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CHAPTER 2

No Difference in Phenotype of the Main Dutch SDHD Founder Mutations

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Abstract

*Background:* SDHD mutations predispose carriers to hereditary paraganglioma syndrome. The objective of this study was to assess the genotype-phenotype correlation of a large Dutch cohort of SDHD mutation carriers and evaluate potential differences in clinical phenotypes due to specific SDHD gene mutations.

*Methods:* Retrospective, descriptive single-center study. All consecutive SDHD mutation carriers followed at the Department of Endocrinology of the Leiden University Medical Center were included. Subjects were investigated according to structured protocols used for standard care, including repetitive biochemical and radiological screening for paragangliomas.

*Results:* 201 SDHD mutation carriers with a mean age at presentation of 42.6 ± 14.4 years and a mean follow-up of 5.8 ± 5.4 years were evaluated. 81% carried the SDHD c.274G>T (p.As92Tyr) mutation and 13% the SDHD c.416T>C (p.Leu139Pro) mutation. No differences in clinical phenotype between these two specific SDHD mutations were found. 91% developed one or multiple paragangliomas in the head and neck region (HNPGLs), of which the carotid body tumor was the most prevalent (85%). Eighteen carriers developed pheochromocytomas, fifteen sympathetic paragangliomas, and nine carriers (4%) suffered from malignant paraganglioma. By end of follow-up, sixteen SDHD mutation carriers (8%) displayed no biochemical or radiological evidence of manifest disease.

*Conclusions:* The two main Dutch SDHD founder mutations do not differ in clinical expression. SDHD mutations are associated with the development of multiple HNPGLs and predominantly benign disease.
Paragangliomas (PGLs) can occur sporadically or as part of a hereditary syndrome (i.e. hereditary paraganglioma syndrome, von Hippel-Lindau disease, multiple endocrine neoplasia (MEN) type 2 or neurofibromatosis type 1).\(^1\) Hereditary paraganglioma syndrome is associated with germline mutations in subunits A, B, C, D or assembly factor 2 of the mitochondrial complex II-succinate dehydrogenase (SDH) gene, which encodes Krebs cycle proteins.\(^3\)-\(^7\) More recently, mutations in TMEM127 and MAX have been found.\(^8\),\(^9\) The various germline mutations have different phenotypic effects. SDHD-linked PGL has an age-related penetrance.\(^10\) Mutation carriers typically present with multifocal, parasympathetic PGLs in the head and neck region (HNPGLs) and sympathetic PGLs (sPGLs; extra-adrenal non-HNPGLs), although they also may develop adrenal PGLs (i.e. pheochromocytomas or PCC).\(^10\),\(^11\) The most common HNPGLs are the carotid body tumor, vagal body tumor and jugulotympanic tumor (i.e. paraganglioma of the temporal bone).\(^12\),\(^13\) PGLs in SDHD mutation carriers are usually benign; the pooled incidence of malignant PGL in populations comprising both asymptomatic mutation carriers and mutation carriers with manifest non-malignant PGL is reported to be 8%.\(^14\) In the Netherlands, the p.Asp92Tyr and p.Leu139Pro founder mutations in SDHD are the most prevalent cause of hereditary PGLs.\(^15\),\(^16\) The high prevalence of Dutch founder mutations is probably due to the fact that until the second half of the 20th century, Dutch society was segregated based on socioeconomic and religious differences, leading to endogamy in isolated populations. This allowed proliferation of the Dutch founder mutations, which have a high prevalence in the Netherlands but are very rare in other series.\(^17\) To the best of our knowledge, potential differences in clinical phenotypes due to specific mutations within the SDHD gene have never been assessed. The high prevalence of founder mutations in the Netherlands gives us the unique opportunity to evaluate a substantial number of carriers of these mutations. The objective of this study was to determine the clinical, biochemical and radiological characteristics of a large Dutch cohort of SDHD mutation carriers, in order to build on the insights into disease manifestations of the specific SDHD mutations.

Materials and Methods

In this retrospective study, we evaluated the clinical, biochemical and radiological data of 201 consecutive SDHD mutation carriers, followed in the outpatient clinic of the Department of Endocrinology of the Leiden University Medical Center (LUMC); a tertiary referral center for patients with PGLs. All carriers were investigated according to structured protocols used for standard care. These included questions focused at tumor- and catecholamine-related signs and symptoms. In order
to detect sPGLs/PCC and HNPGLs, biochemical screening and repetitive head-and-neck magnetic resonance imaging (MRI) were performed at intervals of 2 years (in presymptomatic mutation carriers with intervals of 3 years). Biochemical screening included the measurement of (nor)epinephrine, vanillylmandelic acid (VMA) and dopamine in two 24-h urinary samples. From 2005 onwards, (nor)metanephrine and 3-methoxytyramine (3-MT) were added to these measurements. Urine was collected during 24 hours in duplicate under strict dietary regulations (patients abstained from bananas, nuts, alcohol, coffee, tea and other caffeine containing beverages from two days preceding and during urine collection) and after withdrawal of medications that might interfere with catecholamine secretion for at least one week or after changing antihypertensive medication to doxazosin. In order to ascertain adequacy of collection, urinary creatinine secretion was also measured. 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 was performed to identify potential sources of excessive catecholamine production outside the head and neck region, followed by whole-body $^{123}$I metaiodobenzylguanidine (MIBG)-scans when a suspected lesion was found (Figure 1).

Malignant disease was defined as the presence of metastases, i.e. the presence of chromaffin tissue in nonchromaffin organs or tissues distant from the primary tumor, since there are no reliable histological features to distinguish benign from malignant PGLs.\textsuperscript{18,19} In all surgically resected PGLs, diagnosis was confirmed by pathological investigation.

Screening for $SDH$ mutations was performed in persons who agreed to genetic testing. In case of persons aged between 12 and 16 years, the informed consent of both parents was also required. In index patients, the $SDHD$ gene was scanned for the presence of mutations at the laboratory for DNA diagnostics at the LUMC. All exonic and adjacent intronic regions of these genes were tested by direct sequencing using the Sanger method on an ABI 377 Genetic Analyser (Applied Biosystems, Carlsbad, CA, USA) and multiplex ligation-dependent probe amplification (MLPA) was carried out with the P226 MLPA kit (MRC Holland, Amsterdam, the Netherlands).\textsuperscript{15} Family members of index patients were tested for the family-specific mutation.

Follow-up ended May 1\textsuperscript{st} 2012 or, in case of death, date of death or, when lost to follow-up, date of the last contact with the endocrinologist.

Because the study was an evaluation of routine patient care, the requirements of Dutch law state that it is not necessary to obtain permission from an institutional ethical committee.
Figure 1: Follow-up of SDHD mutation carriers

**Assays**

Urinary secretion of (nor)epinephrine and dopamine in 24-h urine collections were quantified by reversed high-performance liquid chromatography (HPLC) by an electrochemical detector. Inter- and intra-assay coefficients of variations (CV’s) for epinephrine were 4.3-9.0% ranging from high to low levels. For norepinephrine these data were 2.7-3.6% and for dopamine 3.1-4.8%. Urinary secretion of VMA was measured using HPLC with fluorometric detection, with inter- and intra-assay CV’s of respectively 7.4-8.1% and 2.4-9.1%. (Nor)metanephrine and 3-MT were determined by stable isotope mass fragmentography. The CV’s of the 3-O-methylated catecholamine metabolites ((nor)metanephrine and 3-MT) ranged from 1.7 to 4.2.20

Reference ranges were obtained in healthy volunteers. These were: norepinephrine 0.06-0.47 μmol/24h, epinephrine ≤ 0.16 μmol/24h, dopamine 0.46-3.40 μmol/24h, VMA ≤30 μmol/24h,
metanephrine 33-99 μmol/mol creatinine, normetanephrine 64-260 μmol/mol creatinine and 3-MT 45-197 μmol/mol creatinine.²¹

Data analysis
For data analysis, IBM SPSS Statistics version 20.0 (SPSS Inc., Chicago, IL) was used. Results are expressed as mean ± standard deviation (SD). The Shapiro-Wilk test was used to test for normality. The unpaired t-test or, when necessary, the Mann-Whitney U test were used to compare means between specific mutations. Pearson’s chi-square test was used to test whether proportions differed significantly. A p-value < 0.05 was considered significant.

Results
Of the 201 included SDHD mutation carriers included, 105 were men (52%) and 96 women (48%). The mean duration of follow-up was 5.8 ± 5.4 years (range 0-24). Twenty-three persons (11%) were lost to follow-up, as seven persons withdrew from further surveillance and six persons moved away. Ten persons were lost to follow-up for unknown reasons. Four persons died: two deaths were directly related to malignant PGL, one person died due to reasons unrelated to the SDHD mutation and one person for unknown reasons.

Genetics
The details of SDHD mutations are outlined in Table 1. The majority of persons (81%) carried the SDHD c.274G>T (p.Asp92Tyr) mutation, followed by the SDHD c.416T>C (p.Leu139Pro) mutation (13%) and SDHD c.284T>C (p.Leu95Pro) mutation (3%). In our cohort, eighteen individuals (9%) were not molecular genetically tested but were considered to be obligate SDHD mutation carriers, since they had a positive family history with a proven SDHD mutation and a personal PGL diagnosis. The family history of 174 carriers (87%) was positive for PGL.

Table 1: Germline mutations in SDHD mutation carriers

<table>
<thead>
<tr>
<th>cDNA</th>
<th>SDHD sequence variant protein</th>
<th>Number of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.274G&gt;T</td>
<td>p.Asp92Tyr</td>
<td>162 (81)</td>
</tr>
<tr>
<td>c.416T&gt;C</td>
<td>p.Leu139Pro</td>
<td>26 (13)</td>
</tr>
<tr>
<td>c.284T&gt;C</td>
<td>p.Leu95Pro</td>
<td>6 (3)</td>
</tr>
<tr>
<td>del promoter, exon 1 and 2</td>
<td>not applicable</td>
<td>3 (1.5)</td>
</tr>
<tr>
<td>c.242C&gt;T</td>
<td>p.Pro81Leu</td>
<td>2 (1)</td>
</tr>
<tr>
<td>c.337_340delGACT</td>
<td>p.Asp113fs</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>c.34G&gt;A</td>
<td>p.Gly12Ser (unclassified variant)</td>
<td>1 (0.5)</td>
</tr>
</tbody>
</table>
Initial presentation
Fifty-seven persons were referred to the outpatient clinic of the Department of Endocrinology by a clinical geneticist following a positive SDHD mutation test, and 143 persons were referred by an ear, nose and throat (ENT) specialist, a vascular surgeon or general internal medicine specialist following a diagnosis of one or more PGLs. One person was referred because he moved to the Leiden area.

The mean age at presentation at the outpatient clinic of the Department of Endocrinology was 42.6 ± 14.4 years (range 13-75). At presentation, 152 carriers (76%) were diagnosed with PGLs: 151 with HNPGLs and one patient with a sPGL, located in the thymic region and surgically resected. In 43 carriers, radiological imaging to detect PGLs had not yet been performed at time of presentation. In six persons (3%), radiological imaging was performed but no PGLs were detected, i.e. they were asymptomatic mutation carriers.

Follow-up
Clinical characteristics at the end of follow-up of the cohort as a whole and of the specific mutations within the SDHD gene are outlined in Table 2. We aimed to explore potential differences in clinical phenotypes related to the different mutations within the SDHD gene. However, we only compared carriers of the SDHD c.274G>T (p.Asp92Tyr) mutation with carriers of the SDHD c. 416T>C (p.Leu139Pro) mutation. The number of carriers of the other mutations within the SDHD gene was small, and they were not included in our analyses because of a lack of statistical power.

There were no significant differences in sex or age at presentation between the two founder mutations: the group of SDHD c.274G>T (p.Asp92Tyr) mutation carriers comprised 79 females (49%) and had a mean age at presentation of 42.6 ± 14.0 years, while the SDHD c. 416T>C (p.Leu139Pro) mutation group comprised 10 females (39%) and had a mean age at presentation of 40.4 ± 14.5 years. The mean duration of follow-up differed significantly between groups and was 6.3 ± 5.6 years and 3.8 ± 4.2 years, respectively (p = 0.006). Equal numbers from each mutation group were lost to follow-up. Our analyses showed that there were no differences in number and location of HNPGLs, sPGLs or PCC, nor in the occurrence of malignant disease, other tumors or the number of asymptomatic mutation carriers between carriers of the SDHD c.274G>T (p.Asp92Tyr) mutation and carriers of the SDHD c. 416T>C (p.Leu139Pro) mutation.

In the whole cohort, 91% of SDHD mutation carriers had developed one or multiple HNPGLs, of which the carotid body tumor was the most prevalent (in 85% of SDHD mutation carriers). About half of all carriers developed a vagal body tumor and a third a jugulotympanic tumor.
Table 2: Clinical phenotypes of specific mutations within the *SDHD* gene

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Total cohort (n = 201)</th>
<th>c.274G&gt;T (n = 162)</th>
<th>c.416T&gt;C (n = 26)</th>
<th>c.284T&gt;C (n = 6)</th>
<th>del promoter, exon 1 and 2 (n = 3)</th>
<th>c.242C&gt;T (n = 2)</th>
<th>c.337_340delGACT (n = 1)</th>
<th>c.34G&gt;A (n = 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HNPGL (%)</td>
<td>183 (91)</td>
<td>147 (91)</td>
<td>25 (96)*</td>
<td>5 (83)</td>
<td>2 (67)</td>
<td>2 (100)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>- 1 HNPGL</td>
<td>-40</td>
<td>-32</td>
<td>-4*</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
<td>0</td>
<td>-1</td>
</tr>
<tr>
<td>- 2 HNPGLs</td>
<td>-52</td>
<td>-43</td>
<td>-7*</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
<td>0</td>
<td>-0</td>
</tr>
<tr>
<td>- ≥ 3 HNPGLs</td>
<td>-91</td>
<td>-72</td>
<td>-14*</td>
<td>-3</td>
<td>-0</td>
<td>-1</td>
<td>1</td>
<td>-0</td>
</tr>
<tr>
<td>CBT (%)</td>
<td>170 (85)</td>
<td>135 (83)</td>
<td>24 (92)*</td>
<td>5 (83)</td>
<td>2 (67)</td>
<td>2 (100)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>- bilateral</td>
<td>-107</td>
<td>-86</td>
<td>-14*</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
<td>1</td>
<td>0</td>
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<tr>
<td>VBT (%)</td>
<td>95 (47)</td>
<td>76 (47)</td>
<td>15 (58)*</td>
<td>3 (50)</td>
<td>0</td>
<td>1 (50)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>- bilateral</td>
<td>-20</td>
<td>-15</td>
<td>-3*</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>JTT (%)</td>
<td>62 (31)</td>
<td>53 (33)</td>
<td>7 (27)*</td>
<td>1 (17)</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>- bilateral</td>
<td>-5</td>
<td>-5</td>
<td>-0*</td>
<td>-0</td>
<td>-0</td>
<td>-0</td>
<td>-0</td>
<td>-0</td>
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<tr>
<td>HNPGL elsewhere (%)</td>
<td>3 (1)</td>
<td>1 (1)</td>
<td>2 (8)*</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>sPGL (%)</td>
<td>15 (7)</td>
<td>11 (7)</td>
<td>4 (15)*</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>PCC (%)</td>
<td>18 (9)</td>
<td>15 (9)</td>
<td>2 (8)*</td>
<td>0</td>
<td>1 (33)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>- bilateral</td>
<td>-1 (6)</td>
<td>-0</td>
<td>-0</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
</tr>
<tr>
<td>Malignant PGL (%)</td>
<td>9 (4)</td>
<td>7 (4)</td>
<td>0*</td>
<td>1 (17)</td>
<td>1 (33)</td>
<td>0</td>
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<td>0</td>
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<tr>
<td>Asymptomatic (%)</td>
<td>16 (8)</td>
<td>13 (8)</td>
<td>1 (4)*</td>
<td>1 (17)</td>
<td>1 (33)</td>
<td>0</td>
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<tr>
<td>Other tumors</td>
<td>29</td>
<td>23</td>
<td>4</td>
<td>0</td>
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<td>1</td>
<td>1</td>
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<tr>
<td>- urogenital&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-6</td>
<td>-4</td>
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<td>-0</td>
<td>-0</td>
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<td>-1</td>
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<tr>
<td>- breast</td>
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<td>-1</td>
<td>-1</td>
<td>-1</td>
<td>-0</td>
<td>-0</td>
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<td>- hemato-oncological&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-4</td>
<td>-4</td>
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<td>-3</td>
<td>-2</td>
<td>-1</td>
<td>-0</td>
<td>-0</td>
<td>-0</td>
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<td>-0</td>
</tr>
<tr>
<td>- brain&lt;sup&gt;e&lt;/sup&gt;</td>
<td>-3</td>
<td>-3</td>
<td>-0</td>
<td>-0</td>
<td>-0</td>
<td>-0</td>
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<td>-0</td>
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<tr>
<td>- gynaecological&lt;sup&gt;f&lt;/sup&gt;</td>
<td>-2</td>
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<td>-0</td>
<td>-0</td>
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<tr>
<td>- melanoma</td>
<td>-2</td>
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<td>-1</td>
<td>-0</td>
<td>-0</td>
<td>-0</td>
<td>-0</td>
<td>-0</td>
</tr>
<tr>
<td>- thyroid&lt;sup&gt;g&lt;/sup&gt;</td>
<td>-1</td>
<td>-1</td>
<td>0</td>
<td>-0</td>
<td>-0</td>
<td>-0</td>
<td>-0</td>
<td>-0</td>
</tr>
</tbody>
</table>
HNPGL = head and neck paraganglioma, CBT = carotid body tumor, VBT = vagal body tumor, JTT = jugulotympanic tumor
sPGL = sympathetic paraganglioma, PCC = pheochromocytoma, PGL = paraganglioma

* no significant difference (p > 0.05) between carriers of the c.274G>T mutation and carriers of the c.416T>C mutation

a urothelial cell carcinoma (2), prostate cancer (3), testicular cancer (1)
b macroprolactinoma (1), growth hormone (GH)-producing pituitary adenoma (1), non-functioning pituitary adenoma (1), neuro-endocrine tumor of the pancreas (1)
c Morbus Kahler (2), Morbus Hodgkin (1), mantle cell lymphoma (1)
d gastrointestinal stromal tumor (GIST) (2), carcinoma of the oesophagus (1)
e meningeoma (2), oligodendroglioma (1)
f endometrial carcinoma (1), cervical cancer (1)
g papillary thyroid cancer
Three persons developed a HNPGL elsewhere in the head and neck region: one HNPGL was located between the right jugular vein and the right thyroid lobe, one left prevertebral, and one in the region of the left thyroid lobe.

Ninety-nine subjects had been operated on for their HNPGLs. Carotid body tumors were resected in 79 persons, vagal body tumors in six persons, jugulotympanic tumors in 29 persons, and HNPGL located elsewhere in the head and neck region in two persons. Six subjects received radiotherapy for their HNPGLs.

During follow-up, fourteen carriers developed sPGLs, located in the mediastinum (two), paraaortic retroperitoneal (five), aortic arch (four), right paraadrenal (two), and bladder (one). Ten persons were carrier of the $SDHD$ c.274G>T (p.Asp92Tyr) mutation and four persons of the $SDHD$ c. 416T>C (p.Leu139Pro) mutation.

All sPGLs were detected via radiological imaging, which was performed in ten persons due to excessive urinary catecholamine secretion and in three persons due to suspect signs or symptoms. In one person, radiological imaging was performed although (symptoms of) catecholamine excess were lacking; the reason for performing radiological imaging could not be deduced from the medical record.

Hypersecretion of norepinephrine was present in eight sPGL patients (57%) and was the only elevated biomarker in three of these patients. Hypersecretion of epinephrine was present in two patients, as was dopamine. Four patients displayed hypersecretion of VMA, which was the only elevated biomarker in one of these patients. In six patients sPGL was diagnosed before 2005 and therefore measurement of (nor)metanephrine and 3-MT was not performed. Of the other six patients, levels of normetanephrine were elevated in three persons, metanephrine in one and 3-MT in four. In three of these patients, 3-MT was the only elevated biomarker.

In twelve subjects, lesions were resected. In one person, the lesion was surgically difficult to assess and considering the small risk of malignant transformation and the lack of catecholamine excess-related symptoms, it was decided not to operate. Another person did not want to have an operation. All resected lesions were pathologically confirmed as PGLs. In four subjects, urinary hypersecretion of catecholamines persisted post-surgery. One of these persons developed metastases. The other three persons were diagnosed with multiple HNPGLs, which could have accounted for the excessive secretion of catecholamines. Two of the three patients in which 3-MT was the only elevated biomarker were operated on. Although these patients were also diagnosed with HNPGLs, 3-MT levels returned to normal after resection of sPGL.

Eighteen carriers developed a PCC, of which ten persons left-sided, seven persons right-sided and one person, a bilateral PCC. Fifteen persons were carrier of the $SDHD$ c.274G>T (p.Asp92Tyr) mutation, two of the $SDHD$ c. 416T>C (p.Leu139Pro) mutation and one had the promoter region, exon 1 and 2 deleted.
In all subjects, radiological imaging was performed upon detection of excessive urinary catecholamine secretion. Most tumors (83%) had a noradrenergic phenotype. None of the PCC patients displayed elevated levels of epinephrine. Dopamine was elevated in 5 patients and VMA in 11 patients. Of the 10 patients diagnosed with PCC after 2005, four displayed elevated levels of normetanephrine, one of metanephrine and five of 3-MT, which was the only elevated biomarker in two of these patients.

All lesions were resected and pathologically confirmed as PCC. Postoperatively, urinary hypersecretion of catecholamines persisted in nine subjects. These persons were also diagnosed with multiple HNPGLs, which might have accounted for the catecholamine excess. Nine carriers (4%) were diagnosed with malignant PGL, i.e. metastatic disease (Table 3). Since six patients suffered from multiple PGLs, the primary tumor was not unambiguously identifiable in these patients. The mean interval between diagnosis of first PGL and metastatic disease was 9.6 ± 11.6 (range 0-30) years, and metastatic disease was already apparent at time of first PGL-diagnosis in two patients. Sites of metastases included bone (67% of patients), lymph nodes (78%), lung (22%) and liver (11%). Therapy varied between patients, with a “wait-and-scan” policy in two patients lacking symptoms of metastatic disease. Two patients died from progressive disease.

Twenty-nine other tumors were reported in twenty-five carriers, of which urogenital tumors were the most frequent. Four persons were diagnosed with other neuro-endocrine tumors, i.e. a macroprolactinoma, a growth hormone (GH)-producing pituitary adenoma, a non-functioning pituitary adenoma and a neuro-endocrine tumor of the pancreas. Furthermore, four breast cancers, four hemato-oncological tumors (two Morbus Kahler, one Morbus Hodgkin and one mantle cell lymphoma) and three brain tumors (two meningeomas and one oligodendroglioma) were diagnosed. Three carriers suffered from gastro-intestinal malignancies; two persons were diagnosed with a gastrointestinal stromal tumor (GIST) and one with a carcinoma of the oesophagus. Two carriers suffered from gynecologic malignancies and two from melanoma. One carrier was diagnosed with a papillary thyroid cancer.

At the end of follow-up, sixteen SDHD mutation carriers (8%) displayed no evidence of manifest disease, as determined after biochemical and radiological screening for PGLs, i.e. they were asymptomatic mutation carriers.
Table 3: Malignant paragangliomas

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>SDHD mutation</th>
<th>Location PGL</th>
<th>Years since first diagnosed PGL</th>
<th>Location metastases</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>c.274G&gt;T</td>
<td>Right and left CBT, left VBT/JTT</td>
<td>30</td>
<td>Bone, lymph nodes</td>
<td>Radiotherapy, octreotide (intramuscular injections)</td>
<td>Died from malignant PGL at age 59 (2.5 years after diagnosis of malignant PGL)</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>c.274G&gt;T</td>
<td>Mediastinal PGL</td>
<td>0</td>
<td>Bone, lymph nodes</td>
<td>Radiotherapy, $^{131}$I-MIBG therapy</td>
<td>Alive at age 73 (7 years after diagnosis of malignant PGL)</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>c.274G&gt;T</td>
<td>Right and left CBT, right VBT and JTT, left PCC</td>
<td>18</td>
<td>Bone, lung</td>
<td>Wait-and-scan; exceptionally slow progression of disease and patient reports no symptoms</td>
<td>Alive at age 72 (19 years after diagnosis of malignant PGL)</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>c.274G&gt;T</td>
<td>Right and left CBT, left VBT and JTT</td>
<td>2</td>
<td>Lymph node</td>
<td>Resection of lymph node</td>
<td>Alive at age 41 (14 years after diagnosis of malignant PGL)</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>c.274G&gt;T</td>
<td>Right and left CBT, left VBT</td>
<td>24</td>
<td>Lymph nodes</td>
<td>Resection of lymph nodes, lutetium octreotate therapy</td>
<td>Alive at age 58 (2 years after diagnosis of malignant PGL)</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>del promotor, exon 1</td>
<td>Left JTT, right paracaval PGL</td>
<td>5</td>
<td>Bone</td>
<td>Wait-and-scan; patient reports no symptoms</td>
<td>Alive at age 71 (14 years after diagnosis of malignant PGL)</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>c.274G&gt;T</td>
<td>PGL bladder, left VBT</td>
<td>0</td>
<td>Bone, lymph nodes, lung, liver</td>
<td>Radiotherapy, $^{131}$I-MIBG therapy, lutetium octreotate therapy</td>
<td>Died from malignant PGL at age 43 (2 years after diagnosis of malignant PGL)</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>c.284T&gt;C</td>
<td>Right CBT</td>
<td>1</td>
<td>Lymph node</td>
<td>Resection of lymph node</td>
<td>Alive at age 19 (2.5 years after diagnosis of malignant PGL)</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>del promotor, exon 1</td>
<td>Left CBT</td>
<td>6</td>
<td>Bone, lymph nodes</td>
<td>Chemotherapy (cyclophosphamide, vincristine, dacarbazine)</td>
<td>Alive at age 56 (2 years after diagnosis of malignant PGL)</td>
</tr>
</tbody>
</table>
and 2 however development of renal failure: treatment continued with radiotherapy

F = female, M = male, PGL = paraganglioma, CBT = carotid body tumor, VBT = vagal body tumor, JTT = jugulotympanic tumor, JTT = jugulotympanic tumour, PCC = pheochromocytoma

$^{131}$I-MIBG = $^{131}$iodine-metaiodobenzylguanidine
Discussion

The aim of the present study was to assess potential differences in the clinical expression of specific SDHD mutations in a large Dutch cohort of SDHD mutation carriers. However, comparison of the clinical phenotypes of the two most frequent mutations within the SDHD gene in our cohort, the p.Asp92Tyr and the p.Leu139Pro mutation, revealed no significant differences. In our study, the mean duration of follow-up was 5.8 years. A more extended follow-up would fully confirm that no differences are apparent in phenotypic outcome between the two specific SDHD mutations.

At the end of follow-up, ninety-one percent of included SDHD mutation carriers had developed one or multiple HNPGLs. This figure is slightly higher in previous studies assessing genotype-phenotype relations in SDHD mutation carriers, i.e. 79% and 89%.\textsuperscript{10,11} Since SDHD mutations show an age-related penetrance,\textsuperscript{10,11} this small difference may be explained by the fact that our cohort was older and was followed for a longer period than in the previous two studies. Furthermore, the LUMC is a national referral center for patients with HNPGLs; hence a referral bias may be operating.

Nine percent of carriers in our study developed a PCC and 7% a sPGL. Widely varying but substantially higher figures have been reported in literature, with 7% and 53% reported for SDHD mutation carriers developing PCC and 29% and 38% for sPGL development.\textsuperscript{10,11} Again, the LUMC is a national referral center for patients with HNPGLs which may have led to a high prevalence of HNPGLs and a low prevalence of PCC/sPGL. However, the fact that our cohort displayed a low prevalence of PCC/sPGL despite a regular complete endocrinological work-up during multiple years of follow-up may point towards a lower association of the Dutch founder mutations with PCC/sPGL.

In all cases of PCC in our cohort, hypersecretion of catecholamines was present, although catecholamine-related complaints were absent in four patients with PCC. As a result of catecholamine excess, radiological imaging was performed and PCC detected. This stresses the importance of screening all SDHD mutation carriers for the presence of catecholamine excess at regular intervals, regardless of catecholamine-related complaints.

In PCC, elevations of norepinephrine and VMA were found most frequently. Eight patients were diagnosed with PCC before 2005, prior to urinary measurements of normetanephrine and metanephrine (‘metanephrines’), the O-methylated metabolites of norepinephrine and epinephrine, being established in our department. These tests, added from 2005 onwards, provide excellent detection of PCC with a sensitivity of > 97%. Metanephrines have a longer half life and are produced continuously within tumor cells, whereas catecholamines are converted to metanephrines by the high methyltransferase activity of chromaffin tissue.\textsuperscript{22} However, as two patients displayed hypersecretion of norepinephrine without elevated metanephrines and one patient showed hypersecretion of VMA, we believe it is of value to
continue measuring urinary catecholamines and VMA, despite lower sensitivities (86% and 64%, respectively).\textsuperscript{22}

Nine patients with PCC had elevated levels of urinary dopamine and/or its $O$-methylated metabolite 3-MT. It is worth noting that all these patients also had HNPGLs in situ, since these tumors are associated with increased levels of dopamine and/or 3-MT.\textsuperscript{23}

In two patients, 3-MT was the only elevated biomarker. Since these patients were also diagnosed with HNPGLs and hypersecretion of 3-MT persisted after resection of PCC, we cannot exclude the fact that the HNPGLs were responsible for 3-MT hypersecretion and that both PCCs were detected in an early, non-functioning state. In three of the 14 patients diagnosed with sPGL during follow-up, 3-MT was the only elevated biomarker. Although these patients were also diagnosed with HNPGLs, 3-MT levels returned to normal after resection of sPGL in two patients, indicating that sPGLs were responsible for 3-MT hypersecretion. Therefore, we would recommend the incorporation of the routine measurement of 3-MT to the biochemical screening of SDHD mutation carriers.

Four percent of the carriers in our cohort developed malignant PGL. This is in line with a recent systematic review and meta-analysis, which showed that the pooled risk of developing malignant PGL in prevalence-studies comprising both asymptomatic SDHD mutation carriers and SDHD mutation carriers with manifest non-malignant PGL was 4%.\textsuperscript{14} Malignant PGL-related clinical behavior and survival was highly variable. Two patients had a CBT as primary tumor and one a mediastinal PGL. As six patients were diagnosed with multiple PGLs, it was not possible to define the primary tumor in these cases.

Two patients died from malignant disease within 30 months of diagnosis, while another patient is still alive 19 years after diagnosis. The prognosis in malignant PGL is difficult to predict, but is known to be poor. The primary management of patients with malignant PGLs should focus on complete surgical resection of the primary tumor and local or distant metastases.\textsuperscript{24,25} In HNPGL patients, postoperative radiotherapy may slow the progression of residual disease.\textsuperscript{24} Systemic treatment options include radionuclide therapy with $^{131}$I-MIBG or somatostatin analogues and combination chemotherapy of cyclophosphamide, vincristine and dacarbazine.\textsuperscript{26-28} More recently, studies assessing targeted therapies, such as sunitinib, have shown promising results.\textsuperscript{29}

Several additional tumor types were diagnosed in twenty-five carriers (12%). One person, a carrier of the c.274G>T SDHD mutation, was diagnosed with acromegaly due to a GH-producing pituitary adenoma. The presence of a GH-producing pituitary adenoma in a patient with a c.298_301delACTC SDHD mutation and multiple PGLs, which also displayed loss of heterozygosity (LOH) for the SDHD genetic locus, was recently described by Xekouki \textit{et al.}\textsuperscript{30} Another person in our cohort was diagnosed with a non-functioning pituitary adenoma and a third with a macroprolactinoma. Associations between these tumors and PGLs have been reported in literature,\textsuperscript{31-32} although not specifically in association with SDHD mutations.
Two carriers in our cohort were diagnosed with GIST and one with papillary thyroid cancer. The dyad of PGL and GIST, known as ‘Carney-Stratakis syndrome’, has been associated with SDHD mutations,33-35 as has the occurrence of papillary thyroid cancer,11 although a causative relation by genetic studies assessing potential loss of SDHD expression in tumor tissue has not been confirmed. Furthermore, these other cases concerned different specific SDHD mutations than present in our cohort. In our patients, LOH for the SDHD genetic locus was also not tested. Further research is needed to explore if these tumors occur as a consequence of loss of SDHD expression.

In conclusion, the two main Dutch SDHD founder mutations do not differ in clinical expression. Our study confirms previous findings that SDHD mutations are associated with the development of multiple HNPGLs and benign disease. Screening for catecholamine excess can result in the detection of pheochromocytomas and sPGL.
Reference List


