CLINICAL STUDY

Increased prevalence of catecholamine excess and phaeochromocytomas in a well-defined Dutch population with *SDHD*-linked head and neck paragangliomas

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Abstract

Objective: The aim of this study was to identify the prevalence of catecholamine excess and phaeochromocytomas in a well-defined population of people with hereditary head and neck paragangliomas.

Methods: We studied in a prospective follow-up protocol all consecutive patients referred to the Department of Endocrinology, Leiden University Medical Center, Leiden, The Netherlands with documented head and neck paragangliomas and either a positive family history for paragangliomas or a proven *SDHD* gene mutation. Initial analysis included medical history, physical examination and the measurement of excretion of catecholamines in two 24-h urine collections. In the case of documented catecholamine excess iodinated meta-iodobenzylguanidine (¹²³I-MIBG) scintigraphy and magnetic resonance imaging were done.

Results: Between 1988 and 2003, 40 consecutive patients (20 male and 20 female) with documented head and neck paragangliomas were screened. Biochemical screening revealed urinary catecholamine excess in 15 patients (37.5%). In nine of these 15 patients a lesion was found by ¹²³I-MIBG scintigraphy. Exact localization by magnetic resonance imaging revealed phaeochromocytomas in seven of the 15 patients. One of the nine patients had an extra-adrenal paraganglioma. Histopathological examination in a subset of tumors displayed loss of heterozygosity of the wild-type *SDHD* allele in all cases.

Conclusions: The prevalence of catecholamine excess (37.5%) and phaeochromocytomas (20.0%) is high in patients with familial head and neck paragangliomas. Therefore, patients with hereditary head and neck paragangliomas require lifelong follow up by biochemical testing for catecholamine excess.

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Introduction

Paragangliomas are rare hypervascular tumors originating from neural-crest-derived paraganglia that are associated with the autonomic nervous system. These paragangliomas can develop anywhere from the neck to the pelvis in locations parallel to the ganglion chain. Normal paraganglia can excrete catecholamines in response to stress or act as chemical sensors around blood vessels (1).

Paragangliomas traditionally have been divided into two separate categories with respect to anatomical location: tumors that are located in the head-and-neck region and tumors situated in thorax and abdomen (1). In cases where the adrenal medulla is affected they are referred to as phaeochromocytomas. These two categories of paraganglioma also demonstrate

different clinical courses. Paragangliomas located in the head and neck most often lack excessive secretion of catecholamines and are mainly detected by the effects of tumor growth. They are non-chromaffin tumors derived from the parasympathetic system (1–3). On the other hand, phaeochromocytomas may cause paroxysmal symptoms and hypertension due to the excessive secretion of catecholamines (4).

In general, head and neck paragangliomas within a hereditary context were reported in about 10–25% of the cases (5, 6). Furthermore, in patients with a positive family history the tumors were more often found to be multicentric and, in 30% of cases, bilateral as opposed to 5% in sporadic cases (6, 7).

Recently, the major susceptibility genes in hereditary paragangliomas have been identified. Germline mutations were discovered in the genes coding for

subunits of mitochondrial complex II succinate dehydrogenase (SDH); *SDHD* (hereditary paraganglioma type 1 (PGL1)) at chromosome 11q23, *SDHC* (PGL3) at chromosome 1q21 and *SDHB* (PGL4) at chromosome 1p36 (8). In several studies loss of heterozygosity (LOH) of wild-type *SDHD* and *SDHB* alleles in paragangliomas and pheochromocytomas could be demonstrated, implicating these *SDH* genes as tumor-suppressor genes (9–11). Another PGL locus, mapped to chromosome 11q13 (PGL2) in a single Dutch family, remains to be characterized further (12).

A large, multibranch Dutch family with hereditary head and neck paragangliomas in the central western region of the Netherlands was identified previously. Using this pedigree a single ancestral mutation was deemed to be responsible for most hereditary paragangliomas in this region in the Netherlands (13, 14). While seeing all these patients for a protocolized endocrinological screening, we suspected an increased prevalence of catecholamine excess or pheochromocytomas. In addition, several studies have presented data on the prevalence of *SDH* gene mutations among familial and sporadic head and neck paragangliomas and pheochromocytomas, showing significantly more mutations among familial cases (15, 16). Previous reports in the literature demonstrated an increased prevalence of pheochromocytomas among patients with *SDHD*-linked head and neck paragangliomas of only approximately 5% (13). However, this prevalence was based on cases ascertained by medical history only and, therefore, the true prevalence may have been underestimated. The aim of this study was to assess the prevalence of pheochromocytomas in a well-defined population of patients with hereditary head and neck paragangliomas due to a mutation in

the *SDHD* gene. In applicable cases where the index tumor was resected, LOH for the *SDHD* gene in the tumors was investigated.

Materials and methods

This study focuses on the association of pheochromocytomas with familial head and neck paragangliomas due to germline mutations in the *SDHD* gene. For this purpose a prospective follow-up study protocol was used. We analyzed all patients referred to our endocrine clinic at the Leiden University Medical Center with documented head and neck paragangliomas and either a proven positive family history or a proven mutation of the *SDHD* gene since 1 January 1988. We used a protocolized diagnostic approach aimed at detecting catecholamine excess (Fig. 1). *SDHD*-mutation analysis was performed as described previously (14). These patients were part of a large cohort of patients identified with head and neck paragangliomas by the Department of Otorhinolaryngology, Leiden University Medical Center, Leiden, The Netherlands. There was no selection for referral based on clinical signs or symptoms compatible with pheochromocytomas or catecholamine excess or a family history of pheochromocytomas. Our center serves as a tertiary referral center for most of the western part of the Netherlands with frequent referral from regional hospitals. The treatment of hereditary paragangliomas has been centralized in Leiden.

The initial consultation included a thorough medical history, focusing on paroxysmal symptoms of palpitations, headaches and diaphoresis. In addition, the medical and family history was evaluated and all medications were listed. All patients received a full

Figure 1 Flowchart of protocolized endocrinological screening of referred patients with head and neck paragangliomas and/or a *SDHD* (PGL1) germline mutation.

physical examination, focusing especially on hypertension, tachycardia and the presence of paragangliomas.

After this consultation, patients were instructed to collect urine for 24 h on two separate occasions. Drugs known to interfere with the assays were discontinued 4 weeks prior to collection or, if necessary, replaced. Doxazosin (alpha-blocker) was the only anti-hypertensive drug, allowed for 4 weeks prior to collection of the urine. In addition, patients were instructed to follow a diet and to avoid strenuous exercise on days the 24-h samples were collected (17). These samples were used for measurement of 24-h urinary excretion of catecholamines (epinephrine, norepinephrine and dopamine) and vanillylmandelic acid (VMA). In order to ascertain adequacy of collection the urinary creatinine content was assessed as well (18). Epinephrine, norepinephrine and dopamine in urine were detected by reversed-phase HPLC with an electrochemical detector. Inter-assay coefficients of variation (CVs) for epinephrine were 3.4–28.4%, ranging from high to low levels. For norepinephrine these data were 3.9–7.7% and for dopamine 3.4–7.3%. The intra-assay CVs are slightly lower. VMA in urine was measured by HPLC with fluorometric detection. Here, the inter-assay CVs ranged from 2.4 to 9.1% with also slightly lower values for the intra-assay CV (19, 20). The levels above which the 24-h urinary excretion of catecholamines was determined to be increased were: epinephrine, 0.16 µmol/24 h (29 µg/24 h); norepinephrine, 0.47 µmol/24 h (80 µg/24 h); dopamine, 3.40 µmol/24 h (520 µg/24 h); VMA, 30 µmol/24 h (6 µg/24 h). These cut-off values were based on studies in which 24-h urine specimens were assessed for both patients with a proven phaeochromocytoma and patients referred for investigation, but subsequently found not to have a phaeochromocytoma. In case only marginally elevated catecholamine levels were found, the test was repeated (20).

Once the above-mentioned biochemical testing revealed an excess of catecholamine production, additional diagnostic tests were performed. For patients who did not demonstrate elevated urinary levels of catecholamines or their metabolites no additional tests were ordered. After biochemical confirmation of catecholamine excess had been found, iodinated metaiodobenzylguanidine (^{123}I -MIBG) scintigraphy was performed. Imaging was performed 24 h after the intravenous administration of 185 MBq ^{123}I -MIBG (Amersham, Eindhoven, The Netherlands). Whole-body scintigraphy was done with a dual-head gamma camera (Toshiba GCA 7200; Toshiba, Tokyo, Japan), which was followed by single-photon-emission computed tomography (SPECT) of the head and neck region and of the upper abdomen. All images were analyzed visually by two experienced nuclear physicians. This strategy was chosen to avoid missing possible extra-adrenal phaeochromocytomas by an initial radiological localization technique such as magnetic

resonance imaging (MRI). Next to ^{123}I -MIBG scintigraphy, MRI was performed to further localize a possible phaeochromocytoma. A 1.5 T whole-body scanner was used (Gyrosan ACS/NT15 and Intera; Philips, Best, The Netherlands). In case this additional radiological localization showed a lesion suspected to be a phaeochromocytoma, surgery was performed, enabling a confirmation of the diagnosis by histology. During this time, analysis of the head and neck paragangliomas was performed by the Department of Otorhinolaryngology, Leiden University Medical Center. Routine follow-up examinations were performed every 2 years.

LOH analysis

From all patients that were operated upon for their index tumor, available tumor specimens were retrieved from the archives of the Department of Pathology of the Leiden University Medical Center. Tissue specimens were archived and retrieved according to the guidelines of the Dutch Federation of Research Associations (FEDERA).

Only frozen tumor specimens were selected for DNA flow-cytometry analysis as described previously (21). Prior to analysis selected cases were reviewed histologically and tumor percentages in the samples were determined through microscopic examination of 4 µm serial sections. Additional flow sorting was performed with aneuploid and tetraploid tumors based on propidium content on a FACStar flow cytometer (Becton Dickinson Immunocytometry Systems, San José, CA, USA) as described in (9). A minimum of 10 000 nuclei were sorted in microfuge tubes. From available peripheral blood samples, diploid (unsorted) tumors, and sorted aneuploid cases, subsequent DNA isolation was performed as described in (22). Isolated DNA was screened for the Dutch *SDHD* germline mutation Asp⁹² → Tyr (D92Y) by restriction digestion as described in (14). The presence of *SDHD* mutations and allelic imbalance (retention or loss) of the wild-type gene was determined through examination of digestion patterns. LOH was considered present when the ratio between mutant and wild-type alleles was larger than 2, according to restriction intensity measurements. DNA extracted from peripheral blood samples from a patient with confirmed mutations and a non *SDHD*-linked patient served as control samples to validate the PCR process and restriction-digestion analysis.

The software package used for statistical analysis was SPSS for Windows version 10.0.5 (23). The results of statistical tests were considered significant at a level of $P < 0.05$.

Results

Between 1988 and 2003, 40 consecutive patients (20 male and 20 female) with documented head and neck paragangliomas were referred to our endocrine clinic

Table 1 Patients' characteristics (*n* = 40).

	Number (%)
Age (mean)	45.8 years (S.D. $\pm$ 11.8)
Gender	Male, <i>n</i> = 20; female, <i>n</i> = 20
<i>SDHD</i> gene mutation ascertained in case	<i>n</i> = 26 (65.0)
<i>SDHD</i> gene mutation ascertained in family	<i>n</i> = 7 (17.5)
Familial head and neck paragangliomas	<i>n</i> = 39 (97.5)
Type of head and neck paraganglioma	
Glomus caroticum	<i>n</i> = 34 (85.0)
Glomus vagale	<i>n</i> = 19 (47.5)
Glomus jugulotympanicum	<i>n</i> = 13 (32.5)
Multiple head and neck paragangliomas	<i>n</i> = 20 (50)
Follow-up duration (mean)	5.7 years (S.D. $\pm$ 7.8 years; median, 2 years)
Paroxysmal symptoms	<i>n</i> = 12 (30)
History of hypertension	<i>n</i> = 10 (25)
Hypertension	<i>n</i> = 26 (65)

for a protocolized endocrinological screening. The patient characteristics are summarized in Table 1. All but one reported familial cases of head and neck paragangliomas, whereas the *SDHD* gene mutation was ascertained in 26 individuals (25 with the D92Y mutation and one with a Leu¹³⁹ $\rightarrow$ Pro mutation), and documented to be present within a family member in another seven cases. The one patient without a positive family history for head and neck paragangliomas was found positive for the *SDHD* gene

mutation. The total patient population included members from 24 separate families.

Half of the patients observed in this study were found to have multiple head and neck paragangliomas. The most common types of paraganglioma were carotid body tumors (85%) and vagal body tumors (47.5%; Table 1).

A thorough medical history revealed actual paroxysmal symptoms of palpitations, headaches and diaphoresis in 12 patients (30%). Furthermore, 10 patients were previously diagnosed with hypertension and seven of those 10 patients were actually using anti-hypertensive medication. During physical examination another 16 patients were found to have a blood pressure of $\geq$ 140/90 mmHg.

The results of the protocolized screening of the patients are shown in Fig. 2. After collection of two 24-h urinary samples 17 patients (42.5%) were categorized as having catecholamine excess. The urinary tests were normal in 23 patients. All but three patients identified with increased catecholamine excretion were analyzed by ¹²³I-MIBG scintigraphy. In two of these three patients the first measurement yielded only slightly increased values (single norepinephrine measurements of 0.49 and 0.51 μ g/24 h) and repeated measurements revealed normal excretion of catecholamines. In a third patient catecholamine levels were found to have normalized after removal of the head and neck paragangliomas. The remaining 14 patients with increased 24-h urinary catecholamine levels underwent ¹²³I-MIBG scintigraphy.

Figure 2 Flowchart with results of endocrinological screening of referred patients with head and neck paragangliomas and/or a *SDHD* (PGL1) germline mutation.

The results of ^{123}I -MIBG scintigraphy showed a normal distribution in five patients. Four of these patients were found to have no abdominal lesions on MRI suggestive of pheochromocytoma. Three of the four patients analyzed by MRI of the abdomen previously underwent (subtotal) surgery for head and neck paragangliomas (one with bilateral paragangliomas was operated on only unilaterally), but postoperatively continued to show an increased 24-h urinary excretion of catecholamines. Another patient had normalization of the 24-h urinary samples after surgery.

In nine patients with catecholamine excess a lesion on ^{123}I -MIBG scintigraphy was found in the abdomen. MRI investigation revealed a pheochromocytoma in seven patients, which was confirmed histopathologically after surgical removal of the lesion. One patient was discovered with an extra-adrenal paraganglioma. In only one patient did MRI scanning fail to reveal a pheochromocytoma. In this particular patient the increased catecholamine was dopamine.

Thus, in 15 of the 40 patients included (37.5%), sustained urinary catecholamine excess was detected. In six of these 15 patients ^{123}I -MIBG scintigraphy and MRI of the abdomen could not demonstrate a pheochromocytoma and in one patient the excess did subside after removal of the head and neck paragangliomas, after which no further tests were performed. This indicates that head and neck paragangliomas may be responsible for excess catecholamine production in about 17.5% of the patients included in the study ($n = 7$). Although quantitative assessment of catecholamine secretion in 24-h urine samples has a specificity of between 69 and 95%, it seems unlikely that false-positive samples explain the persistent excess catecholamine production (24). Another eight patients (20.0%) were diagnosed with a pheochromocytoma, including one patient with an extra-adrenal tumor.

In a univariate analysis no distinctive parameters could be demonstrated identifying either the 15 patients with catecholamine excess or the eight patients that were diagnosed with (extra-adrenal) pheochromocytoma (Table 2). However, there was a striking,

but not significant, difference in follow-up duration between patients with and without the presence of a pheochromocytoma (10.4 ± 9.3 s.d. versus 6.4 ± 7.1 s.d. years). Indeed, some patients were diagnosed with head and neck paragangliomas early, allowing more biennial visits for screening of catecholamine excess.

LOH analysis

Suitable frozen tumor specimens were retrieved from four of 15 patients with catecholamine excess that were detected after screening. The selected specimens included two adrenal pheochromocytomas, one extra-adrenal pheochromocytoma and one jugulotympanic paraganglioma. Additionally, one extra-adrenal pheochromocytoma was retrieved from a member of a PGL1 family that was not included in the initial patient selection (tumor 5). The clinicopathological characteristics of the tumors are listed in Table 3. All tumors were clinically benign. Histologically the pheochromocytomas displayed proliferations of chief cells embedded in a highly vascularized stroma. The jugulotympanic tumor was organized in a typical Zellballen pattern with clusters of chief cells and sustentacular cells located at the periphery. Flow cytometric analysis revealed aneuploid populations in three tumors and tetraploidy in two tumors. From the aneuploid and tetraploid tumors, different cell populations were subsequently isolated by flow sorting based on their DNA content. Mutation analysis of peripheral blood samples revealed the D92Y founder mutation in exon 3 of the *SDHD* gene in all cases. Subsequent restriction-digestion analysis of peripheral blood samples and sorted tumor fractions displayed LOH of the wild-type *SDHD* allele in all tumors examined (Fig. 3).

Discussion

This is the first study specifically addressing the prevalence of catecholamine excess and pheochromocytomas in a well-defined population with head and neck paragangliomas. In 37.5% of the examined

Table 2 Univariate analysis of possible distinctive parameters for catecholamine excess or (extra-adrenal) pheochromocytomas.

Parameter	Catecholamine excess		Pheochromocytomas	
	Present ($n = 15$)	Absent ($n = 25$)	Present ($n = 8$)	Absent ($n = 32$)
Age (years)	46.4 (s.d. ± 12.8)	47.2 (s.d. ± 11.2)	49.8 (s.d. ± 13.7)	46.2 (s.d. ± 11.2)
Gender (male/female)	9/6	11/14	5/3	15/17
Duration of follow-up (years)	8.7 (s.d. ± 8.2)	6.3 (s.d. ± 7.3)	10.4 (s.d. ± 9.3)	6.4 (s.d. ± 7.1)
Paroxysmal symptoms	4 (26.7%)	8 (32.0%)	1 (12.5)	11 (34.4%)
History of hypertension	3 (20%)	7 (28.0%)	2 (25.0%)	8 (25.0%)
Hypertension	11 (73.3%)	15 (60.0%)	6 (75.0%)	20 (62.5%)
Type of head and neck paraganglioma				
Glomus caroticum	12 (80.8%)	22 (88.0%)	7 (87.5%)	27 (84.4%)
Glomus vagale	6 (40.0%)	13 (52.0%)	4 (50.0%)	15 (46.9%)
Glomus jugulotympanicum	5 (33.3%)	8 (32.0%)	2 (25.0%)	11 (34.4%)

Table 3 Clinicopathological characteristics of tumors selected for LOH analysis. JJT, phaeo, eap, jugulotympanic tumor, phaeochromocytoma, extra-adrenal phaeochromocytoma; a, d, t, aneuploid, diploid, tetraploid; D92Y, *SDHD* germline mutation exon 3; r-d analysis, restriction-digestion analysis (see text); LOH, loss of heterozygosity (see text).

Tumor	Location	Ploidy	Tumor (%)	Mutation	r-d analysis
1	JJT	a	70	D92Y	LOH
2	eap	t	70	D92Y	LOH
3	phaeo	a	70–100	D92Y	LOH
4	phaeo	a	40	D92Y	LOH
5	eap	t	60	D92Y	LOH

Figure 3 Loss of wild-type *SDHD* allele in sorted tumor fraction from an extra-adrenal pheochromocytoma. After PCR amplification of exon 3, digestion was performed with restriction enzyme *RsaI* to detect the Asp92 → Tyr mutation. The wild-type allele contains two restriction sites that will result in three fragments of 23, 106 and 334 bp after digestion (the smallest is not detectable). The mutation creates a third restriction site within the 334 bp fragment resulting in two additional fragments of 202 and 132 bp. The digest from the diploid population shows a wild-type and mutant allele. In the sorted tumour fraction, loss of the wild-type allele is observed. LOH occurs in this fraction since the ratio between the mutant and wild-type alleles is larger than 2 according to restriction-fragment intensity measurements (see text). C, control digestion obtained from a peripheral blood sample with confirmed Asp92 → Tyr mutation; D, normal (diploid) DNA obtained from peripheral blood sample of patient; T, DNA from sorted tetraploid tumor fraction; W and M, wild-type and mutant fragments. In some fractions additional fragments are present due to incomplete digestion.

patients, the catecholamine levels were elevated and a phaeochromocytoma was detected in 20% of the patients. Therefore, this high prevalence of catecholamine excess and phaeochromocytomas is in contrast with those in previous observations (13, 25–27). Furthermore, it is likely that the increased prevalence of phaeochromocytomas documented in the present study is a minimal estimate. As the protocol is repeated every 2 years more patients with catecholamine excess are expected to arise. Therefore, it is likely that longer duration of follow up will also reveal a higher prevalence of phaeochromocytomas.

We studied a specific population of Dutch patients with head and neck paragangliomas due to a *SDHD* mutation, as the prevalence of catecholamine in these patients was suspected to ascend above the previously mentioned prevalence of about 5% (13). In our study 82.5% of patients were found to have a positive family history for head and neck paraganglioma and/or were diagnosed with a *SDHD* gene mutation. The remaining 17.5% only had a positive family history for head and neck paraganglioma and refused genetic testing for different reasons. Since *SDHD* mutations explain the inheritance of head and neck paraganglioma in 97% of Dutch families, we did not exclude these patients from our analysis (14). In addition, we found that in the seven patients without ascertainment of the *SDHD* mutation only two had catecholamine excess and none were diagnosed with a phaeochromocytoma. Therefore, excluding these patients from the analysis will only strengthen the association between hereditary head and neck paragangliomas with a proven *SDHD* mutation and the prevalence of phaeochromocytomas.

Subsequent genetic analysis of extirpated tumors revealed LOH of the wild-type *SDHD* gene in all investigated cases. This finding strongly implicates a role for *SDHD* as a tumor-suppressor gene in the tumorigenesis of phaeochromocytoma as has been described previously in the development of head and neck paragangliomas (9, 28). However, due to the low prevalence of phaeochromocytomas in relation to the prevalence of head and neck paragangliomas in families with *SDHD* gene mutations, one may hypothesize that additional mutations are required for the development of meta- or synchronous phaeochromocytomas in addition to head and neck paragangliomas (29).

A protocolized diagnostic strategy was used to evaluate all patients referred to the endocrinology clinic with documented head and neck paragangliomas and either a positive family history for paragangliomas or a proven *SDHD* gene mutation. Patients were referred to the Department of Endocrinology, Leiden University Medical Center for routine consultation, irrespective of the results of medical history and physical examination by the referring physician. This is further demonstrated by the absence of a clear correlation between catecholamine excess and presenting signs and symptoms.

The longer follow-up time of patients diagnosed with a phaeochromocytoma cannot therefore be explained merely by referral bias. A possible explanation for the observed difference in follow-up may be the time needed for patients to develop a phaeochromocytoma during their life. Possibly, head and neck paragangliomas are detected earlier in life as they are located superficially, whereas phaeochromocytomas are not detectable during physical examination and further analysis through urine sampling is needed to obtain the correct diagnosis. Furthermore, as mentioned above, additional mutations may be required for a patient to develop phaeochromocytomas in addition to head and neck paragangliomas.

Although current standards for biochemical diagnosis of a phaeochromocytoma may dictate a biochemical screening for suspected cases of phaeochromocytomas with plasma free metanephrine and urinary metanephrine levels, these tests are currently unavailable in our laboratory. In particular, plasma free metanephrines have shown sensitivity and specificity of 97% and 96%, respectively, in hereditary phaeochromocytomas (24, 30). However, urinary free catecholamines were found to have a sensitivity of more than 90% (4, 31). Although VMA measurement in urine samples demonstrates a low sensitivity, the specificity is very high with 99% among hereditary cases of phaeochromocytomas (24). We used a temporized approach, starting with the measurement of urinary free catecholamines and VMA. This biochemical evaluation was then followed by MIBG scintigraphy and MRI of the abdomen, allowing for adequate diagnosis. Urinary sampling confirmed the diagnosis of catecholamine excess, whereas anatomical identification of (extra-) adrenal phaeochromocytomas was achieved by MIBG scintigraphy and MRI. Especially in hereditary phaeochromocytomas, MIBG scintigraphy has a high specificity, almost 100%, with a sensitivity approximating 90%, whereas MRI scanning has a higher sensitivity of almost 100%, in particular for extra-adrenal phaeochromocytomas (32–34). Combination of both diagnostic techniques yields the most information. Since the strategy used in this protocol for biochemical testing may be less sensitive we might have underestimated the true prevalence of catecholamine excess. The one patient that was identified as having an increased urinary excretion of dopamine was later found to have a positive MIBG scan, but a negative MRI. A possible explanation may be the fact that dopamine is less specific in detecting phaeochromocytomas, as it is found to be more widespread in the body. An accumulation of dopamine will then appear on MIBG scanning, but not on MRI, as there is no actual phaeochromocytoma. In addition, we cannot exclude the possibility of the presence of adrenal tumors in the patients with negative biochemical screening of catecholamine excess, since we did not perform visualization studies in those patients. However, this possibility does not

invalidate our conclusions with respect to the high prevalence of phaeochromocytomas.

Remarkably, the likelihood of catecholamine excess cannot be predicted by signs and symptoms. This observation is in concordance with previous reports in the literature. Generally phaeochromocytomas are described as a distinct clinical entity, but in clinical practice the symptoms may be misleading or even absent, as is known in other familial syndromes with phaeochromocytomas such as multiple endocrine neoplasia type 2, Von Hippel Lindau's disease and neurofibromatosis 1 (30). Apparently, phaeochromocytomas are detected in an earlier phase in those genetic syndromes by biochemical screening, prior to development of the classical signs and symptoms of phaeochromocytomas. Therefore, in these groups of patients biochemical testing is obligatory. The warning symptoms of catecholamine excess may frequently be lacking, because the observed prevalence of phaeochromocytomas at autopsies is increased almost 500-fold compared with that in the general living population (35, 36). In accordance, even in our study, which focused on the detection of phaeochromocytomas, patients experienced hardly any signs or symptoms classically associated with phaeochromocytomas. Therefore in many cases phaeochromocytomas may not be detected merely from signs and symptoms.

The increased prevalence of catecholamine excess and phaeochromocytomas has grave consequences for the future follow up of patients with head and neck paragangliomas with a documented or suspected *SDHD* mutation. Catecholamine excess may implicate special pre-operative screening in order to minimize hazards of anesthesia and surgery for head and neck paragangliomas or other procedures. Sudden changes in blood pressure and electrocardiographic patterns might be prevented if patients are adequately screened pre-operatively for catecholamine excess. Moreover, the question may be raised of whether an increased prevalence of catecholamine excess is present in asymptomatic carriers of the *SDHD* gene mutation, i.e. without head and neck paragangliomas. This needs to be addressed in future studies.

In conclusion, the prevalence of catecholamine excess and phaeochromocytomas in a well-defined Dutch population with hereditary head and neck paragangliomas due to a germline mutation in the *SDHD* gene is remarkably high. The loss of the wild-type *SDHD* allele in the phaeochromocytomas from patients with germline *SDHD* mutations strongly supports the evidence that these tumors belong to the *SDHD*-associated tumor spectrum.

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