, phase II, international, multicenter study which is dedicated to determine the efficacy of sunitinib on the progression-free survival at 12 months in patients with progressive malignant pheochromocytoma and paraganglioma; HNPGL, head and neck paraganglioma; M, male; MN, metanephrine; NA, not assessed; NE, norepinephrine; NMN, normetanephrine; PGL, paraganglioma; RFA, radiofrequency ablation; VMA, vanillylmandelic acid.

were relatives of index patients (19). This cohort seems to resemble the proportions of our study cohort, and the prevalences of PCC (1.6%) and sPGL (6.5%) found in their study are more comparable to the results in our current

study (2.1% and 13.4% respectively). The low percentages of PCC/sPGLs reported in France and in the present study indicate that the high percentages described in several other studies are likely to be the result of ascertainment



**Table 5** Clinical characteristics of patients with metastatic paragangliomas.

| Case | Sex | <i>SDHB</i> mutation | Location PGL                  | Age <sup>a</sup> (years) | Age <sup>b</sup> (years) | Location metastases          | Treatment malignant disease   | Outcome  |
|------|-----|----------------------|-------------------------------|--------------------------|--------------------------|------------------------------|---|--|
| 1    | M   | c.200+1G>A           | Retroperitoneal (pararenal)   | 42                       | 45                       | Bone                         | Surgery, <sup>131</sup> I-MIBG therapy, radiotherapy                  | Alive at age 52, with disease                                |
| 2    | M   | Exon 3 deletion      | Thoracic (vertebra Th6)       | 10                       | 13                       | Intra-thoracic               | Surgery, chemotherapy radiotherapy <sup>131</sup> I-MIBG therapy, RFA | Alive at age 37, without evidence of disease                 |
| 3    | F   | c.418G>T             | Right tonsil                  | 18                       | 20                       | Lymph nodes, bone (vertebra) | Surgery, radiotherapy   | LTF, follow-up till age 22, alive with disease               |
| 4    | F   | c.725G>A             | Para-adrenal                  | 40                       | 45                       | Lymph nodes, bone            | Surgery and <sup>177</sup> Lutetium octreotate therapy                | Alive at age 51, with disease                                |
| 5    | M   | c.423+1G>A           | Jugular body                  | 48                       | 57                       | Bone (vertebra)              | None  | Died at age 57, due to rapidly progressive malignant disease |
| 6    | F   | Exon 3 deletion      | Carotid body                  | 35                       | 66                       | Lymph nodes, bone            | None (not within study period)  | Alive at age 66, with disease                                |
| 7    | F   | c.653G>C             | Retroperitoneal               | 66                       | 70                       | Lymph nodes, bone            | <sup>131</sup> I-MIBG therapy   | Alive at age 78, with disease                                |
| 8    | M   | Exon 3 deletion      | Retroperitoneal               | 37                       | 38                       | Lymph nodes                  | Surgery   | Alive at age 40, with disease                                |
| 9    | M   | c.325A>C             | Para-aortic abdominal         | 30                       | 39                       | Lymph nodes, bone, lung      | <sup>131</sup> I-MIBG therapy   | Alive at age 46, with disease                                |
| 10   | M   | c.200+1G>A           | Bladder                       | 45                       | 45                       | Lymph nodes, bone, lung      | Surgery, radiotherapy, chemotherapy (CVD)                             | Alive at age 47, with disease                                |
| 11   | F   | c.727T>A             | Retroperitoneal (para-aortic) | 52                       | 55                       | Bone                         | Radiotherapy  | Died at age 63, due to intercurrent disease                  |
| 12   | M   | c.626C>T             | Bladder                       | 42                       | 46                       | Lymph nodes, bone            | Radiotherapy, Firstmappp trial (started June 2014)                    | Alive at age 52, with disease                                |
| 13   | M   | Exon 3 deletion      | Presacral                     | 28                       | 28                       | Bone                         | Surgery, <sup>131</sup> I-MIBG therapy, radiotherapy                  | Died at age 32 due to progressive disease                    |
| 14   | F   | c.654G>A             | Bladder                       | 19                       | 58                       | Lymph nodes, bone            | sunitinib   | Died at age 62 due to progressive disease                    |
| 15   | F   | Exon 3 deletion      | Para-vertebral abdominal      | 33                       | 33                       | Lymph nodes, bone            | Surgery, <sup>131</sup> I-MIBG therapy, radiotherapy                  | Died at age 37 due to progressive disease                    |

<sup>a</sup>Age at diagnosis of paraganglioma; <sup>b</sup>age at diagnosis of malignant disease.

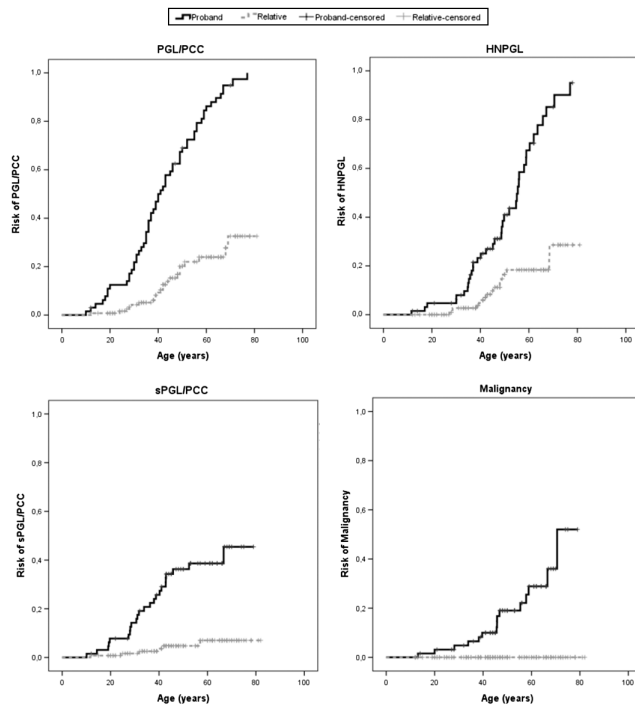
CBT, carotid body tumor; CVD, cyclophosphamide, vincristine, dacarbazine; F, female; Firstmappp, randomized, double-blind, phase II, international, multicenter study which is dedicated to determine the efficacy of sunitinib on the progression-free survival at 12 months in patients with progressive malignant pheochromocytoma and paraganglioma; HNPGL, head and neck PGL; LTF, loss to follow-up; M, male; PGL, paraganglioma; RFA, radiofrequency ablation; Th6, 6th thoracic vertebra.

bias. Furthermore, it should be noted that the percentages mentioned in most studies are calculated using the total number of tumors divided by the total number of patients

with any tumor, thereby taking only disease-affected persons into account. Removal of all unaffected mutation carriers from our cohort (111 subjects) would give a

**Table 6** Clinical characteristics of index patients and relatives.

|                     | Age (mean ± s.d.) | Follow-up (median, year) | HNPGL (%) | PCC (%) | sPGL (%)  | Malignant PGL/PCC |
|---------------------|-------------------|--------------------------|-----------|---------|-----------|-------------------|
| Index patients (65) | 43.6 ± 14.8       | 4.5                      | 38 (58.5) | 3 (4.6) | 21 (32.3) | 15 (23.1)         |
| Relatives (129)     | 45.4 ± 16.6       | 2.0                      | 16 (12.4) | 1 (0.8) | 5 (3.9)   | 0                 |



**Figure 1**

Comparison of age at onset in *SDHB* mutations carriers: index patients (probands) vs relatives. PGL/PCC, risk of (all) paragangliomas/pheochromocytoma; HNPGL, risk of head and neck paraganglioma; sPGL/PCC, risk of sympathetic paraganglioma/pheochromocytoma; malignancy, risk of malignancy.

figure for PCC of 4 in 83 (4.8%) and 26 in 83 (31.3%) for sPGL. Even if we take only disease-affected individuals into account, our figures are substantially lower than in previous studies that have assessed clinical characteristics in *SDHB* mutation carriers. By contrast, we found a relatively high frequency of HNPGLs (27.8%) among *SDHB* mutation carriers compared with other studies (3–31%) (5, 6, 8, 18) and even when compared with that of the French network (14.5%) (19). If only the disease-affected mutation carriers were taken into account, the prevalence of HNPGL was as high as 54/83 (65.1%) in our cohort, nearly double the frequency reported previously in disease-affected subjects (5, 6, 8). This might in part be explained by the observation that in our study the proportion of HNPGL patients with a positive family history (i.e. non-index HNPGL patients) is 29.6% (16/54). The large majority of these patients had no symptoms and had not yet come to medical attention. The genetic testing of relatives and structured follow-up protocols of persons with a *SDHB* mutation in the Netherlands identifies a relatively high number of asymptomatic

mutation carriers, with or without tumors, allowing for a more accurate representation of the phenotype of *SDHB* mutation carriers.

The observation that the majority of *SDHB*-linked patients develop an HNPGL furthermore underlines the importance of radiological screening of the head and neck region in *SDHB* mutation carriers.

Only fifteen patients (7.7%) in the entire cohort, including both disease-affected and unaffected mutation carriers, developed metastatic PGL. In three of these patients (20%) the primary tumor was an HNPGL (including one in the tonsil) and in 12 patients (80%) the primary tumor was an sPGL. Removal of all unaffected mutation carriers (111 subjects) results in a prevalence of metastatic disease by 18.1% (15/83) in PGL/PCC patients. Taking into account only the sPGL patients, the malignancy risk is as high a 46.2% (12/26). For HNPGL patients, this malignancy rate was 5.6% (3/54). This means that the malignancy risk for patients already suffering from an sPGL is high, which has implications for the follow-up of those patients. Srirangalingam *et al.* reported metastatic PGL in five of 16 (31%) disease-affected subjects (8). However, the malignancy rate for the *entire* cohort was 16% (5/32). The rates of malignancy reported in the literature are calculated based on disease-affected subjects and vary from 31 to 97% (5, 6, 7, 8, 9). These reported malignancy rates are however most likely also inflated because of selection bias in referral-based studies. Alternatively, the discrepancy in malignancy rates may also be a result of variable follow-up times (7, 8). A recent systematic review of prevalence studies comprising both asymptomatic *SDHB* mutation carriers and *SDHB* mutation carriers with manifest non-malignant PGL documented a pooled risk for developing metastatic PGL of 13 and 23% respectively (10), also much lower than previously reported (20, 21). In the fifteen patients with metastatic PGL, we found a wide range of time to metastatic disease (0–39.2 years). This is in line with previously published results. Timmers *et al.* found a range from 0 to 17 years (7) and Srirangalingam *et al.* between 1.5 and 25 years (8). Because it is not possible to diagnose malignancy based on histopathology of the primary tumor, only if metastatic disease is present, the current and previously reported wide ranges of time to metastatic transformation underscore the need for an extended follow-up in patients with an *SDHB* mutation, especially in disease-affected mutation carriers. The median duration of follow-up is 2.6 years in this study, which is a limitation of this study. However, the follow-up time is relatively short due to a shorter follow-up of relatives compared to index patients.

Future studies with a longer duration of follow-up are needed to validate our results.

Our findings show a relatively mild phenotype of *SDHB* mutations in the Netherlands. One might hypothesize that this could be associated with the low altitude and therefore relatively high oxygen levels in the Netherlands (22). However, studying a large cohort from a single country provides a more homogeneous study population and the inclusion of unaffected mutation carriers should provide better information on actual tumor risks than series that include mainly index patients (18). The high proportion of unaffected mutation carriers in our study seems to reflect an active testing protocol in the Netherlands of at-risk family members of the index patients, who are advised to undergo genetic counseling and DNA testing for the family-specific *SDHB* mutation. Lower lifetime cancer risks have also been established for other genetic tumor syndromes following the inclusion of unaffected mutation carriers, one well-known example being pathogenic *BRCA1/2* gene variants (23). Lower cumulative lifetime risks of breast cancer followed from analyses that excluded index patients while including first-degree relatives.

In conclusion, in this nationwide study which allowed for the inclusion of *SDHB* germline mutation carriers identified in the Netherlands, we found a lower rate of metastatic disease and a relatively high number of HNPGLs compared with previous reports of referral-based cohorts. This is most probably not a regional phenomenon but the result of the more comprehensive inclusion of unaffected mutation carriers, underlining the importance of including both disease-affected and unaffected individuals in studies that assess the phenotype of germline mutations. It furthermore highlights the importance of thorough tumor screening protocols that include radiology of the head and neck region in *SDHB* mutation carriers.

#### Declaration of interest

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

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#### Author contribution statement

Nicolasine D Niemeijer helped in study design, data collection, data analysis and preparation of the manuscript. Johannes A Rijken helped in

study design, data collection and preparation of the manuscript. Karin Eijkelenkamp, Anouk N A van der Horst-Schrivers and Michiel Kerstens helped in data collection and editing of the manuscript. Carli M J Tops and Anouk van Berkel helped in data collection. Henri J L M Timmers helped in data collection and editing of the manuscript. Henricus P M Kunst, C René Leemans, Peter H Bisschop, Koen M A Dreijerink, Marieke F van Dooren and Jean-Pierre Bayley helped in data collection. Alberto M Pereira helped in data analysis and preparation of the manuscript. Jeroen C Jansen helped in data collection and preparation of the manuscript. Frederik J Hes and Erik F Hensen helped in study design, data collection and preparation of the manuscript. Eleonora P M Corssmit helped in study design, data collection, data analysis and preparation of the manuscript.

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